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Transcriptional profiling of *Bacillus amyloliquefaciens* FZB42 in response to seed and root exudates collected under different nutrient regimes

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1. SUMMARY / ZUSAMMENFASSUNG

1.1. Summary

Plant growth-promoting rhizobacteria (PGPR) live in close association with plants and improve their growth. *Bacillus amyloliquefaciens* strain FZB42 is a prominent plant root-colonizing bacterium that is able to stimulate the growth of maize. To decipher the molecular cross-talk between *B. amyloliquefaciens* and crop plants, an exploratory analysis of the effect of seed and root exudates on the transcriptome of *Bacillus amyloliquefaciens* FZB42 was performed. Root exudates were collected from maize plants grown in an axenic hydroponic system under nutrient sufficiency or under deficient supply of nitrogen (N), phosphorus (P), iron (Fe) or potassium (K). An analysis of primary metabolites in the exudates was carried out, compared between treatments, and correlated with the transcriptional profiles of *Bacillus amyloliquefaciens* FZB42 that were gained after incubation of the bacterial culture with the root exudates. Higher exudation rates of citrate were found under Fe deficiency and greater release of γ-amino butyric acid under P deficiency. Based on a negative correlation observed between the average diffusion coefficient of N, P, K, and Fe in soils and the exudation rates of primary metabolites under conditions of N, P, K, or Fe deficiency, it was hypothesized that the exudation of sugars, amino acids and organic acids may reflect the availability and mobility of plant nutrients in soils. In the presence of seed and root exudates collected from nutrient-sufficient plants, genes involved in spore germination, transport and utilization of nutrients, biosynthesis pathways, multidrug transporters, motility and competence development were differentially expressed. In comparison to P, Fe and K, N-deficient maize root exudates caused a more distinguished change in the transcriptome of bacteria when they were in the logarithmic growth phase. During this growth phase, a number of genes coding for ribosomal proteins were down-regulated by N-deficient maize root exudates, indicating that bacterial activity was repressed. Exclusively in the presence of P-deficient maize root exudates, several genes associated to bacterial motility were induced. Moreover, a gene involved in the biosynthesis of the auxin precursor tryptophan was up-regulated by all deficiency treatments. In the transitional growth phase of *Bacillus amyloliquefaciens* FZB42, several genes were commonly down-regulated in different deficiency treatments. This finding is in agreement with previous studies showing that quorum-sensing and

starvation-sensing are integrated to regulate cell entry into the transient phase. Taken together, this is the first study comparing the effect of different nutrient deficiencies on the composition of primary metabolites in root exudates of one plant species and evaluating systematically the transcriptional response of a Gram-positive PGPR to seed and root exudates collected from plants grown under different nutrient regimes. This analysis provides new information about the early communication between plant roots and PGPR and points to involved genes and processes that merit further investigation.

1.2. Zusammenfassung

Pflanzenwachstumsfördernde Rhizosphärenbakterien (plant growth-promoting rhizobacteria, PGPR) leben in enger Assoziation mit Pflanzen und verbessern deren Wachstum. *Bacillus amyloliquefaciens* FZB42 ist ein prominenter Vertreter wurzelkolonisierender Bakterien, die das Wachstum von Maispflanzen stimulieren. Um einen Beitrag zur Aufklärung des molekularen Signalaustausches zwischen *Bacillus amyloliquefaciens* und Kulturpflanzen zu leisten, wurde der Einfluss von Samen- und Wurzelexsudaten auf die Gesamtheit der exprimierten Gene (Transkriptom) von *Bacillus amyloliquefaciens* FZB42 untersucht. In einer axenischen Nährlösungskultur wurden Wurzelexsudate von Maispflanzen gesammelt, die unter ausreichender Nährstoffversorgung oder unter Mangel an Stickstoff (N), Phosphor (P), Eisen (Fe) oder Kalium (K) angezogen wurden. Die gesammelten Exsudate wurden hinsichtlich ihrer Zusammensetzung an primären Metaboliten charakterisiert und dann in Bezug zu den Transkriptionsprofilen der Bakterien gesetzt. Unter Fe-Mangel kam es zu höheren Exsudationsraten an Citrat und unter P-Mangel zu verstärkter Abgabe an γ-Aminobuttersäure. Auf Grundlage einer negativen Korrelation zwischen dem durchschnittlichen Diffusionskoeffizienten von N, P, K und Fe in Böden und den Exsudationsraten von Primärmetaboliten unter N-, P-, K- oder Fe-Mangel wurde die Hypothese aufgestellt, dass die Abgabe von Zuckern, Aminosäuren und organischen Säuren die Verfügbarkeit und Mobilität dieser Pflanzenährstoffe in Böden widerspiegelt. Einige Gene der Sporenbildung und -keimung, des Nährstofftransportes und Stoffwechsels, verschiedener Biosynthesewege, sowie der Mobilität und Kompetenzentwicklung waren nach Inkubation der Bakterien mit Samen- oder Wurzelexsudaten differentiell exprimiert, wenn die Pflanzen keinen Nährstoffmangel hatten. Im Vergleich zu Exsudaten aus P-, Fe- oder K-Mangelpflanzen, führten Exudate von N-Mangelpflanzen zu einer stärkeren Veränderung des bakteriellen Transkriptoms, wenn sich die Bakterien in der logarithmischen Wachstumsphase befanden. Während dieser Wachstumsphase wurde durch N-Mangelexsudate eine Vielzahl von Genen reprimiert, die ribosomale Proteine kodieren, wodurch sich andeutet, dass die bakterielle Aktivität gehemmt wurde. Nur in Gegenwart von P-Mangelexsudaten wurden bakterielle Gene induziert, die mit der Motilität der Bakterien zusammenhängen. Darüber hinaus wurde durch alle Exsudate von Mangelpflanzen ein Gen induziert, das an der Biosyntheses

der Auxinvorstufe Tryptophan beteiligt ist. In der stationären Wachstumsphase von *Bacillus amyloliquefaciens* FZB42 gab es nur geringe Unterschiede in der Expression von Genen durch die unterschiedlichen Exsudate. Dies ist in Übereinstimmung mit früheren Arbeiten, die zeigen, dass "quorum-sensing" und "Mangel-sensing" integriert werden, um den Eintritt der Bakterien in die stationäre Wachstumsphase einzuleiten.

Diese Arbeit vergleicht zum ersten Mal den Einfluss unterschiedlicher Nährstoffmangelsituationen auf das Profil primärer Metabolite in Wurzelexsudaten in einer Pflanzenart und untersucht systematisch die transkriptionelle Reaktion eines Gram-positiven, wachstumsfördernden Bakteriums auf Samen- und Wurzelexsudate von Pflanzen in unterschiedlicher Nährstoffmangelsituationen. Mit dem Hinweis auf Gene und Prozesse, die am molekularen Signalaustausch zwischen Pflanzenwurzeln und PGPR beteiligt sein könnten, leistet diese Arbeit einen Beitrag zum besseren Verständnis der möglichen Kommunikation der beiden Partner innerhalb ihrer Assoziation.

2. GENERAL INTRODUCTION

2.1. Plant-bacteria interactions

Plants and bacteria have cohabited terrestrial ecosystems for approximately 420 million years (Kenrick and Crane 1997). Plant-microbe interactions are, therefore, extremely diverse and generally poorly understood. Heterotrophic organisms rely on external sources of organic carbon to survive and satisfy this requirement through interactions with other organisms. Soil bacteria are largely heterotrophic and occupy resource limited systems. Dead animal and plant materials constitute important organic carbon inputs to soils which support diverse bacterial communities. These communities are involved in critical ecosystem functions such as decomposition. Plant roots release a wide range of carbon-containing compounds that represent another key input of resources to soils. These root-derived compounds, known collectively as rhizodeposits, act not only as a substrate supply for bacteria, but also as signaling molecules that affect the expression of bacterial genes. Certain bacteria detect these signaling molecules using receptors that elicit complex signal transduction cascades that mediate their response to the environment (Brencic and Winans 2005). Once released from roots, the diffusion of rhizodeposits is limited and varies between compounds. The capacity for signaling compounds to affect bacterial activity is thus restricted to the zone of soil that is influenced by the presence and activities of roots (e.g. release of rhizodeposits). This region is known as the rhizosphere and is characterized by higher bacterial densities when compared with root-free soil (Hiltner, 1904; Lynch, 1990).

Before seeds germinate, their vicinity is colonized by certain soil microorganisms. Generally, the microbial biomass and activity in this region (known as the spermosphere) is influenced by soil, seed types and seed genotypes (Buyer *et al.* 1999; Simon *et al.* 2001; Roberts *et al.* 2009). Similar to the rhizosphere, there is an enrichment of bacteria that are specialized to colonize the niches of this special environment (Buyer *et al.* 1999; van den Broek *et al.* 2005; Child *et al.* 2007). An additional input of nutrients and/or the presence of attracting signaling compounds appears to influence this phenomenon, as observed in some studies involving Gram-negative bacteria, especially *Pseudomonas* sp (Gupta Sood 2003; Matilla *et al.* 2007). Some plant-associated microorganisms have a positive effect on seed germination (Shweta *et al.* 2008), seedling development (Selvakumar *et al.* 2008), and

resistance to plant diseases (Ko *et al.* 2009; Verhagen *et al.* 2010). The manipulation of microbial communities associated to plants has received attention in agriculture with the purpose to protect plants from diseases and increase crop yields (Okon and Itzigsohn 1995; Adesemoye *et al.* 2008).

Plant-associated bacteria are often attached to seeds, which can also be a source of pathogens. The interactions ongoing in the vicinity or surface of seeds represent the first contact between a germinating plant and soil microorganisms. Therefore, these relationships may play a major role in defining the nature of the association, namely whether the effect on plants is beneficial or detrimental. Seed inoculation and the subsequent establishment of beneficial bacteria in the rhizosphere of crop species is regarded as an environmentally friendly practice since it may allow a reduction in the use of fertilizers and/or pesticides (Prasad and Sinha 1977; Dey *et al.* 2004; Correa *et al.* 2007). Root colonization seems to be linked with a successful seed adhesion (Espinosa-Urgel *et al.* 2000; Hinsa *et al.* 2003). However, the biology of the spermosphere has not been intensively studied. The first stage during seed germination is the imbibition, which is a physical process in which water present in surrounding soil moves by osmosis into the seed. This stage is largely governed by: 1) the protein, lipid and starch composition of the seed; 2) the water potential between the inner seed tissues and the outer environment of the seed, and 3) the permeability of the seed coat (Nelson 2004). Seed cell membranes are structurally altered by the rapid influx of water, which results in the leakage of solutes and low molecular weight metabolites into the imbibition solution, and consequently a rupture of the seed coat due to the increasing hydrostatic pressure within the seed (Bewley 1997). Typically, large amounts of seed exudates are released within the first twelve hours after sowing (Nelson 2004). Seed exudates may be involved in the attraction of rhizosphere microorganisms and may modulate important bacterial properties which confer the ability to adhere and grow competitively in the seed vicinity. Indeed, seed exudates collected from two varieties of soybean induced a chemotatic response, supported active cell division and induced biofilm formation in *Bacillus amyloliquefaciens* BNM 339. However, root exudates did not have the same effect (Yaryura *et al.* 2008). It is still unclear what differences between seed and root exudates composition lead to different responses in bacteria.

2.2. Root-derived compounds and mechanisms for their release

A myriad of organic and inorganic chemical compounds are released by plant roots. These compounds include carbohydrates, organic acids, phenolic compounds, amino acids, fatty acids, sterols, vitamins, enzymes, purines/nucleosides and also inorganic and gaseous molecules, such as HCO_3^-, OH^-, H^+, CO_2 and H_2 (Dakora and Phillips 2002; Hartmann *et al.* 2009). The lysis of sloughed-off cells and tissues results in the release of lysates, which, together with secreted root exudates, define the process called organic rhizodeposition (Neumann and Römheld 2007). The identification of the chemical composition of rhizodeposits is extremely important for understanding their ecological functions. Most of the released compounds are common plant constituents derived from plant processes such as photosynthesis. Current evidence suggests that certain components that are present in root exudates are involved in a variety of functions, including the modulation of nutrient availabilities (Cakmak *et al.* 1998; Penaloza *et al.* 2002; Wang *et al.* 2008; Lemanceau *et al.* 2009), increased tolerance to heavy metals (Kidd *et al.* 2001; Osawa and Kojima 2006), or attraction of rhizobacteria (Bais *et al.* 2004; Sanon *et al.* 2009).

The root exudation can occur either passively, by leakage and diffusion, or actively, by secretion from intact root cells. Processes such as plant growth, nutrient and water uptake, rhizodeposition and microbial activity alter the rhizosphere by creating longitudinal and radial gradients (Marschner *et al.* 1996). However, these gradients are eliminated in liquid nutrient cultures by active mixing (von Wirén *et al.* 1993). In this regard, the use of solid media (e.g. soil, sand) for root exudate collection may preserve rhizosphere gradients, but is likely to cause mechanical injury of roots during removal of the root system and thus leakage of intracellular constituents, which may lead to an overestimation of exudation rates. In the case of hydroponic cultures, the integrity of the root system is notoriously preserved. Another great advantage of hydroponics is that it enables a qualitative and quantitative analysis of exudation patterns in response to well-monitored pre-culture conditions (Neumann and Römheld 2007).

The three main mechanisms involved in root exudation are diffusion, transport via ion channels and transport via vesicles (Bertin *et al.* 2003). Low molecular weight substances, such as sugars, amino acids, carboxylic acids and phenolics are released by a passive process along a steep concentration gradient between the cytoplasm of intact root cells and the external soil solution. The physiological state of root cells and the polarity of

root exudates will strongly determine their permeation through the plasma membrane. The controlled release of particularly high amounts of certain carboxylates, such as citrate, malate, and phytosiderophores in response to a particular nutritional deficiency stress or heavy metal toxicity, may be triggered by more specific mechanisms. In this regard, ion channels have been identified as mediators for the export of these compounds out of the cell (Badri *et al.* 2008; Zhang *et al.* 2008). Alternatively, mucilage, which consists of polysaccharides and uronic acids, and exoenzymes are mainly secreted via vesicular transport (Neumann and Römheld, 2007).

Studies using ^{14}C labeling techniques have shown that microbial colonization and the presence of microbial metabolites enhance root exudation (Meharg and Killham 1991; Phillips *et al.* 2004). Therefore, the use of axenic cultures for root exudates collection may underrate exudation. Nevertheless, microorganisms are able to alter root exudates qualitative and quantitatively by degrading exuded organic compounds and releasing microbial metabolites (Meharg and Killham 1991; Karnwal 2009; Muratova *et al.* 2009). For this reason microbial contamination of nutrient solutions can strongly mislead biological interpretations concerning the quantitative and qualitative chemical characterization of root exudates.

2.3. Essential elements in plant nutrition and their functions

Mineral elements that are essential for the growth of all higher plants are nitrogen (N), phosphorus (P), sulphur (S), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), nickel (Ni), molybdenum (Mo), boron (B) and chlorine (Cl). In order to be considered essential, three pre-requisites must be fulfilled: i) the deficiency of the element hinders the completion of the plant's life cycle; ii) the lack of the considered element triggers a specific deficiency, and iii) the element is directly involved in the nutrition of the plant by being part of an essential metabolite or a co-factor for enzyme activity (Kirkby and Mengel 2001). Essential elements can be divided into four main groups along their physiological roles (Kirkby and Mengel 2001). The first group includes N and S, which build up the organic plant material. Nitrogen is a major component of numerous organic compounds that are crucial for plant structure and functioning. Nucleic acids, proteins (enzymes), adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NAD) and flavoproteins are important examples (Lewis 1991). Another group of elements is formed by P and B. They are taken up as

organic anions or acids and may be bound to hydroxyl groups of sugars, forming phosphate- and borate-esters (Kirkby and Mengel 2001). Nucleic acids, phospholipids and ATP are essential compounds for plant metabolism which have P as one of the major elements in their structure. In addition, important processes such as photosynthesis, respiration and regulation of enzyme activities require the presence of P (Raghothama 1999). The third group is composed by K, Na, Ca, Mg, Mn and Cl. They are either adsorbed to nondiffusible organic anions or occur in the free ionic state in the plant cell (Mengel and Kirkby 2001). Potassium takes part in protein synthesis, photosynthesis and activates enzymes. It also helps to maintain the cation-anion balance in the cytosol and vacuole. Additionally, it plays a fundamental role in osmoregulation during stomatal movements, cell expansion and tropisms (Maser *et al.* 2002). Potassium and magnesium are equally important for loading the phloem with sucrose (Cakmak *et al.* 1994a). The forth group consists of Fe, Cu, Zn, Ni and Mo. They are mostly attached to or bound by proteins. For instance, Fe acts as an electron donor and acceptor, taking part in the electron-transport chains of photosynthesis and respiration (Connolly and Guerinot 2002), or in redox processes during nutrient assimilation (Van Hoewyk *et al.* 2007).

2.4. Effect of nutrient deficiencies on the release of root exudates

Plants respond to nutrient deprivation by morphological and/or physiological adaptations. With regard to root morphology, nutrient deficiencies may lead to shorter primary roots, but longer lateral roots and increased number of root hairs. At the physiological level, plants may adapt by an enhanced synthesis of organic compounds that alleviate stress or deficiency symptoms. This is also the case for root exudates that are released to enhance the mobilization of sparingly soluble nutrient elements in the rhizosphere.

Although N is the mineral nutrient required by plants in largest amounts (Epstein and Bloom 2005), very few studies have investigated the effect of N deficiency on the chemical composition of root exudates. Pine roots exuded ten times less amides and amino acids under N-deficient conditions than those grown under N sufficiency (Bowen 1969). Decreased exudation of carboxylates, total sugars and total amino acids was observed in *Phaseolus vulgaris* under low N supply (Haase *et al.* 2007). In addition, nitrogen limitation may also enhance the exudation of strigolactones, which are signals for root parasitic plants and arbuscular mycorrhizal fungi (Yoneyama *et al.* 2007).

Maize roots exposed to low K supply exuded greater amounts of total sugars, organic acids and amino acids (Kraffczyk *et al.* 1984). Whether this reflects an adaptation of the primary metabolism to the unavailability of K in plant cells is not clear. However, K deficiency has been shown to deplete pyruvate concentrations in roots to the benefit of sugars (Amtmann and Armengaud 2009).

Iron is one of the major constituents of soils, where it is mostly present in the oxidized state (Fe^{III}). In general, Fe is poorly available to plants because of its low solubility at neutral to alkaline pH (Neumann and Römheld 2007). It is particularly insoluble in calcareous soils and oxygenated environments. There are two main strategies used by plants for iron acquisition. 'Strategy I' is reduction-based and adopted by dicotyledonous plants and non-graminaceous monocotyledons. In this case, Fe deficiency stimulates proton extrusion by enhanced activity of the plasma membrane ATPase. Therefore, the acidity of the rhizosphere is increased and consequently Fe^{III} is solubilized. The Fe^{III} solubilization is supported by the exudation of chelating compounds, in particular phenoles. They may form complexes with Fe^{III} and to some extent are responsible for its reduction. The other strategy is the chelation-based Fe acquisition – known as 'Strategy II' – which is performed by grasses. Highly effective chelators for Fe^{III}, the mugineic acids, largely known as phytosiderophores (PS), are released and root cells take up Fe^{III}-PS chelates. Thus, differently to the case of Strategy I plants, Fe^{III} is not reduced prior to uptake by the root cells. The phytosiderophores may then be released back into the rhizosphere (Neumann and Römheld 2007).

Phosphorus is often a limiting nutrient in soils because of processes like adsorption, precipitation, or conversion into organic forms. Therefore, more than 80% of phosphorus fertilizers can become unavailable for plant uptake over time (Holford 1997). The two main phosphorus forms in soils are inorganic orthophosphate (Pi) or organic phosphate, which mostly comprise of phytic acid (inositol hexaphosphate). In nutrient-deficient environments, plants utilize two major adaptive strategies to access soil phosphorus: i) maximization of P use by remobilization of internal P, ii) improved P acquisition by enhanced exudation of phosphatases and organic acids, increased expression of Pi transporters, or modification of root growth and architecture (Raghothama 1999).

2.5. Plant-associated bacteria - their effect on plants and use in agriculture

Plant growth-promoting rhizobacteria (PGPR) is the term used to define free living soil bacteria that exert beneficial effects on plants (Kloepper *et al.* 1989). Several plant traits have been documented to be improved by interactions with PGPR, such as increased nutrient acquisition, tolerance to abiotic stress, enhanced yield, shoot and root weight, higher chlorophyll contents or leaf area, accelerated seed germination rate or delayed senescence (Adesemoye and Kloepper 2009). Different classes of bacteria can be distinguished according to the mechanisms that promote plant growth, namely biofertilizers, rhizoremediators, phytostimulators and stress controllers (Lugtenberg and Kamilova 2009). Plants may be directly provided with nutrients by biofertilizers, for instance, reduced N by N_2-fixing bacteria (Vanrhijn and Vanderleyden 1995), or soluble P by phosphate solubilizers (Vassilev *et al.* 2006). Other bacteria are able to degrade soil pollutants using root exudates as their major nutrient source, being known as rhizoremediators (Kuiper *et al.* 2004). Substances produced by phytostimulators improve plant growth (Williams *et al.* 2003; Glick *et al.* 2007). Consequently, PGPR represent an enormous potential for agriculture and horticulture. Market-orientated crop production is still strongly reliant on chemical fertilizers and pesticides. Fertilizers are used annually in the order of approximately hundred million tons worldwide (Glick *et al.* 1999). Under certain circumstances, environmental threats caused by their use may arise. Global climate change due to high energy requirement for the production of nitrogen fertilizers, leaching and run-off of fertilizer-derived nutrients, eutrophication of water resources, and accumulation of fertilizer residues such as Cd from P fertilizers (Menzi and Gerber 2007) are typical examples. In future, the expansion of intensive agriculture and therefore a massive and continuous input of fertilizers is expected to even increase to meet the food needs of a still-growing world population. Undoubtedly, conventional agriculture needs to be improved by sustainable practices. The use of microbial biofertilizers and biopesticides may become a component if they can efficiently replace chemical fertilizers and pesticides. Arbuscular mycorrhizal fungi (AMF) and especially rhizobia are successful examples and have become the most commonly used inoculants in agriculture (Gentili and Jumpponen 2006). However, in the 1990s other products based on PGPR became available in the

market and many of them contain *Bacillus* strains (Kloepper *et al.* 2004). Together with the genera *Pseudomonas*, *Arthrobacter*, *Clostridium*, *Achromobacter*, *Micrococcus*, and *Flavobacterium*, *Bacillus* species are the most common types of bacteria isolated from soil samples (Darbyshire and Greaves 1972; Hallmann *et al.* 1999). Bacilli are also particularly attractive for practical use as inoculants because they form stable endospores, which can survive heat and desiccation that occur during the preparation of bacterial formulations (Piggot and Hilbert 2004). *Bacillus* species are known to exert beneficial effects on various plant species, such as tomato (Choudhary and Johri 2009; Lim and Kim 2009), maize (Oliveira *et al.* 2009), sunflower (Srinivasan and Mathivanan 2009), or strawberry (Essghaier *et al.* 2009), especially by suppressing pathogens. Their ability to sporulate is advantageous for storage and thereby a longer shelf life. Additionally, they are easy to cultivate due to their nutritional versatility (Ross *et al.* 2001; Tiago *et al.* 2004). Especially *Bacillus amyloliquefaciens* ecotypes have been shown to efficiently colonize roots and therefore seem to be able to overcome the antibacterial action of some plant root exudates (Reva *et al.* 2004). *Bacillus amyloliquefaciens* strains have also been proved to promote salt tolerance in eggplants and pepper (Bochow *et al.* 2001), suppress root-knot nematode infections (Burkett-Cadena *et al.* 2008), increase the yield of tomato (Grosch *et al.* 1999; Guel *et al.* 2008) or cucumber (Grosch *et al.* 1999), and enhance the shoot, root weight and length of maize seedlings (Idriss *et al.* 2002). Moreover, it was documented that a *Bacillus amyloliquefaciens* strain has minor impacts on rhizosphere microbial communities (Correa *et al.* 2009).

Bacillus amyloliquefaciens strain FZB42 is a prominent plant root-colonizing bacterium that is able to suppress plant pathogens and stimulate growth of several plant species, including maize (Chen *et al.* 2007; Chen *et al.* 2009a; Chen *et al.* 2009b). A biofertilizer product containing spores of *B. amyloliquefaciens* FZB42 (RhizoVital®42, ABiTEP GmbH, Berlin, Germany) has been distributed in Europe and China. However, the molecular cross-talk between *B. amyloliquefaciens* and crop plants still remains to be deciphered. Maize represents an appropriate model plant to study bacterial responses to plant-released compounds, because it is the third most important cereal crop worldwide (Fageria *et al.* 1997), and its root exudates have been studied for decades (Vancura 1967). Information is available, therefore, to facilitate interpretation of observations. Knowledge on the effects of plant-derived compounds on gene expression of Gram-positive PGPR is poor. *Bacillus* formulations have been successfully applied using seed coating technologies and therefore seed exudates may play a role in the establishment of the bacteria in the

initial stages of the plant-bacteria interaction. Given that the complete genome of *Bacillus amyloliquefaciens* FZB42 is available, approaches involving comparative genomics have been performed to detect genes that may be potentially involved in the plant-associated lifestyle by comparison with the domesticated model strain *Bacillus subtilis* 168 (Chen *et al.* 2007). However, functional genomic analyses have not been performed so far.

2.6. The advantage of microarray analysis for the investigation of plant-bacteria associations

The simultaneous expression of thousands of genes can be investigated under different conditions employing microarrays. This technology allows the comparison of mRNA amounts in biological samples such as tissues or cells. After the RNA is isolated, equal quantities are reverse-transcribed and differently labeled. Probes of DNA sequences corresponding to the genes of interest are spotted onto a grid on a glass slide, a quartz wafer, or a nylon membrane, thereafter known as a chip. The relative quantities of transcripts in the samples are determined by the measured intensity of the label remaining bound to the cDNA (Schena *et al.* 1995; Bowtell 1999). Oligonucleotide microarrays and cDNA microarrays are the most commonly used platforms. The first consists of synthetic probe sequences designed on the basis of sequences accessible in databases, while the latter comprise cloned probe molecules corresponding to partially or pre-characterized expressed sequences (Gershon 2002). The first step of microarray image data analysis includes background elimination, filtration, and normalization, which should eliminate the systematic variation among chips and allow a comparison among treatments. Then, different microarrays are compared to a standard intensity value to achieve normalization. This standard value can be the intensity of housekeeping genes (whose expression is supposed to be constant), spiked targets or the overall intensity of all genes on the microarray. Base 2 logarithmic transformation is frequently used for a better distribution of expression values (Murphy 2002; Hovatta *et al.* 2005).

Microarray experiments result in datasets that comprise thousands of variables, in this case gene expression levels. Interpretation of such data is greatly facilitated by multivariate statistics. Ordination techniques are particularly useful as they summarize the main trends in the variation of the data into reduced dimensional space that can be visualized graphically. ‘Non-constrained’ ordination methods such as ‘Principal

Component Analysis' (PCA) and 'Correspondence Analysis' (CA) represent, in the first few axes, the maximal variation in the data. 'Constrained' ordination methods, on the other hand, represent, in the first few axes, the maximum variation that can be attributed to a constraining variable, such as a factor for a treatment structure (Kenkel *et al.* 2002; Leps and Smilauer 2003). Both PCA and CA are commonly used to interpret microarray data, although CA is thought to provide a better representation of the relationships between samples and genes (Fellenberg *et al.* 2001; Wouters *et al.* 2003). A constrained ordination method, known as 'Between Group Analysis' (BGA), has been successfully applied to microarray data analysis. This approach allows the investigator to enter information regarding the treatment structure of the experiment and to visualize the maximum variation that can be attributed to the treatment groups (Culhane *et al.* 2002; Baty *et al.* 2006). BGA can be based on PCA or CA, in which case the method is equivalent to redundancy analysis (RDA) or canonical correspondence analysis (CCA), respectively. BGA-CA is considered particularly appropriate for analysis of microarray data because correspondences between genes and samples within groups can be visualized, which assists the identification of genes that discriminate between the groupings (Fellenberg *et al.* 2001; Culhane *et al.* 2002). Another more rigorous technique used to identify genes that discriminate between groups in a BGA uses bootstrapping to assess whether the gene contributions are statistically significant (Baty *et al.* 2008). Ordinations may also be constrained by other variables, such as concentrations of root exudate components. Ordinations of this type can be used to assess the impact of particular root exudates on the expression of certain genes within treatment groups. Typically, such interactions are assessed by investigating the strength of correlations between non-constrained ordination scores and additional variables (e.g. root exudates) as fitted vectors. The vectors are superimposed on a non-constrained ordination as arrows which indicate the direction of the change in the constraining variable and its correlation with the ordination is proportional to their length (Oksanen 2010).

Due to statistical problems intrinsic to the microarray technology, results should be validated by independent methods for gene expression measurements, such as real time quantitative PCR, which is very sensitive. The amount of DNA produced in each PCR cycle is proportional to a fluorescent signal that is measured by this assay. A threshold background fluorescence (C_t) set on the PCR cycle at which the fluorescence starts to increase characterizes individual samples. Samples with larger quantities of the target cDNA present lower C_t values (Nolan *et al.* 2006).

2.7. Aims of the thesis

Knowledge of plant-bacteria associations is generally poor, particularly at the molecular level. This knowledge gap limits the scope for hypothesis driven research. Exploratory analyses, such as transcriptomics and metabolomics, facilitate the identification of genes involved in plant-microbe interactions and the compounds that elicit responses. Candidate genes and compounds can then be validated and specific hypothesis concerning metabolic pathways and processes can be tested.

The objectives of this thesis were to: 1) identify bacterial genes involved in the response to seed and root exudates using microarray analyses, and 2) link patterns of gene expression with specific plant metabolites (root exudates). Given that the nutritional status of plants affects root exudation and plant responses to PGPR, roots were exposed to different nutrient deficiencies prior to root exudate collection. The Gram-positive plant growth promoting rhizobacteria *Bacillus amyloliquefaciens* FZB42 and maize plants were used in this study.

The first results chapter reports how seed and root exudates affect bacterial gene expression. An analysis of the dominant primary metabolites of exudates, namely amino acids, organic acids and sugars, is presented. The differentially expressed genes affected by seed and root exudates are described. Additionally, bacterial genes differentially expressed by seed and root exudates are systematically compared.

The second results chapter reports how root responses to different nutritional deficiencies are reflected in the exudation of dominant primary metabolites. A comparison of qualitative and quantitative changes in profiles of maize root exudates collected from axenically-grown plants exposed to N, P, Fe and K deficiencies is presented.

The third results chapter reports how root exudates collected from plants grown under different nutritional deficiencies affect global gene expression of PGPR. A description of bacterial genes differentially expressed by root exudates collected from maize grown under N, P, Fe and K deficiencies is given. A systematic comparison of transcriptional profiles gained after incubation with different exudates is presented. Additionally, candidate primary metabolites that elicit changes in bacterial profiles are identified.

The final chapter summarizes the results and discusses them in the context of plant-bacteria interactions and plant responses to nutrient deprivation. Root-released compounds associated to N-, P-, Fe- and K- deficiencies are identified. Finally, genes and processes potentially involved in bacterial responses to seed and nutrient-sufficient root exudates, as

well as to N-, P-, Fe- and K- deficient maize root exudates, are identified. By analyzing changes in bacterial gene expression and characterizing dominant metabolites in seed and root exudates, this is, to my knowledge, the first study that accounts for both partners in plant-bacteria associations.

3. MATERIAL AND METHODS

3.1. Sterilization of maize seeds

Maize seeds (*Zea mays* L. var. Surprise) were shaken for 3 min in 96% ethanol, 30 min in 3% sodium hypochlorite solution, rinsed twice in sterile distilled water (SDW) and then left to soak in SDW for 4 h at 25°C. Sterility of seeds was confirmed by the absence of microbial growth in liquid Luria-Bertani (LB) and semi-solid Tryptic Soy Agar media (TCA, 0.3% Agar) to which seeds had been added and incubated for seven days at 37ºC.

3.2. Plant growth conditions

Surface sterilized seeds were pre-germinated on solid half-strength Murashige Skoog medium containing 1% sucrose and 0.7% agar (Difco, Becton Diekison) and maintained at 28°C in the dark. Seedlings were transferred to glass bottles designed to facilitate axenic growth conditions (Figure 1) (von Wirén *et al.* 1995).

Figure 1: Vessels used to culture maize in hydroponics up to the forth-leaf stage (15 days after germination) with roots kept under axenic conditions

The hydroponic system was permanently aerated and maintained in a controlled environment chamber at 60% humidity, 8 h darkness at 20ºC, and 16 h light at 280 µmol photons $m^{-2}.s^{-1}$ and 25°C. The composition of the nutrient solution was as follows: 2.0 mM

$Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KCl, 0.1 mM KH_2PO_4, 1.0 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.2 µM $CuSO_4$, 0.01 µM $(NH_4)_6Mo_7O_{24}$ and 100 µM Fe(III)-EDTA.

3.3. Seed exudates collection

One hundred seeds (approximately 36 g) were added to 100 milliliters (mL) of sterile distilled water placed in 500 mL foil-wrapped Erlenmeyer flasks and kept at 28°C for 12 hours on a rotary shaker at 120 rpm. After collection, 100 microliters (µL) of each sample was inoculated to LB plates to check for sterility. Contaminated batches of seeds were discarded. Each replicate was split in two aliquots and freeze-dried. One aliquot was used for the transcriptional profiling assay and the other was used for the chemical analysis.

3.4. Root exudate collection

In the nitrogen, phosphorus, iron or potassium deficiency treatments the corresponding nutrient was omitted from the nutrient solution. To maintain the ion balance of the nutrient solution, $Ca(NO_3)_2$ was replaced by $CaCl_2$, KH_2PO_4 was replaced by $NaH_2PO_4.2H_2O$, and K_2SO_4 by $MgSO_4$. The nutrient solution was changed once in the first seven days and from then on after each root exudate collection. During every nutrient solution replacement, a 100 µL aliquot was removed and spread on a solid LB media to check for sterility. Contaminated vessels were discarded.

As the focus of this study was on primary responses of root exudates to individual nutrient deficiencies, nutrient starvation periods were chosen according to the plant demand for each nutrient. Relative growth rates based on dry weights have been considered a reliable measure of nitrogen stress (Greenwood 1976) and are not altered significantly up to three days of nitrogen starvation (Lee and Rudge 1986), even though nitrogen deficiency responses such as an enhanced expression of ammonium and nitrate transporters are induced within the first 24 h of deficiency (Ono *et al.* 2000; von Wirén *et al.* 2000). Therefore, plants were subjected to two days of nitrogen deficiency. With respect to the relatively high plant demand for K and the rapid induction of K deficiency responses (Marschner 1995), plants were also subjected to two days of K deficiency. In agreement with the relatively lower plant demand for P and the slower induction of typical deficiency responses (Marschner 1995; Nagy *et al.* 2006), the phosphorus starvation period

was set to three days. Plants were subjected to Fe deficiency for six days, which is, according to previous reports (Schaaf *et al.* 2004; Meda *et al.* 2007), a typical time period required to induce Fe deficiency responses. To increase the likelihood of including the peak of exudate release, root exudates were collected over a period of three subsequent days, pooled, freeze-dried and then stored at -20ºC.

Root exudates were collected from all treatments 13, 14 and 15 days after germination (forth-leaf stage). This plant developmental stage was used to ensure that carbon associated with seed reserves was exhausted prior to root exudate collection. Two hours after the onset of the light period the nutrient solution was replaced with autoclaved ultrapure water in which root exudates were collected for six hours. The root system was aerated throughout the collection period to avoid oxygen limitation.

3.5. Chemical analyses of root exudates

The analyses were focused on sugars, amino acids, and organic acids. It was omitted from analysis root exudates that are specifically released under certain nutrient deficiencies, such as phytosiderophores released under Fe deficiency, to ensure comparability of exudate profiles.

Amino acids were determined using a Shimadzu HPLC system equipped with a fluorescence detector. For each sample 40 µL was derivatized by 160 µL OPA (o-phthaldialdehyde) reagent and 20 µL of the resulting mixture was injected and separated on a GROM-SIL OPA-3 column (3 µm, 125 x 4.0 mm) using gradient elution by solvent A (25 mM phosphate buffer pH 7.2 with 0.75 % tetrahydrofuran) and solvent B (methanol: acetonitrile: 25 mM phosphate buffer pH 7.2 (35:15:50) (v:v:v)). Gradient profile: 0-2 min, 0% B; 2-10 min, 0-50% B; 10-15 min, 50-60% B; 15-20 min, 60-100% B; 20-25 min, 100% B; 25-26 min, 100-0% B; 26-35 min, 0% B. The flow rate was 1 ml/min. Subsequent fluorescence detection of the derivatives was performed at an excitation wavelength of 330 nm and 450 nm for fluorescence emission.

Organic acids were determined by ion chromatography (Dionex, Idstein, Germany) equipped with conductivity detector and suppressor ASRS Ultra II. For each sample a 20 µl volume was separated on the Dionex IonPac AS 11 HC column (2 x 250 mm) using gradient elution starting from 4 mM KOH (0-4 min), then a stepwise linear increase to 80 mM over 28 min (4-10 min, 4-15 mM; 10-14 min, 15-25 mM; 14-24 min, 25-80 mM; 24-28 min, 80 mM), followed by re-equilibration to 4 mM for 2 min and 10 min equilibration

by 4 mM KOH. The flow rate was 0.2 ml/min. Organic acids were identified by comparison of retention time with known standards.

Sugars were determined by GC-TOF-MS (Lisec *et al.* 2006). A lyophilized 75 µL aliquot of root exudates was dissolved in 50 µL methoxamine hydrochloride in dry pyridine and derivatized for 2 h at 37ºC followed by 30 min treatment with 50 µL N-methyl-N-trifluoroacetamide at 37°C. A volume of 1 µL was injected into the GC column in a splitless mode.

3.6. Statistical analysis of seed and root exudates constituents

Differences between seed and root exudates in concentrations of amino acids, organic acids and sugars were tested using *t*-test at the 95% confidence interval. The main effect of nutrient deficiencies on the quantity of root exudate components was assessed using ANOVA and differences between individual deficiency treatments were determined using Tukey's Honestly Significant Difference (HSD) test. All significant differences were considered at the 95% confidence interval. These analyses were implemented using the core functions within the R statistical environment (R-Development-Core-Team 2005). To determine whether various nutrient deficiencies led to significantly different exudate profiles, data were first converted to z-scores (the amount of a compound within a sample minus the mean and divided by the standard deviation of that compound over all samples) and then analyzed using ANOSIM with 999 random permutations (Clarke 1993). This analysis was performed in the Primer 6 statistic software (Primer-E Ltd. Plymouth, UK). The structure and composition of exudate profiles (z-score data) were analyzed using 'Principal Component Analysis' (PCA) based on the correlation matrix. To interpret Principal Component (PC) axes, a matrix of Pearson's correlation coefficients was calculated and associated p-values for the latent vectors (loadings) from the PCA analysis and the z-scores for each exudate component. This facilitated interpretation based on significant relationships between exudate components and PC axes only. The abbreviations of chemical compounds were plotted in the graphs to further facilitate data interpretation. To investigate whether total exudation rates were related to the mobility of nutrients in soil, a linear regression was performed using log nutrient diffusion coefficients in soil taken from the literature (Nielsen 2006) and the measured exudation rates for organic acids, amino acids and carbohydrates. The models, PCA, correlation, and regression

analyses were implemented using the GenStat statistical system (GenStat 11th edition, Lawes Agricultural Trust; VSN International, Hemel Hampstead, UK).

3.7. Transcriptional profiling experiments

Two experiments were performed to analyse the effect of seed and root exudates on the global gene expression of FZB42. The first compared the transcriptional response of FZB42 to root exudates collected from maize grown under optimal nutrient conditions, and seed exudates. The samples used for RNA isolation were cultures of FZB42 supplemented with seed or root exudates compared with cultures to which exudates have not been added. The second experiment compared the transcriptional response of FZB42 to root exudates collected from maize grown under different nutrient deficiencies. Transcript levels of bacteria incubated with exudates from nutrient deficient plants were compared with those of bacteria that were exposed to exudates collected from plant grown under optimal nutritional conditions.

3.7.1. Incubation of cells with seed and root exudates

A single overnight colony of *Bacillus amyloliquefaciens* FZB42 was inoculated in a pre-culture medium containing 0.7% tryptone, 0.3% peptone, 0.1% glucose, 0.5% NaCl and glucose 0.1% (1C). When the optical density at 600 nm (OD) of the pre-culture achieved 1.0, an aliquot with volume corresponding to 1% of the main culture was added into the 1C medium supplemented with 10% of soil extract. Root exudates were added into the main culture up to a final concentration of 250 micrograms (µg) dry weight per mL of culture medium, to normalize the added quantity of seed and root exudates between and within samples. The main culture was then incubated at 24^{o}C, under 210 revolutions per minute (rpm). Bacterial cells were harvested at ODs 1.0 and 3.0, which corresponded to the logarithmic and transitional phases, and 3.0, which corresponded to the transitional phase. The initial volumes were 15 mL and 7 mL, respectively. 10 mL of bacterial culture were mixed with 5 mL of killing buffer, composed of 20mM Tris-HCl, 25mM $MgCl_2$ and 20mM NaN_3, and then centrifuged at 5000 rpm, at 4^{o}C, for 4 min. The supernatant was discarded and the pellet was resuspended in 1 mL of killing buffer, transferred to a 1.5 mL eppendorf tube and centrifuged again at 9000 rpm, at 4^{o}C, for 4 min. The pellet was then stored after quick freezing in liquid nitrogen at –80°C.

3.7.2. RNA purification and labeling of cDNA, hybridization and image acquisition.

Total RNA from bacterial cultured cells was isolated as described in the manual of the RNA purification kit NucleoSpin®RNA L (MACHEREY-NAGEL GmbH & Co.KG, Düren, Germany). Starting from 10 to 30 µg of total RNA, random hexamer primers (Qiagen-Operon, Hilden, Germany), Superscript III RT (Stratagene, La Jolla, CA), and 0.5 mM dNTP, dTTP aminoallyl-dUTP (1:4, dNTPs, PeqLab, Erlagen, Germany; aa-dUTP: Sigma-Aldrich, Taufkirchen, Germany) were used to synthesize aminoallyl-modified first-strand cDNA by reverse transcription. The reaction was incubated at 42°C for 90 min. After hydrolysis and clean-up using CyScribe GFX purification columns (GE Healthcare, Munich, Germany), Cy3- and Cy5-N-hydroxysuccinimidyl ester dyes (GE Healthcare) were coupled to the aminoallyl-labeled first-strand cDNA. Uncoupled dye was removed using the CyScribe GFX Purification kit. Microarrays were prehybridized for 45 min at 42°C in Easyhyb hybridization solution (Roche Diagnostics, Mannheim, Germany) supplemented with 5 µg/ml sonicated salmon sperm DNA. Following prehybridization microarrays were washed in Milli-Q water (21°C, 1 min), submerged in ethanol (21°C, 10 s) and centrifuged (185 × *g*, 3 min, 20°C). Hybridization was performed at 42°C for 16 h in Easyhyb hybridization solution (Roche Diagnostics, Mannheim, Germany) supplemented with 50 µg/ml sonicated salmon sperm DNA in a final volume of 65 µl under a cover slip. Before applying the hybridization solution to the microarray, it was denatured for 5 min at 65°C. Microarrays were washed once in 2× SSC, 0.2% SDS (5 min, 42°C), twice in 0.2× SSC, 0.1% SDS (2 min, 21°C) and twice in 0.2× SSC (2 min, 21°C). Then, slides were dried by centrifugation (3 min, 185 x *g*, 20°C) and scanned at a pixel size of 10 µm using the ScanArray 4000 microarray scanner (Perkin-Elmer, Boston, MA, USA).

3.7.3. Microarray Data analysis

Mean signal and mean local background intensities were obtained for each spot of the microarray images using the ImaGene 5.0 software for spot detection, image segmentation and signal quantification (Biodiscovery Inc., Los Angeles, CA, USA). Spots were flagged as 'empty' in case $R \leq 1.5$, where R = (signal mean - background mean)/background standard deviation. The remaining spots were considered for further analysis. The logarithm to the bases 2 of the ratio of intensities was calculated for each spot using the formula $M_i = \log_2 (R_i/G_i)$. $R_i = I_{ch1i} - Bg_{ch1i}$ and $G_i = I_{ch2i} - Bg_{ch2i}$, where I_{ch1i} or I_{ch2i} is the

intensity of a spot in channel 1 or channel 2 and Bg_{ch1i} or Bg_{ch2i} is the background intensity of a spot in channel 1 or channel 2, respectively. The mean intensity was calculated for each spot $A_i=\log_2 (R_iG_i)^{0.5}$ (Dudoit *et al.* 2002). A normalization method based on local regression that account for intensity and spatial dependence in dye biases was applied. Within a print tip group normalization was performed according to (Yang *et al.* 2002), *Mi* = log2 *(Ri/Gi)* → log2 *(Ri/Gi)* − *cj(A)* = log2 *(Ri/*[*kj(A)Gi*]*)*, where *cj*(*A*) is the lowest fit to the MA plot for the *j*th grid only (i.e. for the *j*th print tip group), *j* = 1, . . . , J, and J denotes the number of print tips. A floor value of 20 was introduced before normalization to be able to use logarithmic values in case of negative *Ri* or *Gi* values. Genes significantly up- or down-regulated were identified by t-statistics (Dudoit *et al.* 2002). Genes were regarded as differentially expressed if $P \leq 0.05$, M-values ≥ 0.9 or ≤ -0.9. Normalization and t-statistics were carried out using the EMMA 1.0 microarray data analysis software developed at the Center for Genome Research at Bielefeld University (http://www.genetik.uni-bielefeld.de/emma) (Dondrup *et al.* 2003).

3.7.4. Multivariate analysis of microarray experiments

A BGA-CA was performed for logarithmic (OD 1.0) and transitional (OD 3.0) growth phases to associate genes with pre-defined sample classes (deficiency treatments). Genes were ranked according to their influence on each class modality. Nine hundred and ninety nine bootstrapped samples were built with the purpose of evaluating the empirical distribution of the gene influence within the data set. Newly calculated gene coordinates after the partial bootstrap were illustrated graphically by convex hulls. The proportion of overly unstable genes (with a p-value ≥ 0.05) was estimated by boxplots of gene contributions. The BGA-CA and bootstrapping were performed using the R package *multistab* (Baty *et al.* 2008).

The metabolite composition in root exudates was incorporated into the analysis through the use of bi-plot ordinations. Variables were combined into a second matrix and plotted as vector fits against a 'Correspondence Analysis' of bacterial transcriptional profiles. Before analyses, the secondary matrix of the primary metabolites data was normalized by dividing values within each variable with column totals (*z*-values). Vector fitting of variables within ordinations was performed using the *envfit* function, in the R package *vegan*. Permutation tests (n=999) were used to determine the significance of vector fits with ordination axes, and significant ($p<0.05$) variables were included in the

resulting bi-plots. Confidence ellipses at the 0.95 level for sample treatments were included in ordinations to examine the variability of sample positions within the ordination.

3.7.5. Real-time PCR

Total RNA (1 µg) was reverse-transcribed with RevertAid^{TM} Premium Reverse Transcriptase (Fermentas, St. Leon-Rot, Germany) according to the manufacturer's instructions, using random hexamers as primers. The real-time PCR was carried out using 7500 Fast Real-Time PCR System (Carlsbad, California, USA). The primers used (Table 1) were designed using the Primer Express software, version 3.0 (Carlsbad, California, USA). Each reaction of 5 µL included 1 µL of a dilution of the target cDNA (1:10-1:10000), 500 nM each primer, 2.5 µL SYBR® Green PCR Master Mix (Carlsbad, California, USA). A 40-cycle amplification was performed (95^{o}C for 3 sec, and 60^{o}C for 30 sec) and the final cycles of 95^{o}C for 15 sec, 60^{o}C for 1 min, and 95^{o}C for 15 sec were used. Target cDNA from reference and experimental samples were amplified in triplicate. The length of PCR products ranged from 59 to 80 bp. Normalization of results was executed relative to gene expression levels of *gyrA*, which did not show altered expression under any of the conditions tested in microarrays. Quantification was based on the analysis of threshold cycle (Ct) values as described by Pfaffl (Pfaffl 2001).

Table 1: Sequences of primers used in the real-time PCR

Gene	Forward Primer	Reverse Primer
licH	CCCGGCAAATTGCACTTC	TGGGCGTCGCAAAAGC
iolC	GCGGACGGTTTCATCGTTAC	CGCGTGACGAATTTGACGTA
yvqH	CATTTCAGTCAGCGCCTTTTT	CAGGCTCAGCTTGCTTTCG
dhaS	CCGGCCTTCACCAAGATACA	AAAGCACGCGCTCATGCT
licB	TCCGCTCCGTTGCATGT	GGAGTGCCGGTTGAAGTCA
flgL	CGATTTCTGTACCGATTGCTTTC	TCCGGGACTTGATGGTTCA
fliS	TTCATTTTTGGCTTCAAGGTTGT	CTGCCTGCGATTCATTAAGCT
iolH	CGTGGTCCGGCTTGATTC	GCGACGAACGCCGTTTT
yocH	CAACACCGGTTGCCGTTAC	TGACTGCAACTGCTTACTCTGCTA
clpC	GCGATTGACGCGTCGAATA	CGCGCCGATGCATTG
glmS	GCGCTGGCGACACAAGA	GCTGCGACTTCCTTCACGTT

4. RESULTS

4.1. Characterization of metabolites in seed and root exudates

Previous studies have highlighted different effects exerted by seed and root exudates on different microbial properties (Kato and Arima 2006; Yaryura *et al.* 2008). Therefore, there might be substantial differences in their metabolite composition. The concentration of the most abundant primary metabolites, namely sugars, amino acids and organic acids, were then determined in seed and root exudates. The composition of seed and root exudates concerning amino acids, organic acids and sugars is depicted in Table 2. Lysine was not detected in maize seed exudates (Table 2). Differences in the metabolite composition between seed and root exudates were significant concerning some amino acids (His+Gly, Thr, Ala, Tyr, Val, Phe, Ile, Leu), citrate and all measured sugars (Table 2). These differences were due to higher concentrations of these compounds in seed compared to root exudates. As discussed in the general introduction, plant species affect seed and root exudates, and plant developmental phase and nutrition affect root exudates both quantitatively and qualitatively. To my knowledge, no data comparing maize seed and root exudates composition is available so far; consequently, comparison with other plant species has to be made with caution. Alanine, tyrosine, valine, isoleucine, leucine, galactose and glucose were also found in higher amounts in soybean seed exudates compared to root exudates (Yaryura *et al.* 2008). Likewise, seed exudates of pea and cotton presented larger amounts of citrate (Kovacs 1971). The present results are therefore in agreement with similar findings previously reported for other plant species.

It is important to note that, to be compared, seed and root exudates were normalized per unit of exudate dry weight. Total organic carbon may be a more appropriate reference for normalization. However, as nearly 80% of maize root exudates are water-soluble sugars, amino acids and organic acids (Hütsch *et al*. 2002), dry weight is likely to be representative of total organic carbon. Given that high-molecular weight compounds such as polysaccharides and proteins are also released by roots, they may account, at least partially, for the measured dry weight. Therefore, comparability between root and seed exudates is thus complicated by the choice of the reference for normalization. In natural environments, bacteria are exposed to seed and root exudates, which may both act as mediators for plant-bacterial associations at different developmental stages at the plant.

Characterizing differences between seed and root exudates may help to interpret distinct bacterial responses to these phytocompounds.

Table 2: Chemical composition of seed and root exudates from (*Zea mays* L.)*

Exudates	Seed	Root
Amino acids (µM)		
Aspartate	39.6 (±7.0)	24.8 (±13.9)
Glutamate	59.7 (±15.6)	29.3 (±26.5)
Asparagine	106.4 (±14.2)	96.8 (±28.3)
Serine	43.1 (±12.6)	17.0 (±11.9)
Glutamine	37.7 (±4.8)	55.8 (±14.0)
Histidine + Glycine	80.8 (±16.5) a	7.8 (±5.5) b
Threonine	17.5 (±3.8) a	5.6 (±4.1) b
Arginine	1.7 (±0.4)	3.0 (±3.2)
Alanine	396.1 (±111.8) a	15.5 (±11.3) b
Tyrosine	8.8 (±1.3) a	2.7 (±1.8) b
Valine	31.3 (±4.8) a	9.7 (±7.1) b
Phenylalanine	8.6 (±0.8) a	3.8 (±2.1) b
Isoleucine	19.9 (±3.4) a	6.4 (±4.2) b
Leucine	23.5 (±2.7) a	6.2 (±4.4) b
Lysine	ND	2.3 (±2.3)
γ-aminobutyric acid	168.3 (±30.5) a	4.8 (±5.1) b
Organic acids (µM)		
Malate/Succinate	88.0 (±9.1)	85.2 (±56.5)
Citrate	37.6 (±3.6) a	3.1 (±0.5) b
Cis-aconitate	1.2 (±0.2)	1.6 (±0.8)
Trans-aconitate	0.5 (±0.4)	92.9 (±62.9)
Carbohydrates (RU) †		
Ribitol	63.0 (±18.9) a	3.5 (±0.4) b
Fructose	8593.8 (±194.4) a	488.7 (±129.5) b
Glucose	966.2 (±19.0) a	77.6 (±48.2) b
Sucrose	13936.7 (±294.3) a	615.1 (±391.2) b
Inositol	4389.3 (±246.0) a	47.1 (±48.2) b
Maltose	5868.8 (±329.4) a	7.9 (±5.8) b
Arabinose	66.1 (±18.6) a	3.3 (±1.7) b
Glycerol	3015.4 (±198.2) a	341.3 (±259.6) b
Erythritol	1722.4 (±271.4) a	3.1 (±1.0) b

ND, not detected; *Different letters between treatments denote significant differences ($p < 0.05$). †Values for carbohydrates are depicted in relative units (RU).

4.2. Seed exudates affected greater levels of bacterial transcripts than root exudates

FZB42 cultures were incubated with maize seed or root exudates and the bacterial transcriptional profiles were compared to cultures without supplemented exudates. The incubation with seed exudates altered the expression of 307 (7.8%) and 318 (8.1%) genes in the logarithmic and transitional phases, respectively. However, the incubation with root exudates changed the expression of 109 (2.8%) and 178 (4.5%) genes, in the respective growth phases (Figure 2). In addition, the bacterial cells exposed to seed exudates presented a larger number of repressed genes in comparison to induced genes (Figure 2). Similarly, in response to root exudates, bacteria showed a slightly larger number of down-regulated genes in comparison to the number of up-regulated genes in the logarithmic phase. However, in the transitional phase more genes were induced by root exudates than repressed (Figure 2). The change in transcript levels of FZB42 was therefore greater in response to seed than to root exudates.

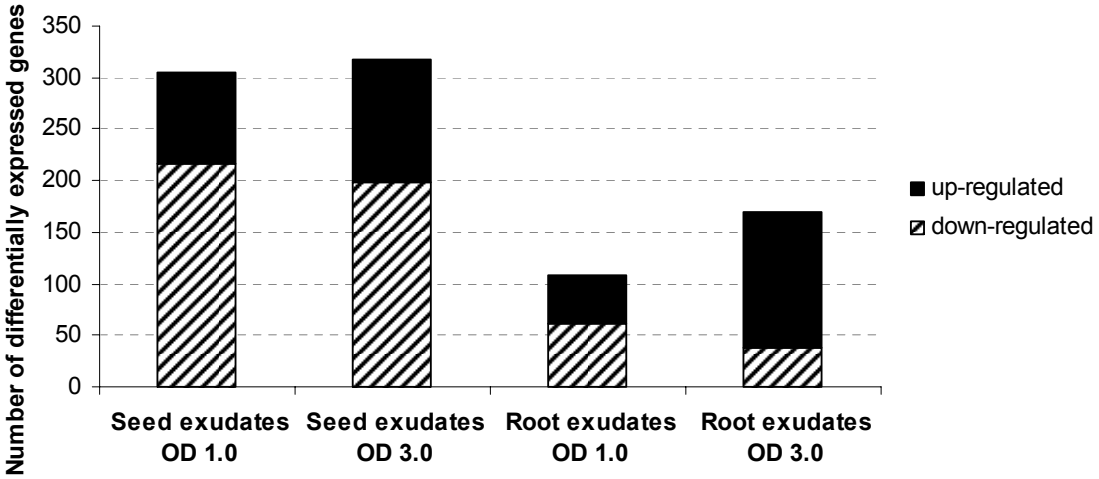

Figure 2: Number of differentially expressed genes in the logarithmic (OD 1.0) and transitional phases (OD 3.0) of *B. amyloliquefaciens* FZB42 cultures in response to maize seed and root exudates.

4.2.1. Transcriptional profiling of *B. amyloliquefaciens* FZB42 in response to seed exudates

4.2.1.1. Differentially expressed genes in the logarithmic phase (OD 1.0)

Fifteen genes involved in uptake and utilization of different compounds, such as substrates and antibiotics, were induced by seed exudates, such as *mtlA* (mannitol uptake), *maeA* (malate utilization), *cimH* (malate and citrate uptake), *ywbN* (iron uptake), *gmuD* (glucomannan utilization), *appB* (oligopeptides uptake), *yxlA* (putative purine/cytosine permease) and *opuBC* (choline transporter) (Appendix I). Genes related to resistance (RBAM_034950 - putative drug resistance transporter), transport (RBAM_030560 - putative multidrug transporter, RBAM_035040 - putative bacitracin ABC transporter permease,) or synthesis of antibiotics (*albG*) were also up-regulated. A nitrite extrusion protein (*narK*), a K^+/H^+ antiporter for K^+ efflux (*yhaT*), an asparagine synthetase (*asnO*) and four genes involved in sporulation (*yraE*, *spsC*, *spoIIID*, *spoIIIAD, safA*) were up-regulated after incubation of the bacterial culture with seed exudates. Two genes (*ymzB*, *katE*) involved in general stress responses, six non-coding RNA and 36 hypothetical proteins, and were also up-regulated (Appendix I).

A very remarkable observation was that, in the presence of seed exudates, nine genes involved in iron uptake and acquisition (*feuA*, *feuB*, *feuC*, *besA*, *dhbA*, *dhbC*, *yusV*, *fhuD*, *fhuG*) were down-regulated (Appendix II). Other transporters were also repressed, such as a low affinity potassium transporter (*ktrC*), a methionine ABC transporter (*metQ*), a putative amino acid transporter (*yhdG*), a cystine ABC transporter (*tcyA*) and a nitrate transporter (*nasA*). Three genes involved in the biosynthesis of folate (*folC*, *folE*, *pabB*), five genes involved in response to stress (*sigB*, *gsiB*, *ysdB*, *ykoL*, *katA*) and eight genes involved in sporulation (*sda*, *spoIISA*, *yabP*, *rapC*, *spo0B*, *sspE*, *cotP*, *spIB*) were also repressed. Seed exudates repressed the expression of three genes related to antibiotics production (*baeB*, *fenE*, *difH*). Genes involved in purine (*ykkE*) and pyrimidine (*pyrD* and *pyrF*) biosynthesis were negatively affected. Two sensor histidine kinases (*lytS*, *yhcY*) were also down-regulated. The gene encoding for the sigma factor SigD, which is involved in the regulation of flagella, motility, chemotaxis and autolysis, was also down-regulated. A large number of hypothetical proteins (72 genes) and three non-coding RNAs were repressed (Appendix II).

In summary, bacterial genes involved in transport and utilization of substrates were mostly induced by seed exudates. Interestingly, a gene encoding for a nitrite extrusion

protein was as well up-regulated and genes involved in the biosynthesis of folate were repressed. Moreover, different genes involved in the same process such as antibiotics production or sporulation were up-regulated and down-regulated. Finally, the gene encoding a sigma factor associated with motility regulation was repressed.

4.2.1.2. Differentially expressed genes in the transitional phase (OD 3.0)

Fourteen genes involved in iron acquisition were induced in *B. amyloliquefaciens* after incubation with seed exudates, such as *feuA*, *feuB*, *feuC*, *dhbA*, *dhbB*, *dhbC*, *dhbF*, *besA*, *ywbN*, *yusV*, *fhuG*, *yclO*, *yxeB* and RBAM_035830 (Appendix III). In *Bacillus* species these genes are usually induced in conditions of iron limitation (May *et al.* 2001; Miethke *et al.* 2006). Interestingly, three genes involved in spore germination were also up-regulated (*gerPB*, *gerM*, *gerPF*). Only one gene encoding a sporulation protein that activates SigG was up-regulated. SigG is the sigma factor associated to the activation of late sporulation genes in the mother cell. A gene related to the control of chemotaxis was also induced (*tlpA*). A putative low affinity inorganic phosphate transporter (*pit*) and a protease (*prsW*), which is involved in the control of SigW, were up-regulated as well. SigW is the sigma plays a role in detoxification and/or production of antimicrobial compounds. Only three genes involved in substrate uptake were up-regulated, such as *yxjA* (purine uptake), *yxlA* (purine-cytosine permease) and *maeA* (malate utilization). A putative drug resistance transporter was up-regulated. Some genes associated to detoxification and responses to stress and were induced, such as *ykuT* (mechanosensitive channel), *yplP* (survival at low temperatures), *yitZ* (putative multidrug resistance protein), RBAM_002410 (detoxification), *yxiS* (survival to ethanol and salt stresses), *bcrC* (resistance to bacitracin and oxidative stress) and *cadA* (cadmium export). Genes involved in the biosynthesis of structural compounds of *Bacillus* were induced, such as fatty acids (*fabI*), polysaccharide (*ytgP*) and phospholipids (*cdsA*). One gene involved in the efflux of arabinose (*ytbD*) was highly up-regulated (6 fold-change). A two-component histidine kinase (*comP*) involved in regulation of genetic competence and quorum sensing was also up-regulated. In addition, sixteen predicted non-coding RNAs and 34 hypothetical proteins were induced (Appendix III).

Thirty genes involved in the uptake, utilization or catabolism of various substrates were down-regulated in the transitional phase (Appendix IV), for instance, genes related to potassium (*ktrC*), methionine (*metQ*), lichenan hydrolysis products (*licA*, *licB*, *licC* and *licH*), inositol (*iolH*, *iolB*, *iolC*, *iolE*), trehalose (*treA*, *treR*), threonine (*tdh*), fructose

(*fruK*, *fruA*), nitrate (*nasA*, *nasD*), ribose (*rbsB*, *rbsC*, *rbsD*, *rbsK*), mannose (*manA*), mannitol (*mtlA*), salicin (*bglH*), beta-glucoside (*bglA*), glycerol (*glpD*, *glpK*), galactose (*galE*, *galT*), gluconate (*gntK*, *gntP*), arginine, ornithine and citrulline (*rocD*), sucrose and glucitol (*ydjE*). Furthermore, the transcription of many regulators was repressed in the presence of the seed exudates, such as *phoP* (regulates phosphate metabolism), *gmuR* (controls glucomannan utilization), *rbsR* (ribose utilization), *lexA* (DNA damage repair), *resE* (aerobic and anaerobic respiration), *rok* (regulation of genetic competence), *degR* (controls DegU activity), *sigD* (controls flagella, motility, chemotaxis and autolysis). One protein involved in motility (*motA*) and four involved in chemotaxis (*yfmS*, *fliE*, *mcpC*, *sigD*) were also repressed. Only three genes involved in antibiotics production were down-regulated (*bmyB*, *lci*, *difH*). Five genes involved in sporulation (*yhaL*, *spoIIIAB*, *spoVG*, *kinE*, *oppA*) and eleven genes involved in adaptation to stress and toxicity (*ywsB*, *ylhF*, *yfhF* – salt, ethanol and low temperatures, *ykoL*, *iseA* – inhibitor of autolysis involved in protection against envelope stresses, *gsiB*, *ytxG*, katA – detoxification of hydrogen peroxide, *ysbB* – antiholin-like protein, *nucA* – DNA uptake) were repressed. Sixteen genes involved in the biosynthesis of compounds such as substrates and structural molecules for bacteria were down-regulated. These compounds included folate (*folC*, *folE*, *pabB*), phosphoglycerolipids (*araM*), citrate (*citA*), phosphoenolpyruvate (*pckA*), biotin (*bioI*), capsule (*capB*), peptidoglycan precursor (*dat*), purine nucleotide (*ykkE*), coenzyme A (*yloI*), lipids and branched-chain amino acids (*lipA*), and arginine (*argB*). Four non-coding RNAs and 58 hypothetical proteins were down-regulated (Appendix IV).

In brief, several genes involved in iron acquisition were, in the transitional phase of bacterial growth, induced after incubation with seed exudates. Three genes involved in spore germination and a putative drug resistance transporter were also induced. Genes associated to utilization and catabolism of substrates were, differently to the logarithmic phase, mostly down-regulated in the transitional phase. In addition, thirteen genes involved in biosynthesis of substrates and structural compounds were repressed. Like in the logarithmic phase, a sigma factor involved in motility (*sigD*) and a motility protein (*motA*) were down-regulated. The induction of genes related to iron acquisition was in fact the opposite of what occurred when *Bacillus* cells were harvested in the logarithmic phase, in which these genes were mainly down-regulated.

4.2.2. Transcriptional profiling of *B. amyloliquefaciens* FZB42 in response to root exudates

4.2.2.1. Differentially expressed genes in the logarithmic phase (OD 1.0)

Two putative two component histidine kinases (*yocF*, *yxdK*), a gene involved in spore germination in the presence of nutrients (*gerBB*) and a protein involved in the control of sporulation initiation (*spoVG*) were up-regulated in the presence of root exudates. A putative spore cortex protein (*ytgP*) and an antagonist of biofilm repression (*ymcA*) were also induced. Genes involved in the transport and utilization of certain compounds, such as urea (*ureB*), inositol (*iolS*), histidine (*hutG*), purine nucleoside (*nupG*), acetoin (*ytrC*) and citrate/malate (*cimH*) were up-regulated. Stress- and detoxification-involved genes, for instance *yceE* (required for survival to ethanol stress and low temperatures), *sodA* (detoxification of oxygen radicals), *katA* (degradation of hydrogen peroxide), *trxA* (oxidative damage) were induced. Seven non-coding RNAs and 12 hypothetical proteins were also up-regulated (Appendix IV).

In the presence of root exudates, genes encoding for an iron-binding protein (*feuA*), three proteins involved in sporulation (*yhaL*, *cotP*, *sspH*) and a polynucleotide phosphorylase necessary for competence development (*pnpA*) were down-regulated. Four genes involved in ABC transport system were repressed, including response regulators (*yxdJ*), a transporter (*oppD*) and putative transporters (RBAM_002400, RBAM_002410). Four other response regulators were also repressed (*degR*, *ycbA*, *rapA1*, *rsbRC*). Three genes involved in survival to stress conditions were also down-regulated, for instance *yjgD* (survival to ethanol stress), *yfhE* (survival to salt/ethanol stresses and low temperatures) and *ykoL* (stress response protein). Genes involved in the synthesis of cell wall constituents, such as phospholipids (*dgkA*) and teichuronic acid (*tuaH*) were down-regulated. One predicted non-coding RNA and 25 hypothetical proteins were repressed (Appendix VI).

In summary, bacterial genes involved in transport of certain compounds that can also be synthesized by bacteria were induced, such as purines and histidines. On the other hand, genes associated to biosynthesis of cell constituents were repressed.

4.2.2.2. Differentially expressed genes at the transitional phase (OD 3.0)

One gene involved in iron acquisition (*dhbC*) was up-regulated. Eleven genes involved in sporulation (*ykoV*, *spo0B*, *ycbE*, *sspM*, *sspE*, *spoVAA*, *spoIIM*, *ykoU*, *yqfD*,

ybaN, *yjaV*) were induced. Twelve genes involved in transport and utilization of substrates were up-regulated, such as *ycbE* (glucarate uptake); *ydiF*, *yfiM* (putative ABC transporter); *ribU* (riboflavin uptake); *glnH* (glutamine uptake); *braB* (uptake of branched-chain amino acids); *dppC* (dipeptide permease); *nasE* (utilization of nitrite); *uxuA* (hexuronate utilization); *kdgA* (utilization of galacturonic acid); *murP* (N-acetyl muramic acid uptake and phosphorylation) and *malS* (malate utilization). Several genes involved in biosynthesis of certain organic compounds were up-regulated. For instance, purine (*purN*, *purF*, *purQ*, *purS*, *purC*, *guaC* – in the last case purine salvage and interconversion), folate (*folC*, *pabB*), fatty acid (*fabF*), branched-chain amino acids (*ilvA*, *ilvB*, *ilvH*), glutamate (*gltA*) phospholipids (*psd*), ketone bodies (*yngG*), serine (*serA*), teichuronic acid (*tuaG*), arginine (*arg*) and histidine (*hisJ*). Seven stress proteins were induced, including *yqhQ*, *csbD*, *gsiB*; *mutT* (oxidative stress); *yfkM*, *yhxD* (salt and ethanol stresses); *mhqD* (protection against methyl-hydroquinone), *yclA* (resistance do salicylic acid) and *hmp* (resistance to nitric oxide). A polynucleotide phosphorylase (*pnpA*) that is necessary for competence development and a translesion synthesis DNA polymerase Y1 (*polY1*) involved in generation of mutations were up-regulated. Seven transcriptional regulators were induced. These are *gmuR*, which is involved in the regulation of glucomannan utilization; RBAM_006180 (putative); *ansR* (negative regulation of the *ansA-ansB* operon), *yodB* (regulation of quinone detoxification), *gabR* (regulation of gamma-amino butyric acid utilization), *glcR* (regulation of sugar metabolism), *ytrA* (regulation of acetoine uptake) and *cggR* (central glycolytic genes regulator). Nine non-coding RNAs and 45 hypothetical proteins were up-regulated (Appendix VII).

Two genes involved in sporulation (*yabQ*, *spsB*) were down-regulated in the presence of root exudates. Some genes involved in transport, uptake and utilization of chemical compounds were repressed, for example, *yxjA* (purine uptake), *yclF*, *yfiB* (putative ABC transporters), *glpD* (glycerol utilization) and *yckE* (utilization of aryl-ß-glucosides). Two genes involved in biosynthesis of riboflavin (*ribD*) and porphyrin (*hemA*) were down-regulated. An efflux pump that confers resistance to arsenite (*ydfA*) was down-regulated. An anti-SigD (*flgM*) was down-regulated (Appendix VIII).

Briefly, genes involved in transport and utilization of substrates were mostly induced. Genes associated to biosynthesis of organic compounds were also up-regulated. In addition, the induction of genes involved in competence development (*pnpA*) and generation of mutations (*polY1*) was observed.

4.2.3. Shared bacterial transcripts in response to seed and root exudates collected from maize plants grown under optimal nutritional conditions

Interestingly, as illustrated in Figure 3, bacterial transcriptomes in response to seed and root exudates shared only few of the differentially expressed genes. The two commonly up-regulated genes between seed and root exudates in the logarithmic phase encode a hypothetical protein (*yoxB*) and a citrate/malate transporter (*CimH*). Among the 15 commonly repressed genes, there is a translation initiation factor I (*infA*); an ABC-transporter for siderophores (*feuA*); two proteins involved in sporulation (*cotP* and *spoIISA*), a stress response protein (*ykoL*), and two hypothetical proteins (*ydeS*, *yjlC*).

In the transitional phase, however, the shared induced genes between seed and root exudates treatments code for a predicted non-coding RNA, a RNA polymerase (*rpoB*), an isochorismate synthase which is involved in siderophore biosynthesis (*dhbC*) and a transcriptional regulator (*glcR*). Two genes were commonly repressed. One is involved in glycerol utilization (*glpD*) and the other encodes an anti-SigD involved in the control of SigD activity (*flgM*).

In summary, few bacterial genes had their expression commonly altered by seed and root exudates. This observation indicates that seed and root exudates may be substantially different. In fact, significant differences in metabolite composition were found (Table 2). These commonly altered transcripts were mainly involved in nutrient transport and utilization (malate, iron and glycerol) and sporulation.

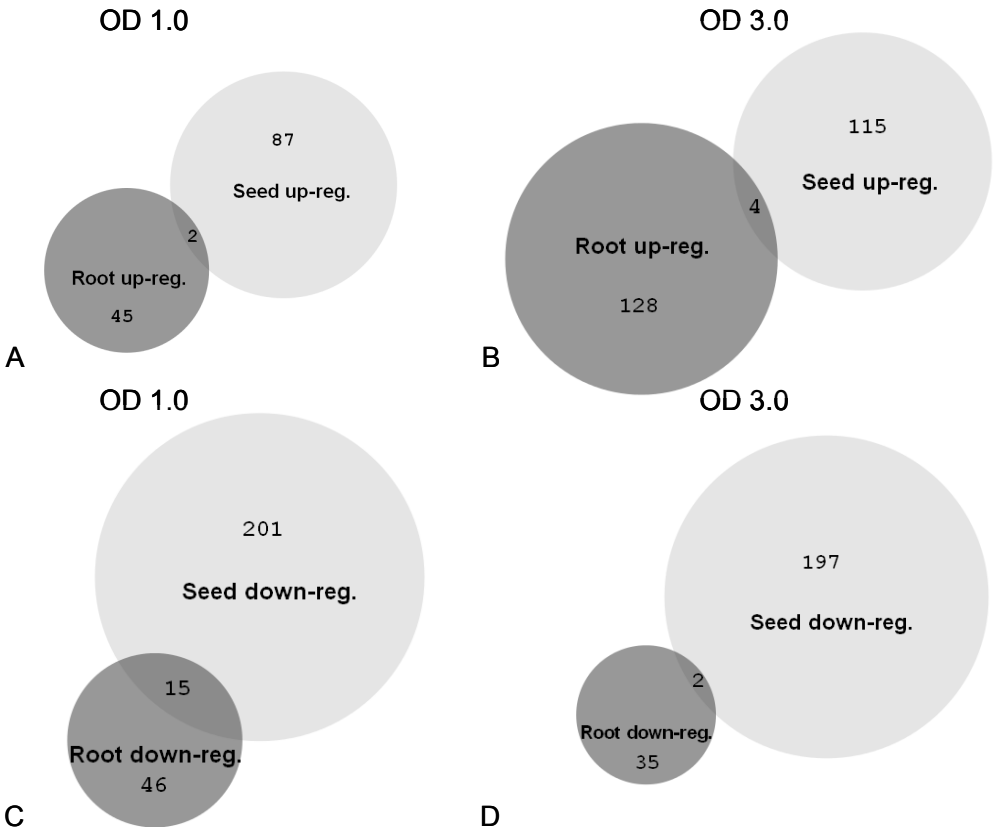

Figure 3: Venn diagrams showing numbers of *B. amyloliquefaciens* FZB42 genes up-regulated by maize seed and root exudates in the logarithmic phase (OD 1.0) (A), down-regulated in the logarithmic phase (OD 1.0) (B), up-regulated in the transitional phase (OD 3.0) (C), and down-regulated in the transitional phase (OD 3.0) (D).

4.2.4. Between Group Analysis comparing bacterial transcriptional responses to seed and root exudates

A 'Between Group Analysis' (BGA) based on 'Correspondence Analysis' (CA) was performed with the bacterial transcriptome data collected in logarithmic and transitional growth phases. The BGA-CA was carried out to associate genes with pre-defined sample classes. Genes were then ranked according to their contribution to their discrimination between class modalities. The class modalities are represented by seed (S) and root (R) exudates treatments. The BGA-CA performed using the differentially expressed genes from the transitional phase was not significant, as evidenced by the Monte Carlo permutation test ($p=0.101$). Transcriptional profiles corresponding to the logarithmic phase differed significantly between treatments ($p<0.05$). For illustration, individual biological replicates of microarray data obtained in seed (S1, S2 and S3) and root (R1, R2, R3)

exudates treatments were plotted on the same discriminating axis to give a visual indication of the degree to which the two groups are separable (Figure 4a). The 10 genes with the most extreme coordinates for each group are displayed in Figure 4b.

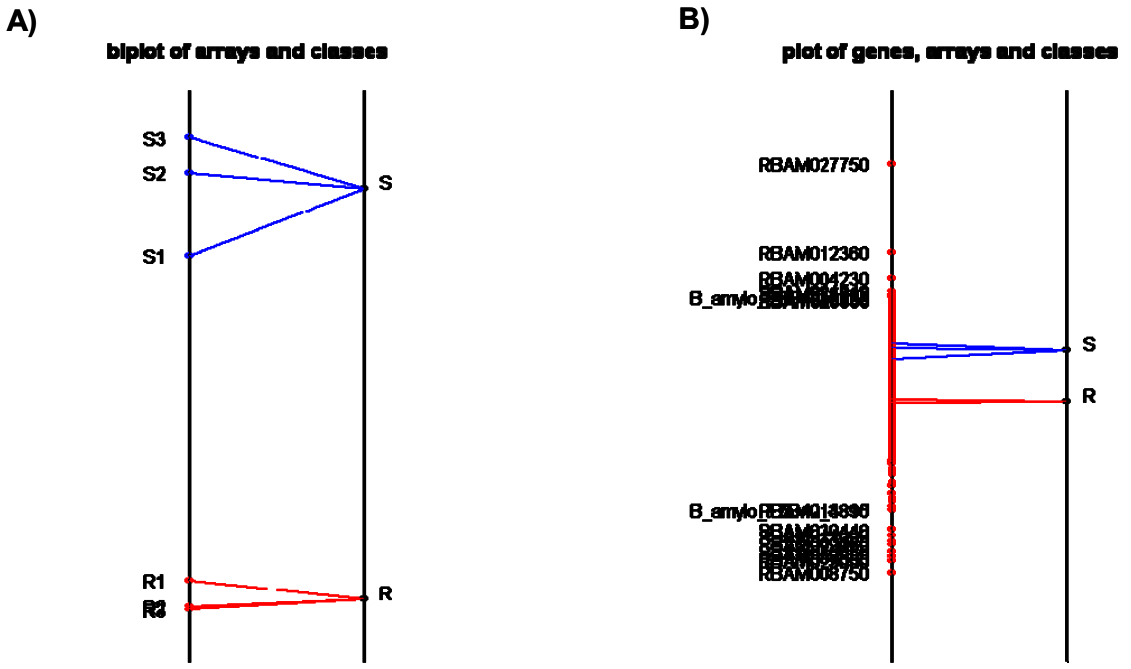

Figure 4: Discrimination between seed and root exudates using BGA-CA (see text). (a) Single axis of the analysis with all replicates plotted. S represents transcriptional response of the bacteria exposed to seed exudates, and R to root exudates; (b) The same analysis as in (a) but with the positions of the 10 most discriminating genes from either end of the axis are labeled.

The genes that contributed to the discrimination between transcriptional responses to seed or root exudates in the logarithmic phase are listed in Table 2.

Table 3: Most discriminating bacterial genes differentially expressed in response to seed compared to root exudates

Gene	*Gene and function*	*Sample*	*M*	*Fold-change*
RBAM_027750	unknown	Seed	0.54	1.45
		Root	-2.76	-6.78
RBAM_004230	*mtlA* - involved in mannitol uptake and phosphorylation, control of MtlR activity	Seed	1.80	3.49
		Root	-0.47	-1.38
B_amylo_FZB42_3965	non-coding RNA	Seed	1.75	3.35
		Root	-0.12	-1.09
RBAM_004240	PTS mannitol-specific enzyme IIA component involved in transport and phosphorylation of mannitol	Seed	1.77	3.41
		Root	-0.02	-1.01
RBAM_034510	*ywhL* - unknown	Seed	1.23	2.34
		Root	-0.57	-1.48
RBAM_034190	*ywkC* - cell division	Seed	1.70	3.24
		Root	-0.07	-1.05
RBAM_010950	*asnO* - biosynthesis of asparagine	Seed	1.46	2.75
		Root	-0.26	-1.20
RBAM_005880	*yraE* - spore coating protein involved in sporulation	Seed	1.27	2.42
		Root	-0.26	-1.20
RBAM_026600	*ywdH* - putative aldehyde dehydrogenase	Seed	1.74	3.35
		Root	0.03	1.02
RBAM_012360	*yjlC* - unknown	Seed	-0.88	-1.84
		Root	-2.55	-5.84
RBAM_008750	*sspE* - involved in protection of spore DNA	Seed	-3.05	-8.30
		Root	-0.05	0.97
RBAM_007300	*yetG* - unknown	Seed	-2.69	-6.46
		Root	0.19	1.14
RBAM_030060	*yusV* - ABC transporter for the siderophores Fe-enterobactin and Fe-bacillibactin (ATPase)	Seed	-2.57	-5.93
		Root	0.26	1.20
RBAM_029050	*dhbA* - involved in siderophore biosynthesis	Seed	-2.83	-7.10
		Root	-0.02	0.98
RBAM_029060	*besA* - trilactone hydrolase involved in iron acquisition	Seed	-2.67	-6.37
		Root	-0.06	-1.04
RBAM_011000	*yisX* - unknown	Seed	-2.44	-5.44
		Root	-0.01	1.01
B_amylo_FZB42_3895	non-coding RNA	Seed	-1.86	-3.64
		Root	0.65	1.57
RBAM_030440	*fhuD* - ABC transporter involved in siderophore uptake	Seed	-2.51	-5.68
		Root	-0.12	-1.08
RBAM_017920	unknown	Seed	-1.58	-2.99
		Root	0.74	1.67
RBAM_032460	required for survival at low temperatures	Seed	-1.74	-3.34
		Root	0.58	1.49

From those genes with annotated functions, two genes were 3.4 times up-regulated in the presence of seed exudates but not differentially expressed in root exudates, *mtlA* (associated to mannitol uptake and phosphorylation) and a PTS mannitol-specific enzyme

IIA component (RBAM_004240). A spore coating protein (*yraE*), a cell division protein (*ywkC*) and a gene involved in the biosynthesis of asparagine (*asnO*) were also induced by seed exudates and not altered by root exudates. Four genes involved in iron acquisition (*yusV*, *dhbA*, *besA* and *fhuD*) were down-regulated by seed exudates and not affected by root exudates.

The annotated functions for most discriminating genes indicate that expression was mostly affected by the presence of nutrients. This observation is evidenced by the induction of genes involved in mannitol transport (*mtlA*, PTS mannitol-specific enzyme) and the repression of genes associated to iron acquisition (*dhbA*, *besA* and *fhuD*). Indeed, significant differences in the composition of primary metabolites between seed and root exudates were found (Table 2), which suggests that there are differences in concentrations of other metabolites that have not been measured.

4.3. Specific responses to nutritional deficiencies in root exudates

Qualitative and quantitative changes in maize root exudate profiles collected from axenically-grown maize exposed to four different nutrient deficiencies were evaluated. In addition to a comparison of exudate profiles for N, K, P and Fe deficiencies, general trends in exudation rates relative to the mobility of the corresponding nutrients in soils were examined.

Relative to the control, increased concentrations of glutamate (Glu), citrate (Cit), ribitol (Rib) and glucose (Glc) were found in exudates collected from Fe-deficient plants (Figure 5).

In exudates collected from P-deficient plants, higher concentrations of γ-aminobutyric acid (GABA) and carbohydrates, such as inositol (Ino), erythritol (Ery), ribitol (Rib), fructose (Fru), glucose (Glc) and arabinose (Ara) were found (Figure 5). Lower concentrations of sugars, including glycerol, ribitol, fructose and maltose, were measured in exudates collected from K-deficient plants; and lower concentrations of amino acids (particularly aspartate, tyrosine, isoleucine and lysine) and maltose were found in exudates collected from N-deficient plants (Figure 5).

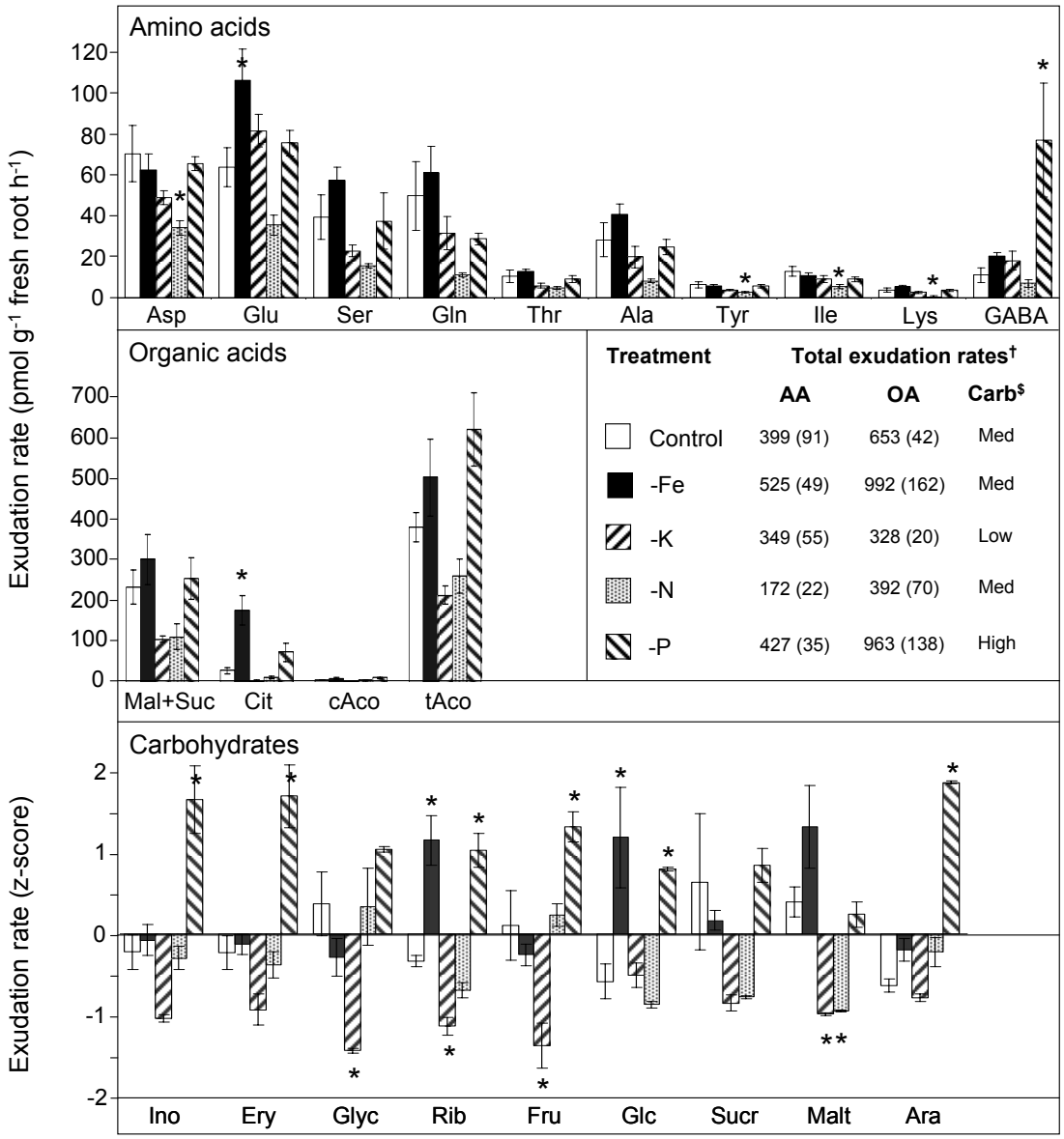

Figure 5: Exudation rates of amino acids, organic acids and carbohydrates released by maize root under iron (-Fe), potassium (-K), nitrogen (-N) or phosphorus (-P) deficiency. For clarity Asn, His+Gly, Arg, Val, Phe and Leu were not displayed because they did not differ between treatments; * denotes treatments that are significantly different to the control ($p<0.05$ in Tukey HSD test). AA denotes amino acids; OA, organic acids and Carb, carbohydrates. †Total exudation rates are displayed in pmol g^{-1} fresh root h^{-1}. ‡Total exudation rates of carbohydrates are displayed in z-scores terms. Bar represent means with standard errors (n = 4).

4.3.1. General responses in root exudation to nutrient deficiencies

The relative proportion of sugars, organic and amino acids differed among plants subjected to different nutritional deficiencies. A similarity analysis (ANOSIM) revealed that all treatments were different from each other ($p<0.001$), except the control and the Fe deficiency treatment ($p=0.114$). The difference between control and K-deficient exudates

was marginal (p=0.057). The main trends in the variation of root exudate compositions among treatments are summarized in Figure 6.

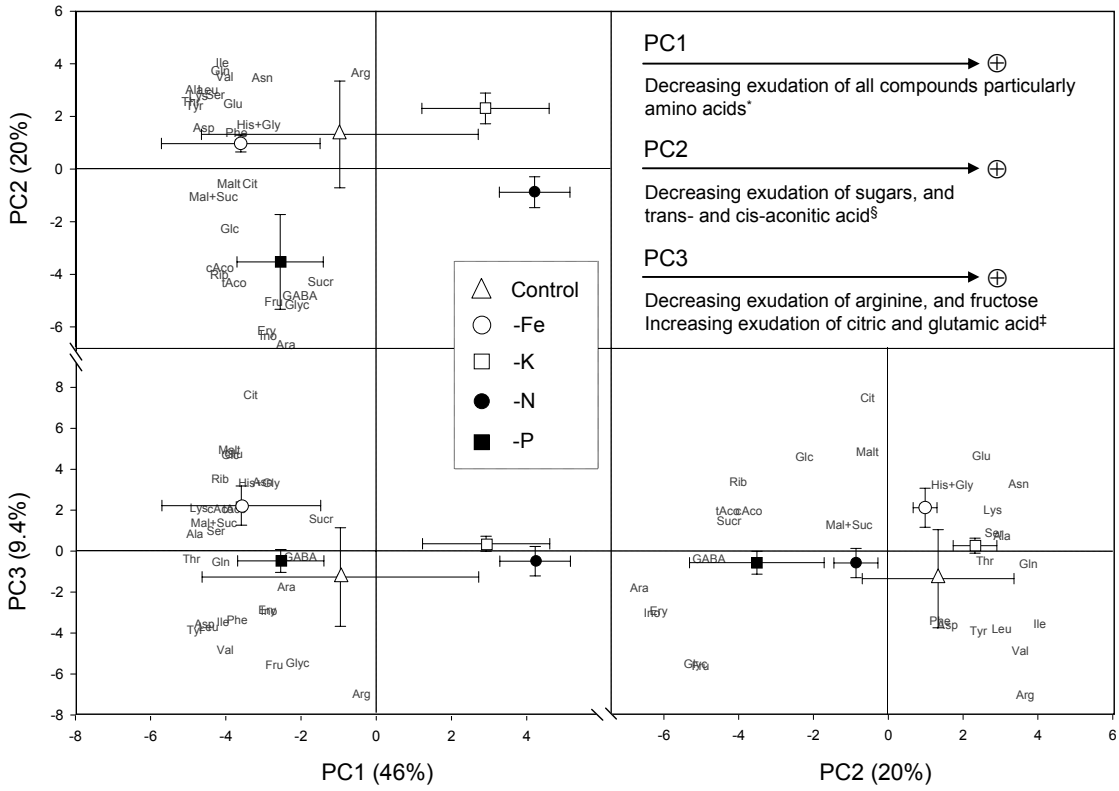

Figure 6: Principal component analysis based on exudation rates of chemical compounds released by plants grown under different nutritional deficiencies. §Negatively-correlated sugars with PC2: Ara, Ino, Ery, Fru, Sucr, Rib. The amino acids Glu and Ile were positively correlated with PC2, other compounds were not significantly correlated. ‡All other compounds were not significantly correlated with PC3.

The first three principal components accounted for 75.4% of the total variation in the dataset. The ordination of the nutrient deficiency treatments along the principal component 1 (PC1) was mostly influenced by total exudation rates of all three metabolic groups, particularly amino acids. As expected, the low amino acid release from N-deficient roots set most apart from that of the other treatments. However, amino acid release was rather low under K deficiency too, which might result from a lower assimilate translocation to the roots. A clear separation became apparent between the Fe or P deficiency versus N or K deficiency treatments, indicating that amino acid release was more prominent under deficiency of those two nutrients with a particular low solubility in soils. Total exudation rates, particularly that of amino acids (AA) and that of organic acids (OA) (Figure 5) support this observation. The principal component 2 (PC2) was mostly affected by differences in the exudation of carbohydrates as well as of *cis*- and *trans*-aconitic acid with

an accumulation of these carbon compounds in the range of negative values (Figure 6). The K deficiency treatment was the one which resulted in the lowest exudation rates of sugars. *Trans*-, *cis*-aconitic acid and GABA also had a significant influence on PC2, all being strongly associated to P limitation (Figure 6). Moreover, GABA was found to be linked with phosphorus starvation. It was mostly citrate and glutamate which largely influenced the ordination of the treatments along principal component 3 (PC3) and separated Fe-deficient root exudates from the rest of the other treatments.

4.3.2. A possible relationship between root exudation and the diffusion coefficient of nutrients in soils

To investigate if there is a relationship between the quantity of root exudation and mobility of the nutrient being in deficiency, exudation rates of amino acids, organic acids and carbohydrates were plotted against the diffusion coefficient of the four nutrients in soil (Figure 7).

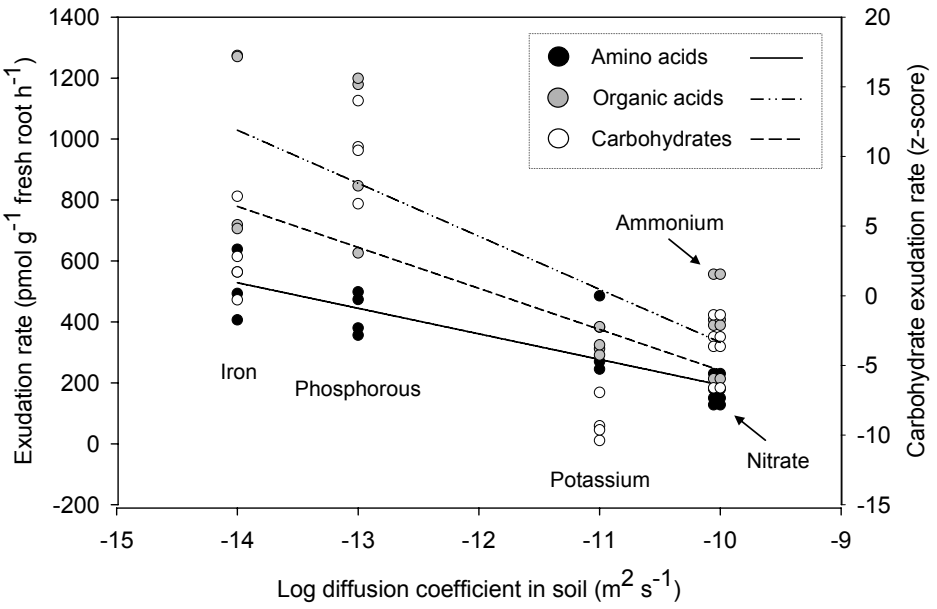

Figure 7: Regression correlating iron, phosphorus, potassium and nitrate diffusion coefficient and exudation rates of total amino acids, organic acids and carbohydrates by plants grown under deficiency of the corresponding nutrients. Effective diffusion coefficients for nutrients in soil were obtained from the literature (Nielsen 2006).

Diffusion coefficients define the mobility of ions (Mengel and Kirkby 2001). The dots represented the total exudation rates of the different metabolic groups (amino acids, organic acids or sugars). Nitrate and ammonium are the main nitrogen forms available to

plants in soils and, although ammonium is less mobile than nitrate (Marschner 1995), their diffusion coefficient appeared to be rather similar in Figure 7 if compared to the other measured nutrients. Interestingly, exudation rates were inversely related to the diffusion coefficient of the growth-limiting nutrient. The strongest correlation between nutrient diffusion coefficients and exudation rates was exhibited by organic acids (gray dots), followed by carbohydrates (white dots) and then by amino acids (black dots) (Figure 7).

4.4. Different nutritional deficiencies distinctively affect the transcriptome of *Bacillus amyloliquefaciens* FZB42

The transcriptomes of *B. amyloliquefaciens* were evaluated in response to root exudates collected from plants grown under N, P, Fe, and K deficiencies. N-deficient maize root exudates affected the greatest number of bacterial genes in both growth phases (Figure 8). These root exudates changed the expression of 143 (3.6%) genes and 183 (4.7%) in log and transitional phases, respectively. In the logarithmic phase, it was followed by P- (28/0.7%), K- (19/0.5%) and Fe- (9/0.2%) deficiencies (Figure 8). Except for the N-deficiency treatment, more bacterial genes were up-regulated than down-regulated in log phase in response to all other deficiency treatments. In the transitional phase, the nitrogen deficiency treatment was followed by iron (157/4.0%), phosphorus (127/3.2%) and potassium (86/2.2%). In this growth phase, the number of repressed genes was higher than the number of induced genes for all treatments (Figure 8). Notably, the effect of root exudates was more pronounced in later stages of bacterial growth, particularly for P, Fe and K deficiency treatments.

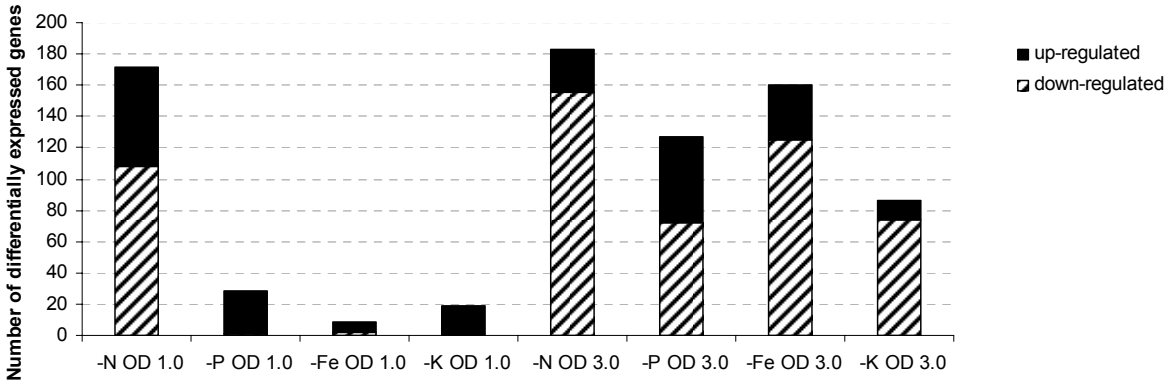

Figure 8: Number of differentially expressed genes of *B. amyloliquefaciens* FZB42 in logarithmic (OD 1.0) and transient (OD 3.0) phases in response to nutrient-deficient maize root exudates treatments. '-N' denotes nitrogen deficiency; '-P', phosphorus deficiency, '-Fe', iron deficiency; '-K', potassium deficiency.

4.4.1. Transcriptional profile of *B. amyloliquefaciens* FZB42 in response to nitrogen-deficient maize root exudates

4.4.1.1. Differentially expressed genes in the logarithmic phase (OD 1.0)

Half of the bacterial genes that were up-regulated by N-deficient root exudates (32 in 64) encoded hypothetical proteins. Four genes involved in the transport/binding proteins and lipoproteins were induced, such as a putative ABC transporter permease (RBAM_007430), a putative cation efflux transporter (*ydbO1*), a glucomannan-specific phosphotransferase system enzyme (*gmuA*) and putative efflux transporter (*ywoD*). A flagellar hook-length control protein (*fliK*) related with chemotaxis and motility was also induced. Four genes associated with sporulation were differentially expressed. Two of them are involved in sporulation repression (*spoIVFA*, *lrpA*) and the others in sporulation activation (*spoIIIAB*, *spoIIIAE*). Two genes related to metabolism of carbohydrates (*ylxY*, *pgm1*) and one related to amino acids transport and metabolism (*yoaD*) were induced. Two genes related to DNA restriction/modification and repair were up-regulated, *dinB*, which is a nuclease inhibitor involved in response to DNA damage, and *ywqL*, a putative endonuclease. Six transcriptional regulators (*gmuR*, *senN*, *ansR*, RBAM_035610, *arfM* and *lrpA*) and one gene involved in tRNA modification were induced. One stress response protein involved in adaptation to atypical conditions *ykoL*, a site-specific recombinase (phage integrase family) (RBAM_01881) and eight non-coding RNAs were up-regulated (Appendix X).

Nearly 25% of the down-regulated genes encode for hypothetical proteins. A penicillin binding protein (*pbpE*) was repressed by N-deficient maize root exudates. Five genes associated with transport/binding of proteins and lipoproteins were down-regulated, such as a manganese uptake protein (*mntH*), a multidrug efflux transporter (*ebrB*), a trigger enzyme involved in uptake of lichenan hydrolysis products (*licB*), an oligopeptide ABC transporter (binding protein) involved in initiation of sporulation (*oppA*), and a citrate/malate transporter (*cimH*). Four genes involved in membrane bioenergetics (electron transport chain and ATP synthase) were repressed (*atpE*, *trxA*, *atpC* and *qoxA*). A flagellin (*hag*) involved in motility and chemotaxis and two genes associated with protein secretion (*secE*, *secY*) were also down-regulated. Four genes involved in metabolism of carbohydrates (*ptsH*, *alsD*, *pgk* and *gapA*), three in metabolism of amino acids (*dat*, *aroA* and *lysC*), one in the metabolism of nucleotides and nucleic acids (*purH*), and one in the metabolism of lipids (*acpA*) were repressed. Three transcriptional regulators (*sinR*, *perR*

and *rsiW*) and three RNA polymerases (*rpoC*, *sigW* and *rpoA*) were down-regulated. Interestingly, a number of 32 genes related to protein synthesis were repressed, being mainly ribosomal proteins. Two genes involved in adaptation to atypical conditions were repressed; one is associated to the regulation of exoenzyme synthesis (*degQ*) and the other a major cold shock protein (*cspB*). Three genes involved in detoxification (*katA*, *yceD* and *yceE*) and one in antibiotics production (*baeI*) down-regulated. Ten non-coding RNAs were also repressed (Appendix XI).

In summary, bacterial genes involved in metabolism of biomolecules such as amino acids, nucleotides or lipids were mostly repressed by N-deficient maize root exudates. Transcriptional regulators were mainly induced. Moreover, processes like protein synthesis and ATP synthesis were repressed, as evidenced by the substantial number of down-regulated genes associated to them.

4.4.1.2. Differentially expressed genes in the transitional phase (OD3.0)

A gene involved in cell wall synthesis (*glmS*) and four genes related to transport/binding of proteins and lipoproteins, such as a putative ABC-transporter integral membrane protein (*mrsE*) and a bacitracin export permease protein (*bceB*) were up-regulated in the transitional phase. Two genes related to membrane bioenergetics were also induced, a quinol oxidase that is involved in respiration (*qoxD*) and an ATP synthase (*atpC*). One gene associated to spore resistance (*cotA*) and one in spore germination (*gerAC*) were induced. Two genes involved in pyrimidine biosynthesis (*pyrD*, *pyrF*), and one gene involved in fatty acid degradation (*yusL*) were up-regulated. Interestingly, 12 ribosomal proteins were induced. Just one hypothetical protein and a non-coding RNA were up-regulated (Appendix XII).

The most representative functional groups of the 155 down-regulated genes at the transitional phase was the hypothetical proteins similar to *B. subtilis* (33 genes), followed by RNA synthesis (18 genes that encode mostly transcriptional regulators), metabolism of carbohydrates and related molecules (14), non-coding RNAs (11) and metabolism of amino acids and related molecules (10). Genes belonging to the metabolism of carbohydrates are mainly related to the utilization of different sugars, for instance trehalose (*treA*), inositol (*iolC*, *iolG*, *suhB*, *iolE*, *iolH*), salicin (*bglH*), mannose (*manA*), beta-glucoside (*bglA*), galactose (*galK1*) and lichenan (*licH*). Genes related to the metabolism of amino acids and related molecules were involved both in biosynthesis and degradation of amino acids. Some among them are genes related to biosynthesis of serine (*serA*),

aromatic amino acids (*aroA*), histidine (*hisJ*), lysine and peptidoglycan (*dapB*), peptidoglycan precursor (*dat*), threonine utilization (*tdh*); protein degradation (*ispA*) and aspartate degradation (*ansB*). Seven genes related to transport/binding of proteins and lipoproteins were induced; two putative multidrug resistance proteins (*ycnB*, *yojI*), a mannose uptake trigger enzyme (*manP*), three genes involved in lichenan uptake (*licA*, *licB*, *licC*) and a ribose ABC transporter (*rbsB*). Four genes associated with sporulation were differentially expressed. A two-component sensor kinase (*kinE*) involved in initiation of sporulation was repressed. Genes involved in utilization of branched-chain keto acids (*bcd*, *buk*) and fatty acid biosynthesis (RBAM_006010, *ymfI*, *fabF*) were down-regulated. Five genes encoding for flagellar proteins were repressed (*fliM*, *fliE*, *flgE*, *fliK*, *fliH*). In addition, four genes related to the antibiotics production (*nrsC*, *bacA*, *baeB*, *bmyA*) were down-regulated. Three genes involved in detoxification were down-regulated, two beta-lactamase precursors, involved in the resistance to beta-lactam antibiotics (*blm*, *penP*), and one vegetative catalase (*katA*). A transcriptional repressor (*rok*) involved in the regulation of genetic competence was also down-regulated (Appendix XIII).

In brief, as previously observed (Figure 8), bacterial gene repression was more pronounced than gene activation in response to N-deficient maize root exudates. Bacteria metabolism in general seem to have been inhibited, as evidenced by the down-regulation of genes involved in transport, catabolism and utilization of substrates, biosynthesis of structural compounds, and motility. Interestingly, in contrast to the logarithmic phase, several ribosomal proteins were induced in the transitional phase.

4.4.2. Transcriptional profile of *B. amyloliquefaciens* FZB42 in response to phosphorus-deficient maize root exudates

4.4.2.1. Differentially expressed genes in the logarithmic phase (OD 1.0)

Twelve out of 27 genes (44.4%) encoding for hypothetical proteins were induced. Three genes involved in sporulation (*yobW*, *spoVFB*, *cgeD*), two putative ABC transporters (RBAM_007430, *ydiF*), two genes related to protein synthesis (*thrS*, *lepA*) and a motility protein (*motA*) were also up-regulated. A putative transcriptional regulator was induced (*arfM*) (Appendix XIV).

The only down-regulated gene was a hypothetical protein (*ywfO*) (Appendix XV).

In summary, compared to other treatments and growth phases, few bacterial genes were affected by P-deficient root exudates in the logarithmic phase. Furthermore, almost half of the differentially expressed genes have unknown function.

4.4.2.2. Differentially expressed genes in the transitional phase (OD 3.0)

A number of 56 bacterial genes were up-regulated in the transitional phase and 11 (19.6%) of these were hypothetical proteins with unknown function. The other predominant functional groups were related to motility and chemotaxis (17.9%), protein biosynthesis (16.1%) and transport/binding of proteins and lipoproteins (12.5%) (Appendix XVI). Examples of genes involved in motility were *hemAT* (haem-based aerotactic transducer), *fliS* (flagellar protein), *flgL* (flagellar hook-associated protein III), *motB* (motility protein), *flgK* (flagellar hook-associated protein I), *fliT* (flagellar protein), *fliD* (flagellar hook-associated protein II) and *flgB* (flagellar basal-body rod protein) (Appendix XVI). Some genes involved in protein synthesis encoded nine ribosomal proteins and one translational initiation factor (*infA*). The functional group transport/binding of proteins and lipoproteins included two ribose ABC transporters (*rbsC*, *rbsD*) that is involved in ribose uptake, a gene involved in mannitol transport (PTS mannitol-specific enzyme IIA component), two ABC type multidrug transporters (RBAM_011880, RBAM_011870), an iron-uptake system permease protein (*feuB*) and a gluconate permease (*gntP*). Interestingly, two transcriptional repressors of sugar operons were up-regulated, *fruR* (fructose operon) and *rbsR* (ribose operon). Genes involved in fructose (*fruK*) and ribose (*rbsK*) utilization were induced. An RNA polymerase (*rpoA*) and genes involved in purine (*purH*) and pirimidine biosynthesis (*pyrD*, *pyrF*) were up-regulated. Genes involved in cell wall synthesis (*glmS*) and serine utilization (*sdaAB*) were also up-regulated. A gamma-DL-glutamyl hydrolase involved in polyglutamic acid degradation and a gene related to protein secretion (*secY*) were induced (Appendix XVI).

A number of 72 genes were repressed by phosphorus deficient maize root exudates and 25% of them encoded for hypothetical proteins with unknown function (Appendix XVII). Seven non-coding RNAs were also repressed. Seven genes belonging to the functional group of metabolism of carbohydrates were down-regulated, such as genes involved in myo-inositol catabolism (*iolG*, *iolH*), hexuronate utilization (*uxaB*), methylglyoxal synthase involved in bypassing of glycolysis (*mgsA*), a 6-phospho-beta-glucosidase involved in beta-glucoside utilization (*bglA*) and a 6-phospho-alpha-glucosidase involved in maltose utilization (*malA*). Seven genes involved in the

metabolism of amino acids and related molecules were repressed. Some are associated with the biosynthesis of different amino acids, such as *dapB* (lysine), *gltA* (glutamate) and *argG* (arginine). Others are related to degradation, for instance *ansB* (aspartate) and *ispA* (protein). A sigma factor (*sigM*) involved in resistance against cell envelope stress, oxidative stress and salt stress was down-regulated. Three regulators were repressed, such as one related to xylan and xylose utilization (*xylR*), and *ccpC*, which is associated to the regulation of tricarboxylic acid branch of the TCA cycle. Genes involved in fatty acids biosynthesis (*fabD*, *fabF*), utilization of branched-chain keto acids (*bkdAA*) and adaptation of membrane fluidity at low temperatures (*des*) were down-regulated. Three genes involved in transport/binding proteins and lipoproteins were repressed; a dipeptide ABC permease involved in the uptake of dipeptides, a cystine ABC transporter (*tcyA*) and a putative ABC-transporter ATP-binding protein (RBAM_029210). A gene associated to biosynthesis of folate (*folC*) and two genes involved in detoxification, one associated to hydrogen peroxide degradation (*katA*) and the other to resistance to beta-lactam antibiotics (*penP*) were down-regulated. Some genes involved in DNA repair and recombination were repressed. Examples are *mutSB* and *recN*, which are related to DNA repair, and *parC*, which is associated to chromosome segregation and compaction. A gene involved in sporulation (*sspM*), a general stress protein (*gsiB*), and a bacillomycin synthetase related to antibiotics production (*bmyA*) were repressed. A glucose-inhibited division protein (*gid*) was down-regulated (Appendix XVII).

Briefly, in the transitional growth phase, the most remarkable finding was the induction of genes involved in motility by P-deficient maize root exudates, which was exclusive for this treatment. Transcriptional repressors for sugar operons were up-regulated, as well as genes involved in sugar utilization. Interestingly, root exudates from P-deficient plants had higher amounts of sugars than exudates from nutrient-sufficient plants (Figure 5). Similarly to the N deficiency treatment in the transitional phase, there appears to be a trend of overall inhibition of bacterial metabolism due to the elevated number of repressed genes involved in transport, catabolism and utilization of substrates and biosynthesis of structural compounds.

4.4.3. Transcriptional profile of *B. amyloliquefaciens* FZB42 in response to iron-deficient maize root exudates

4.4.3.1. Differentially expressed genes in the logarithmic phase (OD 1.0)

Compared to N and P deficiency treatments in the same growth phase, very few genes (9 genes) were differentially expressed by iron-deficient maize root exudates. Two genes related to transport/binding proteins and lipoproteins were up-regulated: a putative praline-specific permease (*ybxG*), and a putative di-tripeptide ABC permease (*yclF*). A gene encoding a hypothetical protein showing 61% identity with a sporulation membrane protein from *B. subtilis* was also induced. Interestingly, an inhibitor of SigG (*csfB*), which is the sigma factor associated to the transcription of sporulation genes, was up-regulated. The other three up-regulated genes encoded hypothetical proteins with unknown function and predicted non-coding RNAs (Appendixes XVIII and LI).

Two genes were down-regulated, one encoding for a hypothetical protein (*yolA1*) and the other is a predicted non-coding RNA. An interesting gene that was down-regulated but its adjusted p-value marginally significant (0.052) was *cimH*, which is involved in citrate and malate uptake (Appendixes XIX and LI).

4.4.3.2. Differentially expressed genes in the transitional phase (OD 3.0)

Eleven out of 34 up-regulated genes (32.3%) encoded hypothetical proteins. The most representative functional groups were transport/binding proteins and lipoproteins and RNA synthesis, with seven genes and four genes respectively. Among the induced genes related to transport were a putative amino acid permease (*yxeN*), a putative 4-aminobutyrate aminotransferase (*gabT1*), an ABC transporter for the siderophores Fe-enterobactin and Fe-bacillibactin that is involved in iron acquisition (*feuB*), a bacitracin ABC export permease (*bceB*), and a cadmium transporting ATPase (*cadA*). The genes related to RNA synthesis were mostly putative transcriptional regulators (*ywtF*, *mgsR*, *yhbI*). Two genes related to protein synthesis (*rpsR*, *fmt*) and one to arabinan degradation (*abn2*) were also induced. A gene that confers resistance to organic peroxide (*ohrB*) and one related to respiration (*qoxD*) were up-regulated. A nutrient receptor (*gerAC*) involved in spore germination in response to L-alanine was also induced (Appendixes XX and LI).

Hypothetical proteins corresponded to 51.4% (37 genes) of the down-regulated genes. The functional group with known function containing the highest number of repressed genes was related to metabolism of carbohydrates and related molecules (16 genes).

Examples of genes belonging to this group are *pckA* (involved in synthesis of phosphoenolpyruvate), *uxaB* (hexuronate utilization), *bglH* (salicin utilization), *lutA* (lactate utilization), *iolC*, *iolG*, *iolH* (myo-inositol catabolism), *treA* (trehalose utilization), *bglA* (beta-glucoside utilization), *licH* (lichenan utilization) and *malA* (maltose utilization). Ten genes related to transport/binding of proteins and lipoproteins were repressed. Instances were cystine ABC transporter (*tcyC*), P-type zinc-transporting ATPase (*zosA*), N-acetyl muramic acid-specific phosphotransferase system (*murP*), galactarate/glucarate transporter involved in glucarate uptake (*ycbE*), a dipeptide uptake permease (*dppC*), an ABC transporter probably melibiose uptake (*msmE*), a probable glucitol transport protein (*gutA*) and a lichenan-specific phosphotransferase system (*licA*) involved in lichenan uptake and phosphorylation (Appendix XXI and LI).

In summary, a gene coding for a bacterial siderophore transporter was induced under Fe-deficient maize root exudates, which suggests that bacteria may be able to sense plant iron starvation. Additionally, a gene involved in spore germination was also induced. Similarly to what was observed in P and N treatments in the transitional phase, a tendency of overall inhibition of bacterial metabolism is again revealed by the elevated number of repressed genes involved in transport, catabolism and utilization of substrates and biosynthesis of structural compounds.

4.4.4. Transcriptional profile of *B. amyloliquefaciens* FZB42 in response to potassium-deficient maize root exudates

4.4.4.1. Differentially expressed genes in the logarithmic phase (OD 1.0)

Nineteen genes were up-regulated when bacterial cells were exposed to potassium deficient maize root exudates. However, 13 (68.4%) of these encoded for hypothetical proteins with unknown function and four were predicted non-coding RNAs. The other two induced genes encoded for putative transcriptional regulator (RBAM_005370) and a L-lactate dehydrogenase involved in overflow metabolism and fermentation (Appendix XXII).

No genes were repressed in this growth phase.

4.4.4.2. Differentially expressed genes in the transitional phase (OD 3.0)

Eleven genes were induced in the transitional phase. Examples are two genes involved in transport. One is a hypothetical transport protein (*yybF1*) with 76% identity with a sugar

transporter superfamily protein (*yybF*) from *Bacillus liqueniformis*. The other encodes a putative 4-aminobutyrate aminotransferase. One gene involved in spore germination (*gerAC*) and one encoding a spore coat protein (*cotA*) were induced. A transcriptional repressor (*fatR*) and a ribosomal protein (*rpsR*) were also up-regulated. A gene involved in the biosynthesis of leucine (*leuD*) and another encoding a UV DNA damage endonuclease (*uvsE*) were up-regulated. The other two induced genes have unknown function and one is a predicted non coding RNA (Appendix XXIII).

A number of 75 bacterial genes were down-regulated in the transitional phase by potassium-deficient maize root exudates. From these, 26 (34.6%) encode hypothetical proteins with unknown function and two are predicted non-coding RNAs. Six genes involved in metabolism of amino acids and related molecules were repressed, such as *ald* (alanine utilization), *argH* (biosynthesis of arginine), *proI* (biosynthesis of proline), *yclM* (aspartokinase III), *dapB* (biosynthesis of lysine and peptidoglycan) and *ansB* (aspartate degradation). Six genes related to metabolism of carbohydrates and related molecules were down-regulated. Instances are *ioG* and *iolC* (involved in myo-inositol catabolism), *glcK* (phosphorylation of the free glucose moiety of di-and oligosaccharides), *uxaB* (hexuronate utilization), *galK1* (galactose utilization) and *malA* (maltose utilization). Some genes belonging to the functional group of metabolism of lipids were repressed, such as *bkdB*, *bkdAA* and *buk* (related to utilization of branched-chain keto acids), *scoB* (lipid metabolism), *fabF* (fatty acid biosynthesis), and *des* (phospholipid desaturase). Five transcriptional regulators associated to different processes such as TCA cycle (*ccpC*) and DNA damage repair (*lexA*) were repressed. Four transporters were down-regulated; a P-type zinc-transporting ATPase involved in zinc uptake (*zosA*), a mannose uptake trigger enzyme (*manP*), a dipeptide ABC permease (*dppC*) and galactarate/glucarate transporter involved in glucarate uptake (*ycbE*). A two-component sensor histidine kinase homolog involved in the initiation of sporulation (*kinE*), a response regulator aspartate phosphatase related to the control of sporulation initiation (*rapA1*), and an anti-SigF (*spoIIAB*) were repressed. SigF is a sigma factor associated to the transcription of sporulation genes. Two genes related to adaptation to atypical conditions were down-regulated, *gsiB* (general stress protein) and *degR* (involved in control of DegU activity). A gene that encodes for beta-lactamase (*penP*) and therefore is involved in resistance to beta-lactam antibiotics was repressed (Appendixes XXIII and LI).

In summary, genes involved in spore germination and protein synthesis were induced. Potassium-deficient maize root exudates repressed a higher number of genes than induced,

like other deficiency treatments (Figure 8). Similarly to N, P and Fe deficiency treatments, a trend of global inhibition of bacterial metabolism seems to have taken place, since many genes involved in transport, catabolism and utilization of substrates and biosynthesis of structural compounds were repressed.

4.4.5. Shared bacterial transcripts in response to root exudates collected from maize plants grown under different nutritional deficiencies

4.4.5.1. Logarithmic phase (OD 1.0) – up-regulated genes

Seventeen up-regulated bacterial genes were shared between different treatments in the logarithmic phase (Figure 9). Most of the shared genes encode for hypothetical proteins or were predicted coding RNAs (Appendix XXV to XXX). Therefore, interpretation of these results is rather difficult. Two hypothetical proteins (RBAM_008260 and RBAM_034640), unique for FZB42, were shared among N, P and K deficiency treatments. A hypothetical protein involved in sporulation (*yobW*) was induced by phosphorus and iron deficiency treatments. The treatments that have more genes in common are N- and P-deficiencies (Appendix XXV to XXX). No genes are commonly down-regulated between treatments in the logarithmic phase.

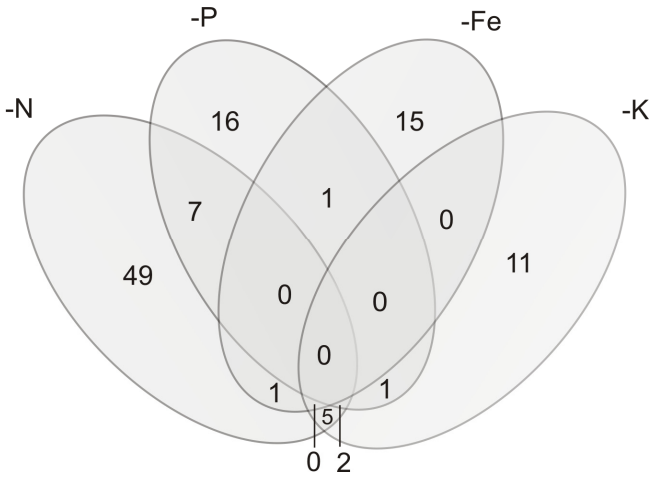

Figure 9: Venn diagram showing numbers of *B. amyloliquefaciens* FZB42 genes up-regulated in the logarithmic phase by different nutrient-deficient maize root exudates. '-N' denotes nitrogen deficiency treatment; '-P', phosphorus deficiency, '-Fe', iron deficiency and '-K' potassium deficiency.

4.4.5.2. Transitional phase – up-regulated genes

Interestingly, a ribosomal protein (*rpsR*) was up-regulated in all deficiency treatments and five ribosomal proteins (*rpsP*, *rpIF*, *rpsH*, *rplE*, *rplN*) were induced by N- and P-

deficient maize root exudates (Appendix XXXI, Figure 10). Five induced genes were shared among N, Fe and K deficiencies (Appendix XXXVI, Figure 10). These are *trpC* (indole-3-glycerol-phosphate synthase involved in tryptophan biosynthesis), *gerAC* (nutrient receptor involved in spore germination in response to L-alanine), *yybF1* (hypothetical transport protein), *cotA* (a spore coat protein involved in resistance of the spore), and *gabT1* (4-aminobutyrate aminotransferase involved in utilization of GABA). An isopropylmalate isomerase involved in biosynthesis of leucine (*leuD*) and a hypothetical protein (*yhcC*) were commonly induced in P-, Fe- and K-deficiency treatments (Appendix XXXVII). An iron-uptake system permease protein (*feuB*) was induced in P- and Fe- deficiency treatments (Appendix XXXIII). Two genes involved in pyrimidine biosynthesis (*pyrD*, *pyrF*) and an enzyme involved in cell wall synthesis (*glmS*) were induced by N and P deficiencies. As observed in the logarithmic phase (Figure 9), the highest number of genes was shared between N and P deficiencies (Figure 10).

It is important to point out that, in the N, Fe and K deficiency treatments, bacterial genes coding for the auxin precursor tryptophan biosynthesis (*trpC*) and a nutrient receptor involved in spore germination (*gerAC*) (Appendix XXXVI) were induced.

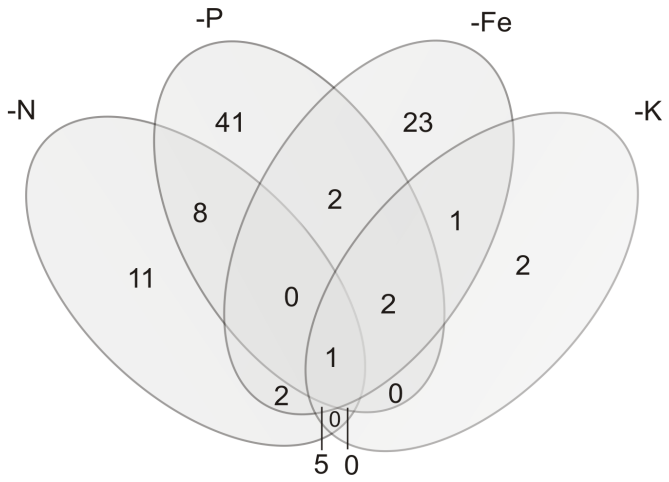

Figure 10: Venn diagram showing numbers of *B. amyloliquefaciens* FZB42 genes up-regulated in the transitional phase by different nutrient-deficient maize root exudates. '-N' denotes nitrogen deficiency treatment; '-P', phosphorus deficiency, '-Fe', iron deficiency and '-K' potassium deficiency.

4.4.5.3. Transitional phase – down-regulated genes

A number of 117 down-regulated genes were shared between transcriptomes of bacteria incubated with different nutrient-deficient maize root exudates (Appendix XL to XLIX, Figure 11). 13 genes were commonly repressed in all deficiency treatments, four of which encoded for hypothetical proteins with unknown function. Others included a general

stress protein (*gsiB*), a beta-lactamase precursor involved in resistance to beta-lactam antibiotics (*penP*), a fatty acid desaturase involved in adaptation of membrane fluidity at low temperatures (*des*), a beta-ketoacyl-acyl carrier protein synthase II involved in fatty acid biosynthesis (*fabF*), a putative cell-wall binding protein (*yocH*), a bifunctional glucosyl transferase/transpeptidase penicillin-binding proteins IA/IB (*ponA*), a dihydrodipicolinate reductase involved in biosynthesis of lysine and peptidoglycan (*dapB*), a myo-inositol 2-dehydrogenase involved in *myo*-inositol catabolism (*iolG*) and a putative transcriptional regulator (RBAM_006180) (Appendix XLIX, Figure 11). N, Fe and K deficiency treatments shared seven down-regulated bacterial genes. Three of them are involved in substrate utilization, such as maltose (*malA*), hexuronate (*uxaB*) and branched-chain keto acids (*bkdAA*). The others are involved in the uptake of dipeptides (*dppC*), prophage-mediated lysis (*xlyB*) and thioredoxin reduction (*trxB*) (Appendix XLVI). Three genes were commonly repressed in N-, P- and K-deficiencies. These are *parC*, which encodes for a DNA topoisomerase involved in chromosome segregation and compaction; *ansB*, which is involved in aspartate degradation; and *folC*, which is associated to the biosynthesis of folate (Appendix XLVII). A number of 20 genes and a non-coding RNA were commonly repressed in N, Fe and K deficiency treatments, eight of which have unknown function (Figure 11). Three genes are involved in utilization of compounds, such as galactose (*galK1*), threonine (*kbl*) and branched-chain keto acids (*buk*). A penicillin-binding carboxypeptidase (*dacC*), a hydrolase involved in cell wall metabolism (*cwlS*), an anti-sigma factor F involved in control of sporulation initiation (*spoIIAB*), a gene involved in mannose uptake (*manP*) and a hypothetical protein involved in protection against daptamycin (RBAM_030250) were also down-regulated (Appendix XLVI). N, P and Fe deficiency treatments shared seven repressed genes and one predicted non-coding RNA (Figure 11). Instances are a gene associated to detoxification of hydrogen peroxide (*katA*), and another to antibiotics production (*bmyA*). Two genes involved in protein (*ispA*) and poly-glutamate capsules (*ggt*) degradation were repressed. A 6-phospho-beta-glucosidase associated to beta-glucoside utilization (*bglA*) and an inositol utilization protein related to *myo*-inositol catabolism (*iolH*) were also repressed in these three treatments (Appendix XLV). Eleven genes were commonly down-regulated in Fe and K deficiency treatments (Figure 11). Four encodes four hypothetical proteins. The others included *ycbE* (involved in glucarate uptake), *zosA* (zinc uptake), *bkdB* (utilization of branched-chain keto acids), *lutC* (utilization of lactate), *scoB* (lipid metabolism), *argH* (biosynthesis of arginine) and *med* (regulation of competence) (Appendix XLIV). P and K deficiency treatments shared

five repressed genes, being the two with known function - a transcriptional repressor (*ccpC*) and a leucyl-tRNA synthetase involved in translation (*leuS*) (Appendix XLIII). Two genes were down-regulated in Fe- and P-deficiency treatments, one that encodes for a repressor protein involved in xylan and xylose utilization (*xylR*) and another associated to resistence against paraquat (*yqjL*) (Appendix XLII). A number of 29 genes and two predicted non-coding RNAs were commonly down-regulated in N and Fe deficiency treatments (Figure 11). Seven genes encode hypothetical proteins with unknown function. Among the ones with known function, some are involved in uptake or utilization of different compounds, such as trehalose (*treA*), branched-chain keto acids (*bcd*), lichenan (*licA*, *licH*) and salicin (*bglH*). Five transcriptional regulators involved in different processes were repressed, such as fatty acid and phospholipid biosynthesis (*fapR*), biofilm formation (*ymcA*), inhibition of AbrB (*abbA*), phosphate metabolism (*phoP*) and protein degradation (*mcsB*). Other genes included *clpC* (class III stress response-related ATPase involved in protein degradation), *blm* (beta-lactamase II precursor), *rodZ* (morphogenic protein required for cell shape determination) and *resA* (thiol-disulfide oxidoreductase involved in cytochrome c biogenesis) (Appendix XL). N and P deficiencies shared mostly hypothetical proteins and three non-coding RNAs (Appendix XXXIX).

In summary, a strikingly large number of repressed genes in FZB42 (117 genes) were shared among deficiency treatments (Figure 11). Mainly processes associated to transport and utilization of substrates and biosynthesis of structural compounds seem to have been commonly hindered by N, P, Fe and K- deficient maize root exudates.

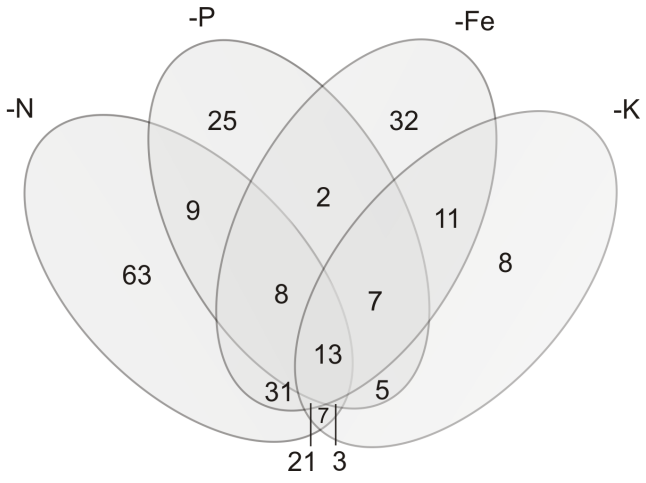

Figure 11: Venn diagram showing numbers of *B. amyloliquefaciens* FZB42 genes down-regulated in the transitional phase by different nutrient-deficient maize root exudates. '-N' denotes nitrogen deficiency treatment; '-P', phosphorus deficiency, '-Fe', iron deficiency and '-K' potassium deficiency.

4.4.6. Real time for validation of the microarray analysis

To validate results from the microarrays, some genes were arbitrarily selected for confirmation with real-time PCR. Most of the selected genes were confirmed, except for *trpC* (Table 4). Very approximate values were obtained by some genes, but for some a higher fold-change was found. However, the tendency of up- or down- regulation was corroborated. This trend was also observed in other studies (Jenson *et al.* 2003; Hamidi *et al.* 2008; Biller *et al.* 2010), due to the fact that generally microarrays are less sensitive for quantitative detection of differential expression of genes.

Table 4: List of genes for which real-time PCR was performed to validate the microarray analysis (values are shown in fold-change)

Treatment	Gene ID	Gene	Microarray	Real-time
-Fe	RBAM_035760	*licH*	-6.6	-187.6
-Fe	RBAM_036760	*iolC*	-2.5	-21.3
-Fe	RBAM_030250	*yvqH*	-4.3	-9.6
-Fe	RBAM_019060	*dhaS*	-3.2	-3.6
-Fe	RBAM_035790	*licB*	-3.3	-111.0
-P	RBAM_032550	*flgL*	2.4	2.8
-P	RBAM_032490	*fliS*	2.5	2.7
-P	RBAM0_36710	*iolH*	-2.5	-9.9
-P	RBAM_018960	*yocH*	-3.2	-2.7
-N	RBAM_001110	*clpC*	-2.0	-2.2
-N	RBAM_036710	*iolH*	-4.7	-28.5
-N	RBAM_002320	*glmS*	2.2	2.3
-N	RBAM_018960	*yocH*	-2.0	-1.9
-K	RBAM_019060	*dhaS*	-2.1	-68.6
-K	RBAM_036760	*iolC*	-2.1	-244.0
-K	RBAM_018960	*yocH*	-2.7	-22.1
-K	RBAM_030250	*yvqH*	-5.5	-305.8
C*	RBAM_006890	*purQ*	2.0	67.0
C	RBAM_006910	*purF*	3.1	24.6
C	RBAM_018620	*gltA*	3.6	20.3

Iron deficiency treatment is represented as '-Fe', phosphorus deficiency as '-P', nitrogen deficiency as '-N', potassium deficiency as '-K' and *C depicts the treatment in which bacterial transcriptional profiles in response nutrient-sufficient maize root exudates were compared with no exudate addition.

4.4.7. Between group analysis to identify the most discriminating genes between deficiency treatments

BGA-CA was performed to evaluate if changes in the global gene expression of FZB42 after incubation with different nutrient-deficient maize root exudates could be

distinguished and to identify the most discriminating genes for each deficiency treatment. Similarly to the results obtained by the comparison between seed and root exudates, the BGA-CA was significant for data from the logarithmic phase of bacterial growth ($p < 0.05$), but not from the transitional phase. Therefore, only data from the log phase were used in the subsequent analysis. After performing a BGA-CA with different subsets of bacterial genes from the logarithmic phase (OD 1.0), the threshold of the signal ratio (M) chosen for further analysis was 0.8 (Figure 12). This procedure allowed the selection of 218 out of 3933 genes through the elimination of the ones that did not have altered transcription in any of the treatments, and therefore could represent 'noises' in the statistical analyses.

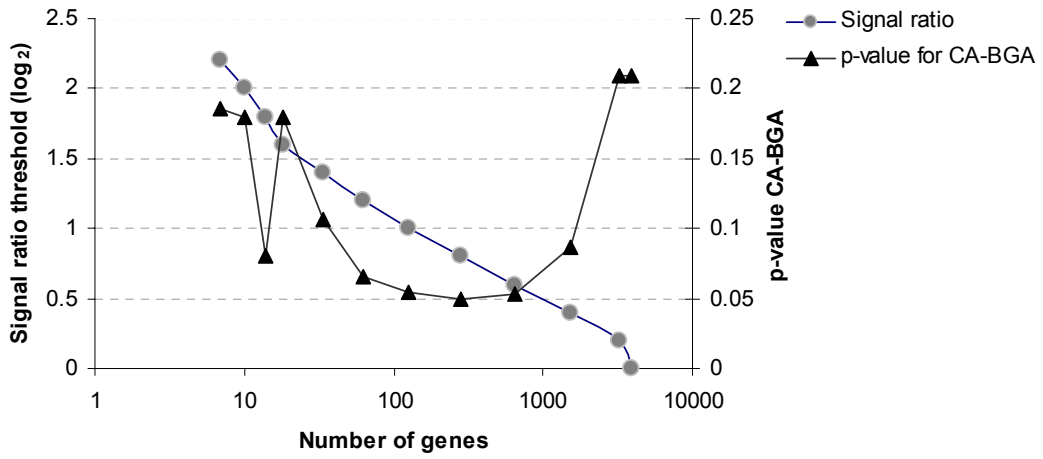

Figure 12: Effect of the signal-ratio threshold on the number of differentially expressed genes (left Y axis) and of the signal-ratio threshold on the BGA-CA Monte Carlo p-value (right Y axis).

A clear separation between the N deficiency treatment and all other treatments (-P, -Fe and –K) along the x-axis of the BGA-CA was observed (Figure 13).

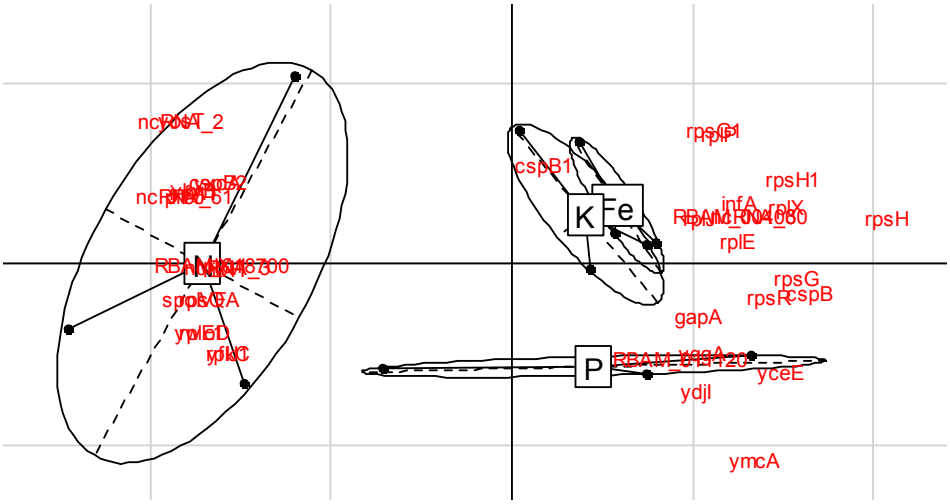

Figure 13: Discrimination of bacterial transcriptional responses to root exudates collected under four different deficiency treatments. The first two axes of a BGA using CA are shown. The four treatments are nitrogen deficiency (N), phosphorus deficiency (P), iron deficiency (Fe) and potassium deficiency (K). Biological replicates grouped in a deficiency treatment were represented by an ellipse. The ten most discriminating genes in each treatment were labeled.

In the y-axis of the BGA, a separation between P deficiency and Fe/K was evidenced (Figure 13). Transcriptional responses to Fe and K deficiencies were not discriminated by either of the BGA axes. The ten most extreme genes associated with the different deficiencies were identified based on the loadings obtained after the BGA-CA. Details of their function are listed in Appendix L.

All first ten most discriminating genes for N deficiency were up-regulated in this treatment and not affected by the P, Fe and K depletion (Appendix L). Six of them encoded for hypothetical proteins with unknown function. The others were involved in the regulation of glucomannan utilization (*gmuR*), synthesis of proline (*proJ*), resistance to osmotic downshock (*yfkC*) and control of SigK (sporulation-specific sigma factor) (*spoIVFA*).

As the P, Fe and K starvation treatments were quite similar to each other, as evidenced by their overlap in the x-axis of the BGA-CA, they shared some of the most discriminating genes. Except for one in P and another in Fe deficiencies, all genes were mainly not differentially expressed in those treatments and down-regulated in the N deficiency treatment. Most of them encoded for ribosomal proteins involved in translation. Others included an antagonist of biofilm repression involved in regulation of biofilm formation (*ymcA*), a hypothetical protein associated to survival to ethanol stress and at low temperatures (*yceE*), a major cold-shock protein involved in RNA chaperone activity (*cspB*), a catabolic enzyme in glycolysis (*gapA*), and an acetolactate synthase involved in

biosynthesis of branched-chain amino acids (*ilvH*). A hypothetical protein with unknown function was induced exclusively in P (RBAM_011120) and another in Fe (RBAM_004030) deficiency treatments (Appendix L).

Indications about the gene stability were given by the sizes of convex hulls in Figure 14. The distance from the center of the BGA axes to the hull locations provides the discriminative power of genes (Baty *et al.* 2008). The specificity of the gene discrimination is indicated by the degree of hull overlap. Therefore the N treatment had the highest specificity and discriminative power of genes.

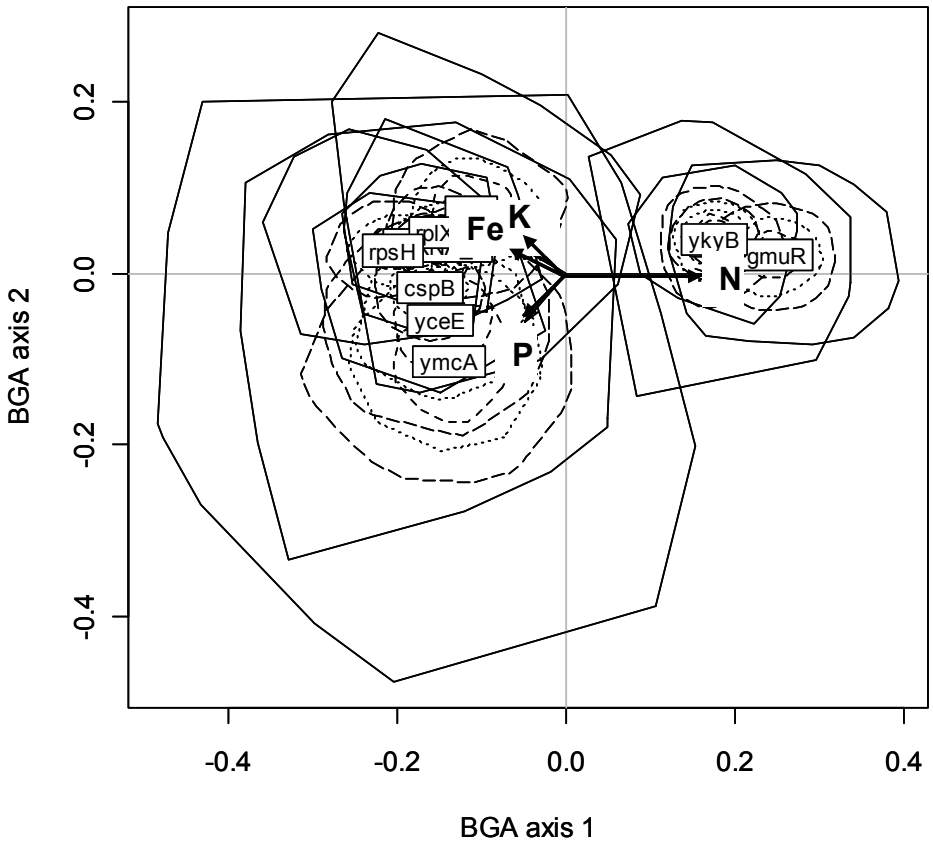

Figure 14: Uncertainty plots represent coordinates of the ten most discriminating genes after partial bootstrap (999 repetitions) in the first two axes of the BGA. The spread of gene coordinates are represented by convex hulls containing 25%, 50%, 75% and 100% of the points. Arrows represent the directions of class centroids.

The boxplots of gene contributions indicates the proportion of overly unstable genes which is normally referred to as false positive rate (FDR) (Baty *et al.* 2008). All treatments showed similar FDR, N deficiency had 27%; P, 28%; Fe, 25% and K (30%), which can be

considered quite high (Baty *et al.* 2008). However, in the highest gene ranks (up to 30) this proportion was very low (Figure 15).

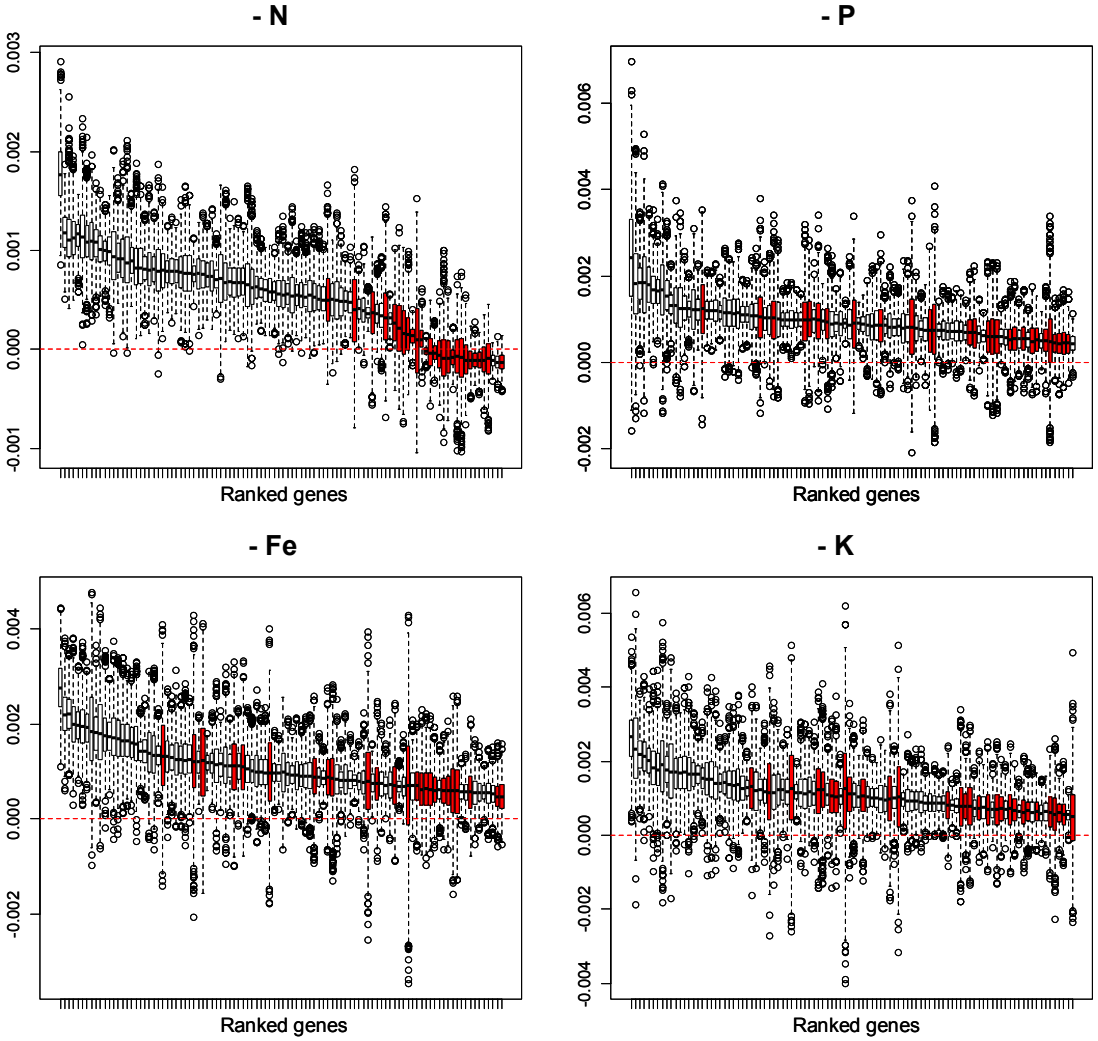

Figure 15: Sensitivity boxplots illustrate the distributions of gene contributions. The zero threshold is represented as a dashed line. Gene distributions where more than 5% of values are below 0 are represented as red boxplots. '-N' denotes nitrogen deficiency; '-P', phosphorus deficiency; '-Fe', iron deficiency and '-K', potassium deficiency.

4.4.8. Interpretation of changes in gene expression using the chemical composition of root exudates

The application of vector fitting was used to identify if changes in root exudates were significantly correlated to the ordination of treatment groups and genes (see Methods). The compounds in root exudates that showed significant correlation with the ordination were aspartate (Asp), valine (Val) and glutamate (Glu) ($p<0.05$) (Figure 16). Therefore, from all

29 measured dominant metabolites in root exudates, only three amino acids were significantly correlated with bacterial transcriptome changes associated to nutrient deficiency treatments. This observation suggests that overall changes in the bacterial transcriptome attributed to responses to different exudates could not be linked to most of the measured dominant metabolites. By observing the direction of the arrows that illustrate the changes in concentration of compounds, it is notable that these compounds mostly explain the separation in the first axis of the CA (CA1). This axis, as also observed in the BGA-CA (Figure 13), shows the separation between the N deficiency treatment from P, Fe and K (Figure 16). Since N-deficient maize root exudates had lower concentration of amino acids (Figure 5), this observation suggests that changes in transcriptional profiles can be partially attributed to differences quantities of Asp, Val and Glu between treatments.

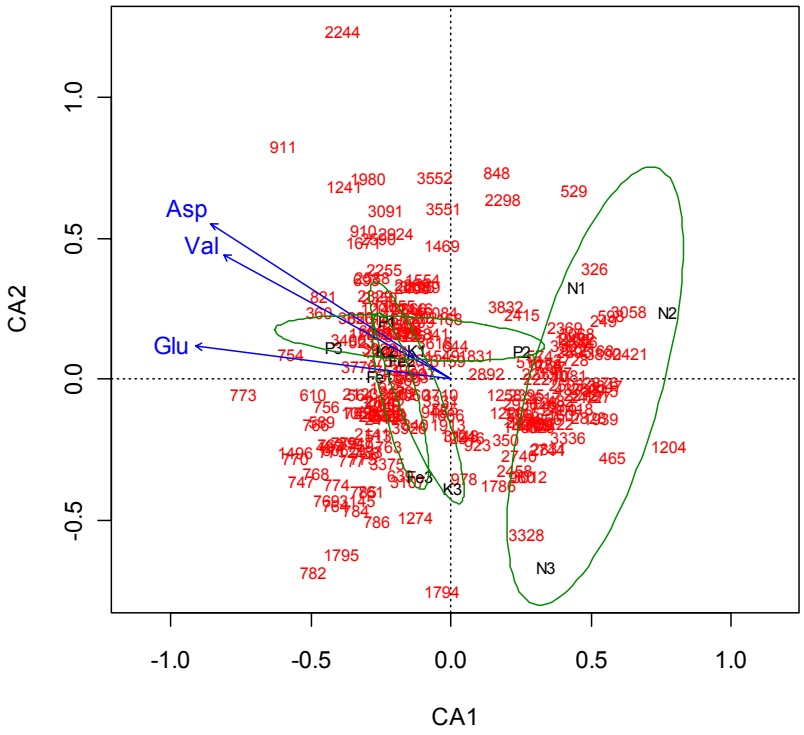

Figure 16: Vector fitting showing the chemical compounds that showed a correlation with the gene expression dataset.

5. GENERAL DISCUSSION

5.1. Transcriptional responses of *B. amyloliquefaciens* to seed and root exudates reflect bacterial adaptations to altered substrate and ion availabilities

Few studies have investigated the influence of plant-derived compounds on the transcriptional profiles of PGPR. The transcriptional profiling of *Pseudomonas* spp. in response to root exudates (Mark *et al.* 2005; Matilla *et al.* 2007) and effect of seed extracts on the induction of *Azospirillum brasilense* genes (Pothier *et al.* 2007) are the main examples. To unveil communication pathways between a Gram-positive PGPR and a crop species, the transcriptional response *B. amyloliquefaciens* FZB42 to maize-derived compounds was investigated in the present work. Root exudates collected from maize plants grown under different nutritional conditions and seed exudates were then incubated with bacterial cultures and RNA was isolated from cells harvested in the logarithmic and transitional phases. Repression of bacterial genes by seed exudates was more pronounced than induction (Figure 2). In the case of root exudates, the number of up- and down-regulated genes was nearly the same in the logarithmic phase. Likewise, root exudates collected from two varieties of sugarbeet up-and down-regulated similar numbers of genes in *Pseudomonas aeruginosa*. Between 8.1-9.3% of the transcriptome was significantly affected by these root exudates (Mark *et al.* 2005). The proportion of genes affected by maize root exudates in *B. amyloliquefaciens* was lower, ranging between 2.8 to 4.5%. However, seed exudates affected a larger proportion of the transcriptome (7.8-8.1%). When Matilla *et al.* (2007) carried out a transcriptional profiling of the root-colonizing bacterium *Pseudomonas putida* KT2440 in the rhizosphere of maize, one of their main observations was that gene activation was more pronounced in the rhizosphere lifestyle than gene repression. Similarly, gene induction by root exudates was more prominent than gene repression in FZB42 during transitional growth phase (Figure 2).

The observed induction of bacterial genes involved in transport and utilization of nutrients in the presence of either seed or root exudates may have occurred because the metabolites released by either plant roots or seeds represent an additional source of carbon or compounds that would be otherwise synthesized, such as purine/cytosines and cholines.

This is thought to be one of the reasons why higher bacterial densities are found in the rhizosphere compared to the bulk soil (Bertin *et al.* 2003; Morgan *et al.* 2005; Matilla *et al.* 2007). *Bacillus* species maximize their efficiency in metabolizing carbon-based energy sources by using a regulatory mechanism called 'Carbon Catabolite Control', which is composed of 'Carbon Catabolite Activation' (CCA) and 'Carbon Catabolite Repression' (CCR). In addition to catabolism, certain genes and operons are involved in particular anabolic processes, for instance the synthesis of secondary metabolites and extracellular enzymes. In such cases, when preferred sources of carbon and energy are available, they are not expressed. In this way transcriptional control of catabolic operons is performed by global regulators. Also the assimilation of the preferred carbon source plays a role in the modulation of the intracellular availability of specific inducers of genes involved in catabolism (Stulke and Hillen 2000; Fujita 2009). Therefore, the repression of some genes involved in the utilization of certain substrates may be related to CCR. Many genes involved in the catabolism of complex compounds are subjected to CCR. At the early stages of bacterial growth, preferred sources of carbon were available, due to the presence of easily metabolizable substrates in the growth medium. Bacterial cultures grown in the presence or absence of seed exudates may then have been affected by CCA and CCR. However, at a later stage, the carbon sources from the control without exudates may have been exhausted earlier than in the treatments with exudates. Therefore, the CCR may last longer in the presence of seed/root exudates due to the availability of additional sources of carbon.

Bacterial genes coding for iron transporters and siderophores by seed exudates were repressed during logarithmic growth (Appendix I). Micronutrients such as Fe, Mn and Zn are found in mature grains of maize and can be leaked during imbibition (Bityutskii *et al.* 2001; Bityutskii *et al.* 2002). Since at least two (Fe, Mn) of these three divalent cations are able to ligate to microbial siderophores (Duckworth *et al.* 2009), it is possible that their presence affects the expression of genes related to their transport. Nevertheless, in this study, Fe concentrations measured in seed exudates were lower than in root exudates (data not shown). Indeed, another study documented no difference in Fe contents of unsoaked maize seeds compared to soaked seeds. In the same report, however, the Zn content decreased significantly, and this reduction after soaking may be attributed to leaching of Zn ions (Lestienne *et al.* 2005). Therefore, Zn or Mn may be exuded in higher amounts than Fe by maize seeds, and bacteria in the spermosphere may promptly sense the available cations. Consequently, in the logarithmic phase, when the compounds released by the

seeds have not yet been exhausted by intense cell growth, genes involved in siderophore production and/or transport were repressed. However, in the transitional phase, 10 genes associated to siderophore biosynthesis and transport were induced. This observation suggests that, at this stage, the divalent cations previously available by the addition of seed exudates were getting exhausted from the medium, and the bacteria switched on a powerful mechanism for Fe and Mn acquisition, which is the production of siderophores (Guerinot 1994).

Seed exudates largely repressed several genes associated to the biosynthesis of compounds in the log and transitional growth phase (Appendix II and IV). Biosynthetic pathways are repressed when the end product is not needed or can be readily obtained by uptake from the environment (Kim and Gadd 2008). For instance, seed exudates repressed the expression of genes related to the biosynthesis of folate in both logarithmic and transitional phases. Folate can be found at a concentration of 0.19 microgram per gram in maize seeds (Bekaert *et al.* 2008). As seed exudates are mainly released by passive leakage, seeds may release folate, which could be absorbed by bacteria. This process would be energetically more efficient than de-novo folate synthesis. Folates are B vitamins, which act as cofactors for C1 metabolism. Cofactors are generally required in very small quantities. The C1 pathway includes nucleotide biosynthesis, amino acid metabolism and the methylation cycle. That may be also the case in the treatment with root exudates since genes related to compounds commonly found in root exudates such as phospholipids (*dgkA*) (Lucas Garcia *et al.* 2001; Schlichting and Leinweber 2009) were repressed at logarithmic growth phase. However, in the transitional phase many genes related to purine, folate, fatty acid and branched amino acid biosynthesis were induced by root exudates (Appendix VII). This observation indicates that, in the presence of root exudates, the cells were metabolically more active, even when the cell density was reasonably high (OD 3.0). This may occur possibly due to the extra input of energy sources, provided by the addition of root exudates into the medium.

The enhanced expression of a putative multidrug ABC transporter and a hypothetical protein showing 57% homology with bacitracin ABC transporter permease was observed in response to seed exudates at logarithmic phase (Appendix I). Multidrug ABC transporters have been related to extrusion of toxic substances, such as drugs or antibiotics (Dawson and Locher 2006; Locher 2009). Spermosphere competence is the increased ability to colonize seed surfaces and their surrounding soil by successful competition with other microbes (Lugtenberg and Kamilova 2009). Being able to survive in the presence of

antibiotics produced by bacterial cells and toxic substances possibly present in seed exudates (Harrison *et al.* 2008) may confer to *B. amyloliquefaciens* this capacity. A high spermosphere competence of *Bacillus* explains at least partially their success in bacterial formulations commonly used in seed treatments (Schisler *et al.* 2004).

A major limitation of DNA microarray studies is their inability to distinguish how many of the cells in the population were expressing a differentially expressed gene. Sporulation tended to be repressed after incubation with seed or root exudates. Maybe nutrients in seed and root exudates offered conditions that allowed the bacteria to remain metabolically active instead of sporulating. Nonetheless, some genes involved in the activation of sporulation were induced. It is noteworthy mentioning that bacterial cell populations of *Bacillus* contain subpopulations that may respond differently to stimuli. It has been already documented that *Bacillus* cultures can occur in a physiologically heterogeneous state (Chung and Stephanopoulos 1995; Kearns and Losick 2005), also concerning their sporulation ability (Veening *et al.* 2005; Morohashi *et al.* 2007). This may explain why, in both growth phases, irrespective of whether FZB42 was supplemented with seed or root exudates, some bacterial genes associated to sporulation were induced and others repressed.

A gene that encodes a nitrite extrusion protein (*narK*) was up-regulated in the presence of seed exudates (Appendix I). In low amounts, nitrite released by bacteria has been documented to cause phytohormonal effects in graminaceous species, being even more active than IAA in some root assays (Zimmer *et al.* 1988; Bothe *et al.* 1992; Didonet and Magalhães 1993). It has been suggested that ascorbate associated with nitrite also plays a role in the enhanced formation of root hairs and lateral roots (Zimmer *et al.* 1988; Bothe *et al.* 1992). Under acidic conditions, nitrite forms nitrous acid (HNO_2) and nitric oxide (NO) (Lundberg 2008). It was documented that non-enzymatic NO-releasing substances enhanced root tip expansion in a dose-dependent manner and even employ similar signal transduction pathway than IAA (Gouvea *et al.* 1997). It is tempting to speculate that also in the case of *B. amyloliquefaciens* the extrusion of nitrite may be an additional mechanism involved in its plant growth promotion. Further experiments need to be performed to test this hypothesis.

A key observation was the induction of genes related to the germination of spores, either by seed or root exudates. The term ‘germinants’ is used to define nutrients that can elicit spore germination. Some molecules such as amino acids, sugars, purine nucleosides are known germinants. However, different combinations of nutrients, such as asparagine,

glucose, fructose and K^+ can also trigger germination, e.g. in *Bacillus subtilis* (Paidhungat and Setlow 2002). Nutrient-independent germination takes place in the presence of lysozyme, pyridine-2, 6-dicarboxylic acid (dipicolinic acid) associated to Ca^{2+}, cationic surfactants, salts and elevated pressure (Gould 1969). Indeed, ordinary compounds known to play a role in germination were found in root and seed exudates, such as asparagine, glucose, or fructose (Table 2). Alanine is also known as a powerful germinant. Its concentration in seed exudates was strikingly high (Table 2).

Bacterial genes involved in motility and chemotaxis were generally repressed in the presence of seed exudates. The expression of the RNA polymerase sigma factor SigD involved in the regulation of flagella, motility and chemotaxis genes, and a motility protein (MotA) were decreased when cells were exposed to seed exudates, in both growth phases (Appendix II and IV). Possibly bacteria are able to sense compounds typically present in the spermosphere and, as the surface area of seeds is generally small, there would be no advantage in being highly motile. However, in the transitional phase, genes related to control of chemotaxis and motility were down-regulated, such as *mcpC* (methyl accepting chemotaxis protein) and *flgM* (anti-SigD). At this point, as the cell density was getting higher, the availability of nutrients was reduced, and hence motility may have been encouraged, as documented in other studies (Jurgen *et al.* 2005). Indeed, SigD has been proven to be active in the transitional phase of *B. subtilis* cultures (Marquezmagana and Chamberlin 1994). In the present experimental conditions, an evident effect of root exudates on genes related to motility and chemotaxis could not be observed.

Root exudates induced bacterial genes related to competence development (*pnpA*) and error-prone DNA synthesis (*polY1*) during the transitional phase (Appendix VII). Bacterial evolution is largely dependent on mutations and horizontal transfer of genetic material (Ochman *et al.* 2000; Novichkov *et al.* 2004). Fluctuations in environmental conditions can be overcome by the flexibility to adapt provided by such mechanisms. Indeed, there are indications that mutations and subsequent rhizosphere selection are directly associated to increases in competitiveness for root colonization (Martinez-Granero *et al.* 2006). A very important mechanism for the horizontal transfer in soils is natural transformation (Paget and Simonet 1994; Draghi and Turner 2006), through which competent bacteria take up free DNA. Typical compounds present in root exudates were reported to increase the competence for natural transformation in *Acinetobacter* sp., being organic acids the most efficient (Nielsen and van Elsas 2001). Hence, at least part of the performance displayed by *Bacillus amyloliquefaciens* FZB42 as an unspecific and competitive root colonizer may

be attributed to its genetic versatility, which may have conferred its great plasticity to adapt to hostile enviroments such as the rhizosphere.

5.2. Nutrient deficiencies affect the composition of primary metabolites in maize root exudates

The metabolite composition of maize root exudates was investigated to identify plant responses to different nutrient deficiencies. Higher concentrations of glutamate, citrate, ribitol and glucose were observed in Fe-deficient maize root exudates (Figure 5). An enhanced exudation of glutamate under Fe deficiency has also been observed in barley roots (Fan *et al.* 1997). Glutamate has been characterized as a strong bacterial attractant (Wood and Hayasaka 1981; Barbour *et al.* 1991) and ribitol and glucose are readily utilized C sources by most bacteria. A major mechanism evolved by microbes for Fe acquisition is the biosynthesis of siderophores, which are low-molecular weight molecules showing a high affinity for ferric Fe (Guerinot 1994). However, microbial siderophores do not appear to be direct sources of Fe for Strategy II plants, such as maize (Crowley *et al.* 1992). A ligand exchange between microbial siderophores and phytosiderophores may occur (Yehuda *et al.* 1996; Hördt *et al.* 2000) or, after degradation of microbial siderophores, solubilized Fe^{3+} can be captured by phytosiderophores (Barness *et al.* 1992). As microbial siderophores may increase the mobility of Fe in the rhizosphere (Hördt *et al.* 2000), the enhanced release of glutamate, glucose and ribitol may be a strategy to attract microorganisms and thus to cope with Fe deficiency. Fe limitation was also linked to a higher exudation of citrate (Figure 5 and Figure 6). A higher efflux of citrate has been previously reported in nutrient-deficient maize plants (Jones and Darrah 1995). Other monocots such as barley also exhibited higher exudation rates of organic acids under Fe deficiency, in particular of malate (Fan *et al.* 1997). Ultimately, Fe mobility in soils is enhanced by the presence of organic acids either directly by the formation of Fe-complexes that are suitable for Fe acquisition by plant roots (Jones *et al.* 1997) or indirectly by the formation of labile $Fe^{(III)}$-complexes with organic acids that facilitate subsequent ligand exchange (Kraemer *et al.* 2006).

Root exudates from P-deficient maize plants contained larger amounts of GABA and several sugars compared to those from nutrient-sufficient plants (Figure 5). Glutamate decarboxylases are known to catalyze the conversion of L-glutamate to GABA, which

have been reported to accumulate in various plant tissues under a variety of stress conditions (Shelp *et al.* 1999; Kinnersley and Turano 2000). Although the role of GABA in plants is still unclear, a stress signaling function has been convincingly suggested (Bouche and Fromm 2004). An increased exudation of sugars under P deficiency was also observed in other plant species, such as *Sorghum vulgare* and *Citrus aurantium* (Ratnayake *et al.* 1978; Schwab *et al.* 1983). This phenomenon has been associated with a decrease in phospholipid levels and a higher permeability of the plant cell membrane (Ratnayake *et al.* 1978; Graham *et al.* 1981). Since amino acids and organic acids are present as anions with low plasma membrane permeability under typical pH (7.1-7.4) (Bertin *et al.* 2003), and carbohydrates are known to accumulate in root tissues under P starvation (Cakmak *et al.* 1994b), sugars are very likely to be the most diffusible group of substances when the integrity of the membrane is affected. Consequently, an additional input of carbohydrates in the rhizosphere may stimulate germination and growth of PGPR, or of symbiotic microorganisms such as mycorrhizal fungi, which are known to improve P acquisition (Ratnayake *et al.* 1978; Graham *et al.* 1981; Schwab *et al.* 1991; Tawaraya *et al.* 1994). Qualitative differences in the exudation profile of sugars have also been reported in P-deficient plants. For instance, a greater proportion of pentoses relative to glucose and sucrose was released by *Zea mays*, *Brassica napus*, and *Pisum sativum* roots (Schilling *et al.* 1998). As a consequence, the mobilization of phosphate from $Ca_3(PO_4)_2$ by PGPR such as *Pantoea agglomerans* may be increased (Schilling *et al.* 1998). A higher exudation of ribose under P deficiency was also revealed, but there was no significant difference in sucrose release compared to the control (Figure 5). Dicotyledonous plants are generally reported to respond to P deficiency by increasing the root exudation of carboxylates (Neumann and Römheld 2000), and this response is often observed at later stages of P deficiency (Johnson *et al.* 1996). An enhanced release of carboxylates was observed for white lupin and chickpea, but not for tomato and wheat (Neumann and Römheld 1999). In the present study, there was no significant difference in the exudation of organic acids by maize in the early stages of P deficiency. Nonetheless, relative to the controls, the concentration of *cis*-aconitic acid was higher in root exudates from P-deficient plants. Interestingly, in a comparison of maize genotypes that differed in their tolerance to P deficiency, a higher organic acid exudation by P-starved plants was evidenced only in low-P tolerant maize genotypes (Gaume *et al.* 2001; Li *et al.* 2008).

Lower amounts of several sugars were exuded by K-deficient maize roots (Figure 5). The only study that has documented changes in root exudation by maize under K

deficiency reported an increase in sugars, amino acids and organic acids (Kraffczyk *et al.* 1984). In this case, however, plants were exposed to K deficiency for 10 and 15 days, which is a much longer duration of starvation than we used in this study (2 d). It is therefore likely that the observations of Kraffczyk *et al.* (1984) represent secondary responses to K deficiency. Furthermore, the allocation of photosynthates to roots is inhibited under K-deficient conditions (Hartt 1969; Cakmak *et al.* 1994a; 1994b) due to impaired phloem loading (Marschner *et al.* 1996). Therefore, given that the exudation of carbohydrates occurs mainly through passive diffusion (Jones *et al.* 2009), a lower amount of sugars in K-deficient root tissue as a consequence of impaired translocation might explain the low carbohydrate release observed under K-deficient growth conditions.

A lower release of amino acids from N-depleted plants (Figure 5) has also been reported for pine (Bowen 1969) and bean (Haase *et al.* 2007). This suggests that the lower amount of amino acids found in N-deficient root exudates is a direct consequence of the lower amount of amino acids being produced in N-deficient roots (von Wirén *et al.*, 2000) rather than the retrieval of previously released amino acids under N deficiency (Jones *et al.* 2004).

Apart from control plants, the order of treatments with the highest exudation rates of sugars and the two mentioned organic acids was P deficiency > N deficiency > Fe deficiency > K deficiency. As mentioned previously for root exudation by P-deficient roots, the release of carbohydrates in the rhizosphere may be a strategy to stimulate the growth and activity of rhizosphere microorganisms, such as mycorrhizal fungi (Wasaki *et al.* 2005; van Scholl *et al.* 2006), phosphate-solubilising (Vyas and Gulati 2009; Zaidi *et al.* 2009), associative nitrogen-fixing (Perin *et al.* 2006; Mehnaz *et al.* 2007) and siderophore-releasing bacteria (Guerinot 1994; Dey *et al.* 2004). The K deficiency treatment was the one which resulted in the lowest exudation rates of sugars. In the principle component analysis (PCA), *trans*-, *cis*-aconitic acid and GABA also had a significant influence on PC2, all being strongly associated with their enrichment under P limitation (Figure 6). *Trans*-aconitate is the predominant organic acid in grasses (Stout *et al.* 1967), which is in agreement with the present findings in maize (Figure 5). The release of *trans*-aconitic acid was also reported to be important in P-deficient maize lines when grown in acid soils (Gaume *et al.* 2001). Moreover, GABA was found to be linked with phosphorus starvation (Figure 6). Besides its role in stress signaling, the breakdown of GABA generates succinate. As some microorganisms are able to produce enzymes involved in this reaction (Priefer *et al.* 2001), it is possible that GABA might be used by

rhizosphere microorganisms as a precursor for the generation of organic acids, which improve P mobilization. However, additional data on the conversion of root-derived substances to organic acids by microbial activity are needed to prove this hypothesis.

The negative correlations between the average diffusion coefficient of nutrients in soils and root exudation rates under deficiency of the corresponding nutrients held true for each of the metabolic groups (amino acids, organic acids and carbohydrates) (Figure 7) and were in agreement with an increased exudation of organic acids as has been demonstrated in many investigations involving P- and Fe-limiting growth conditions (Ohwaki and Sugahara 1997; Neumann and Römheld 1999; Sas *et al.* 2001; Zocchi *et al.* 2007), whereas a lower root exudation of carboxylates, sugars and amino acids has previously been observed in N-deficient bean plants (Haase *et al.* 2007). With the exception of citrate and malate, this inverse correlation mainly builds on exudate components for which a positive effect on nutrient mobilization has not yet been demonstrated. Thus, the release of root exudates may reflect a non-specific response to nutrient deficiencies with exudation rates increasing at decreasing nutrient solubility. Therefore, a hypothesis is proposed, in which this negative correlation reflects an ancient adaptation strategy evolved before the release of specific nutrient-mobilizing exudates (e.g. phytosiderophores). In such an early stage of adaptation, plant roots may just have released more exudates the lower the solubility of the required nutrient was. Due to the lack of a time-dependent analysis of root exudate profiles for each nutrient, which would be required to precisely identify the maximum peaks of root exudation under any of the investigated nutrient deficiencies, the current study cannot yet prove but rather provide experimental evidence for raising and further testing this hypothesis. The molecular mechanism behind this strategy remains unclear, but plant growth regulators, such as indol-acetic acid (IAA), zeatin or kinetin may play a role. There is a long series of studies showing that these and other phytohormones in roots change with the nutritional status of plants (Marschner 1995; Lopez-Bucio *et al.* 2002; Shin and Schachtman 2004; Seguela *et al.* 2008; Argueso *et al.* 2009). In turn, phytohormones affect ion leakage from cell cultures of winter wheat (Filek *et al.* 2004) or the membrane permeability of rice suspension cells (Grossmann *et al.* 1986). Assuming that the unspecific release of amino acids, organic acids and carbohydrates is mainly mediated by passive diffusion (Jones *et al.*, 2009), phytohormone-induced changes in the permeability of the plasma membrane are then likely to affect the release of those root metabolites that were accumulating at highest levels.

5.3. Root exudates from nutrient-deficient plants affect differently the transcriptome of *Bacillus amyloliquefaciens* FZB42

Many of the differentially expressed genes by seed (33.65%) or root exudates (35%) coded for hypothetical proteins whose specific functions are yet to be elucidated. Likewise, nutrient-deficient root exudates led to altered bacterial transcription of genes encoding several hypothetical proteins (29.7 % for N, 27.9% for P, 30.7% for Fe e 38.1% for K). Similar studies were also confronted with this problem (Mark *et al.* 2005; Matilla *et al.* 2007). *In silico* methods based on protein-protein interactions, comparative genomics, functional assignment based on 3D structures, clustering approaches, genome context methods and other approaches have been used to predict protein function (Sivashankari and Shanmughavel 2006). Nevertheless, laboratory experiments are still needed to assure that those hypothetical proteins are translated and do not represent pseudo-genes (Pawlowski 2008). Clearly, additional efforts have to be made to reveal the functions and thus the mechanisms enclosed in the 'black box' of the hypothetical proteins.

Relative to other treatments, exudates from N-deficient roots affected the highest numbers of transcripts in *B. amyloliquefaciens*, particularly in the early stages of bacterial growth (Figure 8). The main trends observed in the chemical analysis of dominant compounds in these root exudates were: 1) lower concentrations of amino acids and 2) a generally lower exudation of all other analyzed compounds (Figure 5). A number of 32 genes associated to protein synthesis, especially coding for ribosomal, and other proteins involved in electron transport or ATP synthesis (*atpE*, *trxA*, *atpC* and *qoxA*) were down-regulated in the logarithmic phase (Appendix XI). This observation indicates that bacterial activity was lower after incubation with root exudates from N-deficient plants than with root exudates from nutrient-sufficient plants .Therefore, a lower availability of primary metabolites may have affected the global bacteria metabolism. It is worth mentioning, however, that since the release of exudates from maize plants is suggested to promote microbial immobilization (Qian *et al.* 1997) and roots are able to outcompete microorganisms for N (Wang and Bakken 1997; Hu *et al.* 2001), in conditions of N limitation roots may release more substances that inhibit bacterial activity, such as antimicrobial compounds. Indeed, a stress response protein was induced by N deficiency (*ykoL*) (Appendix XI). Furthermore, antimicrobial substances are reported to be released in root exudates (Rumberger and Marschner 2004; Bais *et al.* 2006). However, studies on the

quality and quantity of those substances in root exudates, particularly for different nutritional status of plants, have not yet been performed. Interestingly, in the transient growth phase, a number of 12 ribosomal proteins and two genes involved in membrane bioenergetics were up-regulated (*qoxD* and *atpC*). This might have occurred because antimicrobial substances are commonly unstable with time and can be inactivated through several processes (Jefferys 1952), therefore bacterial activity would no longer have been repressed.

Particularly in the transitional phase, P-deficient maize root exudates induced several bacterial genes involved in motility and chemotaxis. The P forms obtained by plants from the soil solution are orthophosphate anions (predominantly as HPO_4^{2-} and $H_2PO_4^-$). Because of their low concentration in most of the soils, orthophosphate is rapidly depleted in the immediate vicinity of plant roots and consequently a large concentration gradient is created between the bulk soil and the root surface. Nevertheless, due to the low diffusion rates of orthophosphate in soils, plant uptake of P is often limited (Richardson *et al.* 2009a). Consequently, plants have developed morphological strategies to overcome P limitation, such as increase of root elongation/root to shoot biomass ratio, root branching in surface soils or nutrient-rich regions, and increased density of root hairs (Richardson *et al.* 2009b). Among the physiological adaptations, acidification of the rhizosphere is an effective strategy initiated by the release of organic anions and protons, which can increase the solubility of sparingly-soluble inorganic compounds (Neumann and Römheld 2007). The major constituent of the soil organic P pool is comprised by inositol penta- and hexaphosphates (phytates) and their derivatives (Anderson 1980). Microorganisms that are able to mineralize organic P and solubilize inorganic P in soils play a significant role in increasing P availability to plants (Richardson *et al.* 2005). *Bacillus amyloliquefaciens* FZB42 was shown to secrete phytase during the transitional phase, which it is believed to contribute to their plant growth-promoting activity (Idriss *et al.* 2002). Interestingly, a gene involved in protein secretion was up-regulated (*secY*). Under conditions of P limitation, triggering *Bacillus* motility in the rhizosphere may improve root access to sparingly available P. A significant association between metabolites measured in P-deficient maize root exudates and bacterial genes involved in motility was not found. This remark suggests that the mediating compound was not measured in this study. Nonetheless, the chemical analysis of the root exudates from plants exposed to P deficiency evidenced larger concentrations of GABA and several sugars, such as inositol, erythritol, ribitol, fructose, glucose and arabinose in comparison to the control. Since GABA has been associated to

signaling under stress conditions (Bouche and Fromm 2004), it is a candidate for acting as a signaling compound when maize is P-starved. Furthermore, sugars are capable of eliciting chemotaxis responses in bacteria (Thoelke *et al.* 1990). Therefore, they may have played a role in inducing genes associated to motility in FZB42. In fact, an enhanced transcription of genes involved in sugar uptake and utilization, such as *rbsC*, *rbsD*, *rbsK* and *fruK*, was observed in *B. amyloliquefaciens* when exposed to P- deficient maize root exudates (Appendix XI).

An increased concentration of citrate was observed in root exudates from plants exposed to Fe deficiency (Figure 5). Interestingly, a gene associated to citrate/malate uptake was down-regulated in the logarithmic growth phase, although the significance was marginal ($p = 0.052$) (Appendix XIX). CimH functions as citrate/L-malate symporter and was reported as a high affinity, low capacity citrate transporter and a low affinity, high capacity L-malate transporter (Krom *et al.* 2003). High affinity transporters are often repressed when a compound is present in sufficient amounts (Atwell *et al.* 1999). It is then tempting to speculate that the increase in citrate amounts derived from Fe deficient maize root exudates may have caused the reduced expression of CimH.

Either in the case of comparison between seed and root exudates, or between exudates from different nutrient deficiency treatments, fewer genes were shared among deficiency treatments in the logarithmic phase of bacterial growth (Figure 9) compared to the transitional phase (Figure 10 and Figure 11). In addition, the Monte Carlo permutation test after the BGA-CA revealed that differences between treatments in the logarithmic phase were more prominent ($p < 0.05$). A genome-wide analysis of gene expression of the root-colonizing bacterium *Pseudomonas putida* KT2440 in the rhizosphere of maize revealed that many genes encoding ribosomal proteins are induced in the rhizosphere compared to cells in stationary phase and it was hypothesized that active growth and metabolism was occurring at least in a subpopulation of cells (Matilla *et al.* 2007). Therefore, it is possible that the differences between root exudates are more evident in the logarithmic phase because primary transcriptional responses to the added plant-derived compounds may occur when most of these compounds haven't yet been modified, degraded or consumed by bacteria. The microbial transcriptomes from the transitional phase may hence reflect bacterial decomposition products rather than seed or root exudates. Furthermore, bacteria-derived secondary metabolites that accumulate at high population densities (Johnson *et al.* 2005; Barnard *et al.* 2007) may lead to secondary transcriptional responses.

As a general trend, in the logarithmic phase, bacterial transcriptional profiles in response to N-deficient maize root exudates were the most divergent compared to other deficiencies, followed by P deficiency (Figure 13). Many of the most discriminating genes were down-regulated under N depletion and not altered in the others (P, Fe or K) (Appendix L). Several of them code for ribosomal proteins. Transcriptional profile studies have indicated that up-regulation of genes encoding ribosomal and adhesion-related proteins, and repression of flagella-associated genes are frequent responses upon biofilm formation (Lazazzera 2005). Biofilm formation is commonly associated with root colonization, which is the first step in many plant-microbe interactions (Espinosa-Urgel *et al.* 2002; Ramey *et al.* 2004). An antagonist of biofilm repression (*ymcA*) was down-regulated only under N deficiency. This observation indicates that under N-limitation plants may try to hinder root colonization. As discussed previously, plants and microorganisms can compete for N sources (Wang and Bakken 1997) and therefore to avoid bacterial colonization may be a strategy adopted by plants to outcompete microorganisms. In addition, *ymcA* was found to be one of the genes that correspond to differences in aspartate concentrations of nutrient deficient root exudates by using vector fitting (Figure 16).

Aspartate was found in lower concentrations in N-deficient maize root exudates (Figure 6). Its decrease coincided with the induction of a sporulation gene (*spoIIIAE*), evidenced by the vector fitting (Figure 16). This amino acid may then be involved in the bacteria-plant interactions as a cue metabolite for plant nutrient deficiency. However, further experiments are required to clarify this issue. Interestingly, proline biosynthesis appeared to be induced under N deprivation, as its encoding gene (*proJ*) was one of the ten most discriminating genes for the N deficiency treatment (Appendix L). Proline has been referred as a stress protectant in bacteria and plants (Takagi 2008), however in bacteria it is mostly associated to adaption to osmotic stress (Csonka 1989). Additionally, a mechanosensitive channel (*yfkC*) involved in resistance to osmotic downshock was also induced only by N-deficient maize root exudates. Since it is very unlikely that root exudates would shift the osmolarity of the soil solution from high to low, it is possible that a common regulator related to general stress may be affecting the expression of those genes as well. Indeed, SigB represent a potential candidate for such a function, since it is a general stress regulator that also regulates other MscS-type putative channel-forming proteins (YkuT) (Hoffmann *et al.* 2008).

The measured primary metabolites are ubiquitous in soils and can be used as nutrient sources by bacteria. The differences between transcriptional responses of FZB42 in the log phase to P, Fe and K deficient-maize root exudates were mild (Figure 13), possibly because compounds necessary to sustain 'normal' bacterial growth were present. The major trend shown by the vector fitting and the CCA was that the main transcriptional differences occur along a gradient of exudate quantity (Figure 16). Therefore, there is little evidence to suggest that any of the measured compounds were linked to specific genes.

At the transitional phase, a gene encoding for a ribosomal protein (*rpsR*) was induced in all deficiency treatments and five (*rpsP*, *rplF*, *rpsH*, *rplE*, *rplN*) under N and P deficiencies (Figure 10). As previously stated, induction of ribosomal proteins has been associated to biofilm formation (Lazazzera 2005). A non-specific mechanism for induction of biofilm formation may be triggered by nutrient deficiencies, as indicated by up-regulation of ribosomal proteins. This observation suggests that plant exudates may promote colonization by *Bacillus amyloliquefaciens* FZB42 under conditions of nutrient deprivation. This would only be the case if antimicrobial substances in root exudates are not present in inhibiting amounts, as hypothesized for N-deficient maze root exudates in the logarithmic phase of bacterial growth. Several reports have documented that nutrient deficiencies favor the action of PGPR, such as growth promotion by *Pseudomonas* strain GRP3A in iron-deprived mung bean (Sharma and Johri 2003), higher IAA levels by *Azospirillum brasilense* SM under certain macronutrient limitations (Malhotra and Sriuastava 2009), or plant growth stimulation in nutrient-deficient soil by *Pseudomonas alcaligenes* PsA15, *Bacillus polymyxa* BcP26 and *Mycobacterium phlei* MbP18 (Egamberdiyeva 2007). Therefore, an evaluation of plant growth promotion effects of *Bacillus amyloliquefaciens* FZB42 on plants grown under different nutrient deficiencies is urged.

Many studies have attributed the plant growth-promoting effect of PGPR, at least partially, to the production of indol acetic acid (IAA) (Huddedar *et al.* 2002; Kannan and Sureendar 2009; Malhotra and Sriuastava 2009), including *Bacillus amyloliquefaciens* FZB42 (Idris *et al.* 2004). Tryptophan is the main precursor in the IAA biosynthesis pathway in bacteria (Spaepen *et al.* 2007). Plant growth, promoted by FZB42, has been reported to increase in a tryptophan-dependent manner (Idris *et al.* 2007). Additionally, a relatively high amount of IAA was detected in culture filtrates even without the addition of tryptophan (29 $ng.ml^{-1}$) (Idris *et al.* 2007), which indicates that *B. amyloliquefaciens* don't depend on external supply of this amino acid to produce IAA. Interestingly, a gene

encoding for a tryptophan operon RNA-binding attenuation protein (TRAP) (*mtrB*) was up-regulated when *B. amyloliquefaciens* was exposed to nutrient-sufficient maize root exudates (Appendix III). TRAP negatively regulates the tryptophan operon in response to high intracellular levels of L-tryptophan (Gollnick 1994). Therefore, it is possible that bacteria were induced by maize root exudates to biosynthesize or to take-up tryptophan to produce IAA. In spite of the fact that tryptophan has been measured in root exudates of different plants (Kamilova *et al.* 2006), it could not be detected in samples of maize root exudates in the present study. This may be because the amount exuded was below the detection limit of the system used or tryptophan was indeed not released by roots. A gene involved in the biosynthesis of tryptophan (*trpC*) was induced by all maize-deficient root exudates (Appendixes XII, XXI and XXV). However, the significance was marginal in the case of P deficiency treatment ($p = 0.077$). Other PGPR such as *Azospirillum brasilense* SM produced higher IAA amounts under N and P depletion (Malhotra and Sriuastava 2009). Plant characteristics commonly observed as outcomes of IAA action such as increased length and density of root hairs, and elongation of lateral roots are observed in conditions of P (Gahoonia and Nielsen 2004; Akhtar *et al.* 2009), K (Brouder and Cassman 1994), Fe (Schmidt *et al.* 2000; Lopez-Bucio *et al.* 2003), or N (Chun *et al.* 2005; Schachtman and Shin 2007) deficiencies. Therefore, there is evidence to suggest that induction of bacterial growth regulators may be a strategy to overcome nutrient deficiencies. However, the induction of *trpC* could not be confirmed by real-time PCR (data not shown). A possible explanation is that the *trpC* transcript of *B. amyloliquefaciens* is too unstable. One of the difficulties in validating microarray results is attributed to the short half-life of mRNAs. Although a wide range of stabilities can be observed, approximately 80% of all mRNAs in *E. coli* have half-lives between 3 and 8 min (Bernstein *et al.* 2002). It is worth mentioning other intrinsic errors of both techniques that can affect validation of results. In microarray analysis, these errors are dye biases (Yang *et al.* 2002) and non-specific/cross hybridizations of labeled targets to array probes (Chuaqui *et al.* 2002). In real time PCR experiments there are amplification biases (Chuaqui *et al.* 2002), exponential amplification of errors (Freeman *et al.* 1999), mispriming or formation of primer dimers (Bustin 2002) and changes in efficiency at later cycles (Freeman *et al.* 1999). Finally; fundamental differences in normalization of both techniques (Morey *et al.* 2006) may also lead to non-matching results. Therefore, an independent experimental approach should be taken to test this hypothesis, for instance the measurement of IAA

production by *B. amyloliquefaciens* when exposed to nutrient-deficient maize root exudates.

Interestingly, the induction of a bacterial gene involved in spore germination (*gerAC*) was also observed as a rather general response to different nutrient-deficient maize root exudates (-N, -Fe, -K). The *gerA* operon is relatively ubiquitous among spore formers and is required for germination in L-alanine (Hudson *et al.* 2001). However, nutrient-deficient maize root exudates did not show differences in alanine amounts in relation to control exudates (Figure 5). Therefore, it is unlikely that this compound was responsible for altered expression of *gerAC* in FZB42. This observation suggests the existence of another, yet uncharacterized germinant exuded by maize under nutritional stresses.

Bacterial genes involved in *myo*-inositol catabolism were induced in the logarithmic growth phase in the presence of nutrient-sufficient maize root exudates (Appendix V). However, these genes were repressed in the transitional phase by plant nutrient-deficient maize root exudates (Appendixes XIII, XVIII, XXI and XXV). In the present study an enhanced root exudation of inositol under P limitation was observed and no significant differences were found between other deficiency treatments and the control (Figure 5). These observations contradict the idea that lower amounts of inositol in nutrient-deficient maize root exudates would cause the down-regulation of bacterial genes involved in inositol catabolism. Different functions have been attributed to *myo*-inositol as a modulator compound in plant growth and development. Examples of such processes are auxin storage and transport, phosphatidylinositol (PI) signaling pathway, phytate and cell wall biosynthesis and production of stress-related molecules (Loewus and Murthy 2000; Stevenson *et al.* 2000; Perera *et al.* 2006). Inositol derivatives present in root exudates may be acting as general signaling molecules for plant nutrient limitation. Additional studies on the characterization of inositol signaling in response to nutrient supplies are urged. Nonetheless, the induction of inositol degradation genes in FZB42 by root exudates collected from plants under adequate nutrient supply may have occurred in response to the additional input of inositol, which was indeed detected in the metabolite analysis (Figure 5).

Several genes involved in the degradation of compounds, their uptake, and biosynthesis were commonly down-regulated by different nutrient deficient-maize root exudates in the transitional phase (Appendixes XIII, XVIII, XXI and XXV). Bacteria respond to high population densities with quorum sensing. Acylated homoserine lactones are signaling molecules utilized by Gram-negative bacteria for the regulation of quorum

responses and they accumulate extracellularly as cell density increases (Fuqua and Greenberg 1998). In contrast, quorum responses in Gram-positive bacteria, such as *Bacillus subtilis*, are mediated by signaling released peptides which elicit a regulatory response in a concentration-dependent manner (Bischofs *et al.* 2009; Lopez and Kolter 2010). Plant nutrient deficiencies are reflected in root exudates (Figure 5) and root-colonizing bacteria may adapt their metabolism accordingly. When bacterial densities are high, such as in the transitional phase of bacterial growth, microbial genes associated to certain metabolic pathways may be repressed. These metabolic pathways are likely to be mainly associated with catabolism and transport of substrates, to prolong survival of the bacteria in case of nutrient limitation. Indeed, it has been suggested that quorum-sensing and starvation-sensing are integrated to regulate cell entry into stationary phase (Lazazzera 2000). However, it is worth mentioning that this may happen only in a subpopulation of cells, since distinct differentiation pathways are triggered by sensing extracellular signals (Lopez and Kolter 2010).

Interestingly, at least one bacterial hypothetical protein was identified as a discriminating gene specific for N, P, or Fe deficiency treatments (Appendix L). Plant nutrient deficiencies are usually reflected in root exudates before visible symptoms become apparent in plants shoots. Consequently, the use of *B. amyloliquefaciens* FZB42 as nutrient starvation sensing bacterium could be useful for early detection of nutrient limitation in soils. The expression of bacterial genes associated exclusively with a certain plant nutrient deficiency (N, P or Fe) could be measured. Gene expression could then be monitored via real-time PCR, as already suggested for other processes such as hydrocarbon degradation activity (Beller *et al.* 2002).

Several non-coding RNAs had altered transcription by *B. amyloliquefaciens* FZB42 when exposed to different maize root exudates. Non-coding RNAs are also called small RNAs, to which regulatory functions have been attributed in all three domains of life. They vary in sizes from approximately 50 to 600 nucleotides and in bacteria they generally modulate changes in cellular metabolism in response to environmental changes, especially under suboptimal or stressful growth conditions (Wassarman 2002; Pichon and Felden 2008). Detecting regulatory pathways in which specific non-coding RNAs are involved was not within the scope of this study, but these findings may be used as an initial reference for candidate small RNAs modulating bacterial responses to plant nutritional deficiencies.

5.4. Correlation between metabolite composition of root exudates and bacterial gene expression

From all measured primary metabolites, only glutamate, valine and aspartate showed a significant correlation with changes in bacterial gene expression (Figure 16). These amino acids explain mostly the separation of deficiency treatments in the x-axis of the BGA-CA. This axis showed a clear separation between the N deficiency treatment and all other deficiencies (P, Fe and K) (Figure 13). As observed in the primary metabolite analysis (Figure 5), root exudates from N-deficient plants showed lower concentrations of several amino acids. The expression of the most discriminating genes may be associated with decreases in metabolite concentrations in root exudates. There were two major trends. One was the repression of genes involved in translation (ribosomal proteins), biosynthesis of branched chain amino acids (*ilvH*) and response to ethanol stress (*yceD*, *yceE*) by N-deficient maize root exudates (Appendix L). The other was the induction of genes associated with the control of sporulation (*spoIVFA*) and biosynthesis of proline (*proJ*). The lower concentration of amino acids in the N-deficiency treatment may have been an environmental cue for nutritional stress and, as a consequence, the bacteria slowed down their metabolism, which was reflected by the down-regulation of the ribosomal proteins. In addition, amino acids have been reported to serve as signaling molecules for bacteria (Shapiro 1998). They function as communication molecules in the initiation of fruiting body formation in *Myxococcus xanthus* (Kim *et al.* 1992; Kaplan and Plamann 1996) and particularly glutamate and aspartate play a role in during autoaggregation in chemotatic *E. coli* (Budrene and Berg 1991; 1995). Indeed, a genome-wide analysis of *B. subtilis* transcriptional responses induced by glutamate, valine and glutamine pulses revealed that the metabolism of the bacteria was reprogrammed and showed both similarities and dissimilarities between amino acid pulses (Ye *et al.* 2009). However, interpretation over the expression of thousands of genes based on 29 dominant primary metabolites present in a complex mixture of chemical compounds like root exudates has to be made with caution. Anyway, such observations can give important insights to start understanding complicated systems such as molecular plant-microbe interactions. In root exudates of *Arabidopsis thaliana*, a number of 289 possible metabolites were detected, and differences in quantity and quality of certain compounds between treatments confirmed that roots have distinct

responses under different stress conditions (Walker *et al.* 2003). Moreover, only the most dominant primary metabolites in root exudates were considered in this study. However, secondary metabolites released by roots such as flavonoids and strigolactones (Steinkellner *et al.* 2007; Yoneyama *et al.* 2008) are typically associated to signaling in plant-microbe interactions in conditions of plant nutrient limitation. Due to their potential unspecificity, it is rather unlikely that ubiquitous compounds in soils like amino acids, organic acids and sugars may act as signals for plant nutrient starvation. Therefore, ideally, a careful investigation should include all detectable metabolites (both primary and secondary) by a sensitive technique (Krishnan *et al.* 2005) and a multivariate statistical approach may be best suited to correlate metabolites with transcriptional profiles and detect the most important compounds that trigger gene expression.

By combining metabolite analyses of seed and root exudates with bacterial responses at the transcriptome level the present thesis shed light on a few novel aspects in plant-microbe interactions, such as the possible role of seed exudates as inducers of multidrug ABC transporters in the spermosphere, or root exudates as inducers of genes involved in the generation of mutations and in the development of rhizosphere competence. Additionally, gene expression analysis further pointed to a role of seed and root exudates as spore germinants. As seed and root exudates differentially regulated the expression of many bacterial genes, mainly those involved in catabolic or anabolic processes, they are likely to transmit information to the bacterial partner that depends on the developmental stage of the plant/seed. Even though further investigations are needed to understand to what extent the quality or quantity of seed/root exudates determined the transcriptional response in *Bacillus*, and which exudates components were responsible for this differential expression, the present study provides a starting point for subsequent studies aiming at uncovering prominent effects or individual exudates components on the expression of the described bacterial genes. In future studies, promoters of these genes could be fused to reporter genes to characterize developmental or spatial gradients in the root-microbial communication in the rhizosphere. Moreover, these promoter-reporter fusions might be employed to monitor the presence of exudates components that are relevant for the signal exchange in chemically fractionated exudates up to their ultimate purification and identification.

Regarding the effect of the plant's nutritional status on the composition of root exudates, the present thesis found that, in deficiencies of key nutrients, the exudation rates of the most abundant primary metabolites (sugars, amino and organic acids) negatively

correlated with the average diffusion coefficient of these nutrients in soils. This allowed setting up the hypothesis that the release of these primary metabolites in root exudates may reflect an ancient adaptation strategy to solubilize poorly mobile nutrients in the rhizosphere, which probably evolved long before plants started to synthesize and release specific nutrient-mobilizing exudates. To substantiate this hypothesis, however, more extended metabolite analyses are required and quantitative effects of the exudates need to be revisited.

In general, root exudates from N-deficient maize plants had a greater impact on the transcriptome of *Bacillus amyloliquefaciens* FZB42, which was mainly caused by the repression of bacterial activity. In contrast, P-deficient maize root exudates induced the expression of genes associated with motility. Among all 29 analyzed primary metabolites, only changes in aspartate, valine and glutamate were significantly correlated to bacterial transcriptomes differences that could be attributed to different nutrient-deficient maize root exudates. Therefore, these amino acids may serve as rhizosphere cues for plant nitrogen starvation. Further studies with different plant and/or bacteria species designed in rhizosphere environments are urged to better understand plant-bacteria associations in a molecular perspective.

Taken together, these findings present novel knowledge about the early communication between plant and associative-bacteria and identify associated genes and processes that deserve continuing research. This is the first study comparing the effect of different nutrient deficiencies on the composition of primary metabolites in root exudates of one plant species and evaluating systematically the transcriptional response of a Gram-positive PGPR to seed and root exudates collected from plants grown under different nutrient regimes.

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7. APPENDIX

APPENDIX I: Bacterial up-regulated genes in the logarithmic phase by seed exudates

Gene ID	Gene and function	M	Fold-change	p-value
RBAM_026750	Hypothetical protein RBAM_026750	2.12	4.34	4.2E-05
RBAM_008220	Hypothetical protein RBAM_008220	1.95	3.86	2.7E-02
RBAM_004230	*mtlA* - mannitol-specific enzyme IIABC component of phosphotransferase system (PTS)	1.80	3.49	6.3E-04
RBAM_026780	Hypothetical protein RBAM_026780	1.78	3.44	9.6E-03
RBAM_004240	PTS mannitol-specific enzyme IIA	1.77	3.41	1.2E-06
RBAM_030880	*yrdF*- putative ribonuclease inhibitor	1.77	3.40	1.2E-02
RBAM_036010	*cydA* - cytochrome d ubiquinol oxidase (subunit I) involved in respiration	1.76	3.40	4.2E-02
B_amylo_FZB42_3965	predicted ncRNA	1.75	3.35	6.7E-04
RBAM_026600	*ywdH* - putative aldehyde dehydrogenase	1.74	3.35	8.5E-05
B_amylo_FZB42_3861	predicted ncRNA	1.74	3.35	3.7E-02
RBAM_036390	*yxiA* - conserved hypothetical protein	1.74	3.33	2.9E-03
RBAM_034950	putative drug resistance transporter	1.73	3.32	1.9E-02
RBAM_034190	*ywkC/racA* - cell division protein	1.70	3.24	3.9E-03
RBAM_030520	putative two-component response regulator	1.69	3.22	1.0E-02
RBAM_019720	*yoqH* - hypothetical protein [Bacteriophage SPBc2]	1.58	2.99	1.7E-03
RBAM_033620	*spoIIID* - stage III sporulation protein D (SpoIIID)	1.52	2.86	4.9E-02
RBAM_013340	*mtnK* - methylthioribose kinase involved in methionine salvage	1.51	2.85	1.9E-02
RBAM_034210	*maeA* - NAD-dependent malate dehydrogenase involved in malate utilization	1.47	2.78	5.4E-04
RBAM_010950	*asnO* - asparagine synthetase [glutamine-hydrolyzing]	1.46	2.75	1.1E-02
RBAM_037040	putative xanthine dehydrogenase	1.44	2.72	4.1E-02
RBAM_034470	*narK* - nitrite extrusion protein	1.43	2.70	2.6E-02
RBAM_031030	opuBC - choline ABC transporter (choline-binding protein) involved in compatible solute transport	1.39	2.62	7.8E-03
RBAM_035960	*yxlA* - putative purine-cytosine permease	1.39	2.62	2.9E-03
RBAM_024890	*safA* - spoVID associated morphogenetic protein involved in spore coat formation	1.37	2.59	3.1E-02
RBAM_018730	*yoxB* - hypothetical protein	1.37	2.58	2.7E-02
RBAM_036540	*yxeD* - hypothetical protein	1.36	2.56	1.2E-02
RBAM_028520	*yugF*- hypothetical protein	1.31	2.49	4.7E-05
RBAM_031910	*yvdB* - putative anion transporter	1.31	2.48	1.8E-02
RBAM_011650	*yjbQ* - putative Na+/H+ antiporter involved in the pH and Na+ cellular homeostasis	1.28	2.43	1.3E-02

RBAM_034630	*pbpG* - bifunctional glucosyltransferase/ transpeptidase	1.28	2.43	3.0E-03
RBAM_005880	*yraE* - spore coat protein	1.27	2.42	1.4E-02
RBAM_035530	*ywbN* - elemental iron uptake system (binding protein)	1.25	2.38	1.7E-02
RBAM_029720	hypothetical protein	1.25	2.37	3.1E-03
RBAM_022730	*spoIIIAD* - stage III sporulation protein AD	1.24	2.37	4.3E-04
RBAM_031820	conserved hypothetical protein	1.24	2.36	2.3E-03
RBAM_034510	*ywhL* - conserved hypothetical protein	1.23	2.34	1.8E-05
RBAM_024160	putative hydrolase	1.21	2.32	2.2E-04
RBAM_024790	*yrbG* - conserved hypothetical protein	1.20	2.29	2.5E-02
RBAM_024680	*yrzK* -hypothetical protein	1.15	2.23	4.6E-05
RBAM_035900	*gmuD* - phospho-beta-mannosidase involved in glucomannan utilization	1.15	2.22	1.1E-02
RBAM_037720	hypothetical protein	1.13	2.18	3.1E-02
RBAM_010100	*yhaT* - K+/H+ antiporter for K+ efflux	1.11	2.16	5.4E-02
B_amylo_FZB42_3943	predicted ncRNA	1.11	2.15	4.2E-02
RBAM_030400	*yvrL* - anti-Sig(YvrI-YvrHa) involved in the control of Sig(YvrI-YvrHa) activity	1.10	2.14	8.6E-06
RBAM_017060	*ymzB* - hypothetical protein involved in survival of ethanol and salt stresses	1.09	2.13	6.7E-03
RBAM_034800	hypothetical protein	1.08	2.12	1.9E-02
RBAM_036290	*yxxG* - hypothetical protein	1.07	2.10	3.9E-04
RBAM_036020	*cimH* - citrate/malate transporter	1.03	2.05	2.9E-02
RBAM_010880	*gerPB* - spore germination protein	1.03	2.04	2.0E-02
RBAM_034500	*albG* - antilisterial bacteriocin subtilosin biosynthesis	1.01	2.02	1.3E-03
B_amylo_FZB42_3804	predicted ncRNA	1.01	2.02	4.5E-05
RBAM_020430	*yppD* - hypothetical protein	1.00	2.00	2.3E-02
RBAM_035140	*spsC* - spore coat polysaccharide synthesis protein	1.00	2.00	2.0E-06
RBAM_035200	*ywdI* - hypothetical protein	1.00	1.99	9.9E-03
RBAM_036170	*katE* - catalase involved in the degradation of hydrogen peroxide	0.98	1.98	1.2E-02
RBAM_033030	*ywtA* - poly-gamma-glutamic synthesis	0.98	1.97	8.2E-06
RBAM_036030	*yxkH* - conserved hypothetical protein	0.98	1.97	1.5E-04
RBAM_021770	*yqkD* - hypothetical protein	0.96	1.95	4.9E-03
RBAM_024800	*yrzE* - hypothetical protein	0.95	1.94	1.4E-06
RBAM_024640	*yrvN* - conserved hypothetical protein	0.95	1.93	2.0E-02
RBAM_017310	hypothetical protein	0.95	1.93	2.8E-03
RBAM_028440	conserved hypothetical protein	0.94	1.92	4.2E-04
RBAM_005540	*cotR* - conserved hypothetical protein	0.94	1.92	7.2E-03
RBAM_034390	hypothetical protein	0.94	1.92	3.5E-05

RBAM_029600	*bsn* - putative extracellular ribonuclease precursor	0.94	1.91	1.3E-02
RBAM_037000	*fbp* - fructose-1,6-bisphosphatase involved in gluconeogenesis	0.94	1.91	1.4E-07
RBAM_014610	hypothetical protein	0.93	1.91	1.3E-03
RBAM_030710	*yvaA* - putative oxidoreductase	0.93	1.91	6.0E-07
B_amylo_FZB42_3812	predicted ncRNA	0.91	1.89	1.7E-07
RBAM_030560	putative multidrug ABC transporter, permease	0.91	1.88	2.1E-02
RBAM_037660	*levB* - endolevanase involved in levan degradation	0.91	1.87	1.8E-02
RBAM_033520	*glcR* - transcriptional regulator (DeoR family)	0.90	1.87	6.6E-04
RBAM_011390	*appB* - oligopeptide transport system permease protein involved in the uptake of oligopeptides	0.90	1.87	1.8E-04
RBAM_032540	*yviE* - hypothetical protein	0.90	1.86	1.6E-06
B_amylo_FZB42_3997	predicted ncRNA	0.87	1.83	4.5E-05
RBAM_033750	*ywnG* - hypothetical protein	0.87	1.83	7.4E-07
RBAM_036200	*licT* - transcriptional antiterminator (BglG family) involved in substrate dependent induction of bglP-bglH and bglS (sugar catabolism)	0.86	1.81	2.4E-06
RBAM_002620	*ybeF* - hypothetical protein	0.85	1.81	1.2E-02
RBAM_035040	hypothetical protein showing 57% homology with bacitracin ABC transporter permease	0.85	1.80	2.6E-07
RBAM_012730	*ykcB* - conserved hypothetical protein	0.84	1.78	2.3E-02
RBAM_034330	*ywjF* - conserved hypothetical protein involved in fatty acid degradation	0.84	1.78	6.5E-05
RBAM_012570	*xtrA* - phage-like element PBSX protein	0.83	1.78	1.7E-05
RBAM_033340	hypothetical protein	0.83	1.77	9.2E-04
RBAM_030530	two-component sensor histidine kinase	0.82	1.77	4.0E-07
RBAM_000640	*mfd* - transcription-repair coupling factor	0.82	1.76	5.7E-07
RBAM_035950	*yxeI* - putative hydrolase involved in desulfurization of organic sulfur compounds	0.82	1.76	8.8E-05
RBAM_038100	*gidB* - methyltransferase (Glucose inhibited division protein B)	0.82	1.76	7.1E-07
RBAM_030610	*yvgR* - putative sulfite reductase (NADPH2) flavoprotein involved in sulfide reduction	0.81	1.76	5.4E-05
RBAM_036680	*mrsE* - putative ABC-transporter integral membrane protein	0.81	1.75	2.3E-03

APPENDIX II: Bacterial down-regulated genes in the logarithmic phase by seed exudates

Gene ID	Gene and function	M	Fold-change	p-value
RBAM_002120	*feuA* - iron-binding protein	-3.62	-12.29	1.0E-06
RBAM_025130	*folC* - folyl-polyglutamate synthetase involved in the biosynthesis of folate	-3.07	-8.40	1.7E-03
RBAM_008750	*sspE* - putative small acid-soluble spore protein involved in protection of spore DNA	-3.05	-8.30	5.5E-05
RBAM_014250	*ktrC* - low affinity potassium transporter	-3.03	-8.18	1.5E-07
RBAM_006640	*sigV* - RNA polymerase ECF (extracytoplasmic function)-type sigma factor	-3.01	-8.06	7.7E-05
RBAM_029050	*dhbA* - siderophore 2,3 dihydroxybenzoate/bacillibactin synthesis	-2.83	-7.10	1.7E-09
RBAM_013450	*motA* - motility protein	-2.76	-6.78	9.1E-04
RBAM_007300	*yetG* - conserved hypothetical protein	-2.69	-6.46	7.2E-06
RBAM_005560	conserved hypothetical protein	-2.69	-6.44	2.8E-04
RBAM_029060	*besA* - trilactone hydrolase involved in iron aqcuisition	-2.67	-6.37	5.0E-06
RBAM_029810	*metQ* - methionine ABC transporter	-2.63	-6.19	1.9E-05
RBAM_030060	*yusV* - ABC-transporter for the siderophores enterobactin and bacillibactin (ATPase)	-2.57	-5.93	2.8E-04
RBAM_030440	*fhuD* - ferrichrome ABC transporter (ferrichrome binding protein) involved to siderophore uptake	-2.51	-5.68	1.7E-04
RBAM_008600	*yfhH* - conserved hypothetical protein	-2.50	-5.64	2.5E-05
RBAM_009310	*yhcC* - hypothetical protein	-2.48	-5.57	2.5E-05
RBAM_011000	*yisX* - conserved hypothetical protein	-2.44	-5.44	7.3E-05
RBAM_022210	*yqjE* - conserved hypothetical protein	-2.40	-5.27	3.3E-04
RBAM_006400	*ydiF* - putative ABC transporter ATP-binding	-2.38	-5.20	4.7E-03
RBAM_004350	*yczI* - hypothetical protein	-2.33	-5.03	2.1E-03
RBAM_014310	*ykyA* - hypothetical protein	-2.22	-4.67	2.1E-05
RBAM_037310	conserved ypothetical protein	-2.21	-4.64	6.0E-03
RBAM_017930	*ynfC* - hypothetical protein	-2.18	-4.53	4.7E-03
RBAM_002100	*feuC* - iron-uptake system permease protein	-2.17	-4.50	1.7E-05
RBAM_009270	*yhbI* - putative transcriptional regulator (MarR family)	-2.11	-4.31	3.2E-04
RBAM_002110	*feuB* - iron-uptake system permease protein	-2.02	-4.06	4.9E-09
RBAM_023990	*sda* - sporulation inhibitor	-1.96	-3.89	3.0E-03
RBAM_005420	*yrkD* - conserved hypothetical protein	-1.91	-3.76	9.2E-04
RBAM_009300	*yhcB* - conserved hypothetical protein	-1.89	-3.71	1.9E-04
B_amylo_FZB42_3895	predicted ncRNA	-1.86	-3.64	3.4E-06

RBAM_029040	*dhbC* - isochorismate synthase involved in siderophore biosynthesis	-1.84	-3.57	1.3E-07
RBAM_021050	*ypfA* - hypothetical protein	-1.83	-3.55	5.8E-04
RBAM_004120	*ycnE* - conserved hypothetical protein	-1.79	-3.45	1.4E-02
RBAM_030050	*yusU* - hypothetical protein	-1.75	-3.36	5.5E-05
RBAM_020930	*folE* - GTP cyclohydrolase IA involved in biosynthesis of folate	-1.74	-3.35	3.7E-03
RBAM_034670	*ywhB* - putative tautomerase	-1.74	-3.34	4.5E-03
RBAM_032460	*yvyD* - conserved hypothetical protein	-1.74	-3.34	3.8E-03
RBAM_009090	*katA* - vegetative catalase involved in detoxification of hydrogen peroxide	-1.72	-3.30	6.6E-04
RBAM_013730	hypothetical protein	-1.70	-3.25	1.9E-02
RBAM_005840	*ydeS* - conserved hypothetical protein	-1.68	-3.21	8.0E-04
RBAM_023970	*yqeH* - GTPase	-1.67	-3.18	2.0E-02
RBAM_017910	*parC* - DNA topoisomerase IV subunit A (ParC)	-1.66	-3.16	6.5E-03
RBAM_000390	*yaaR* - conserved hypothetical protein	-1.66	-3.15	6.1E-06
RBAM_012680	*spoIISA* - stage II sporulation protein SA (Killer protein) involved in programmed cell death	-1.64	-3.12	6.0E-03
RBAM_013930	*ykuO* - conserved hypothetical protein	-1.60	-3.04	1.8E-04
RBAM_017920	hypothetical protein	-1.58	-2.99	2.8E-02
RBAM_004820	*ydbJ* - putative ABC transporter (ATP-binding protein)	-1.53	-2.89	1.6E-03
RBAM_000460	*abrB* - transcriptional regulator	-1.51	-2.86	5.3E-07
RBAM_012920	*ykkE* - formyltetrahydrofolate deformylase involved in purine biosynthesis	-1.51	-2.85	7.1E-05
RBAM_009560	*glpD* - glycerol-3-phosphate dehydrogenase	-1.51	-2.85	3.0E-03
RBAM_010710	*sipV* - type I signal peptidase	-1.51	-2.84	1.9E-02
RBAM_008590	*yfhG* - conserved hypothetical protein	-1.50	-2.84	5.5E-05
RBAM_020360	*yprB* - conserved hypothetical protein	-1.49	-2.80	5.0E-02
RBAM_012000	hypothetical protein	-1.48	-2.79	2.0E-03
RBAM_019210	*rsbRC* - RsbR paralog involved in the control of SigB activity	-1.48	-2.79	1.5E-02
RBAM_018900	*yocA* - conserved hypothetical protein	-1.48	-2.78	3.5E-03
RBAM_023430	*yqfT* - hypothetical protein	-1.47	-2.78	2.0E-02
RBAM_005520	*ybfA* - conserved hypothetical protein	-1.47	-2.77	9.9E-03
RBAM_013690	*splA* - transcriptional regulator	-1.47	-2.77	1.1E-02
RBAM_031210	hypothetical protein R	-1.44	-2.71	2.6E-03
RBAM_008710	*yfhP* - conserved hypothetical protein	-1.44	-2.70	2.1E-02
RBAM_027410	*ytzC* - hypothetical protein	-1.40	-2.64	2.1E-03
RBAM_029460	*yunD* - conserved hypothetical protein	-1.40	-2.63	9.6E-04
RBAM_032010	*trxB* - thioredoxin reductase	-1.40	-2.63	7.0E-05
RBAM_026650	hypothetical protein	-1.36	-2.57	7.0E-03

RBAM_001650	*rpmJ* - ribosomal protein L36 (ribosomal protein B)	-1.36	-2.56	2.3E-03
RBAM_029890	*yusI* - conserved hypothetical protein	-1.36	-2.56	5.5E-04
RBAM_014710	*ftsW* - cell division protein	-1.35	-2.54	1.9E-02
RBAM_015370	*pyrD* - dihydroorotate dehydrogenase (catalytic subunit) involved in pyrimidine biosynthesis	-1.34	-2.53	4.4E-05
RBAM_035760	*licH* - 6-phospho-beta-glucosidase involved in lichenan utilization	-1.34	-2.53	6.4E-03
RBAM_017720	*ynzD* - hypothetical protein	-1.34	-2.53	9.8E-03
RBAM_011360	*appD* - oligopeptide transport ATP-binding protein	-1.34	-2.52	1.2E-03
RBAM_008790	*ygaE* - conserved hypothetical protein	-1.33	-2.52	1.5E-02
RBAM_004540	*ydaO* - conserved hypothetical protein	-1.33	-2.51	2.4E-02
RBAM_009720	*yhdG* - putative amino acid transporter	-1.32	-2.50	2.2E-05
RBAM_027770	*menE* - O-succinylbenzoic acid-CoA ligase	-1.32	-2.50	2.3E-03
RBAM_021700	*ansB* - aspartate ammonia-lyase involved in aspartate degradation	-1.31	-2.48	2.3E-04
RBAM_025900	*ysdA* - conserved hypothetical protein	-1.31	-2.48	7.9E-04
RBAM_007880	*yflH* - hypothetical protein	-1.30	-2.47	5.4E-04
RBAM_026150	*phoP* - alkaline phosphatase synthesis transcriptional regulatory protein	-1.30	-2.47	2.7E-02
RBAM_014500	*yktA* - hypothetical protein	-1.28	-2.44	7.2E-03
RBAM_004110	*ycnD* - putative NADPH-flavin oxidoreductase	-1.28	-2.43	2.8E-03
RBAM_021750	*yqkF* - conserved hypothetical protein	-1.28	-2.43	3.3E-03
RBAM_022140	*rpmGA1* - 50S ribosomal protein L33 type 1	-1.26	-2.40	1.2E-03
RBAM_011560	*yjbH* - adaptor protein involved in stimulation of Spx degradation	-1.25	-2.38	9.6E-05
RBAM_003780	*tcyA* - cystine ABC transporter (binding protein)	-1.25	-2.38	4.9E-05
RBAM_004060	*yclO* - putative ferrichrome ABC transporter (permease)	-1.25	-2.37	6.1E-05
RBAM_008430	*catE* - catechol 2,3-dioxygenase essential for the viability in the presence of catechol	-1.25	-2.37	4.8E-05
RBAM_015810	*ffh* - signal recognition particle-like (SRP)	-1.23	-2.35	3.2E-03
RBAM_004960	*acpS* - holo-acyl carrier protein synthase	-1.22	-2.33	4.2E-02
RBAM_021630	hypothetical protein	-1.22	-2.33	1.4E-03
RBAM_019380	*ctpA* - carboxy-terminal processing protease	-1.22	-2.32	4.2E-04
RBAM_016510	*ribC* - riboflavin biosynthesis protein	-1.21	-2.32	1.2E-04
RBAM_000280	*yaaL* - hypothetical protein	-1.21	-2.32	5.4E-03
RBAM_001660	*rpsM* - ribosomal protein S13	-1.21	-2.31	3.6E-03
RBAM_034550	putative transcriptional regulator	-1.21	-2.31	2.2E-03
RBAM_016900	*baeB* - hydroxyacylglutathione hydrolase involved in antibiotics production	-1.21	-2.31	3.3E-02
RBAM_011610	*yjbM* - (p)ppGpp synthetase	-1.20	-2.30	5.0E-05
RBAM_023780	*grpE* - heat-shock protein	-1.19	-2.29	1.2E-06
RBAM_024310	*yrhF* - conserved hypothetical protein	-1.19	-2.29	3.2E-02

RBAM_018880	*yobW* - hypothetical protein Y	-1.18	-2.27	4.0E-02
RBAM_034560	hypothetical protein	-1.18	-2.27	7.5E-03
RBAM_030420	*fhuG* - ferrichrome ABC transporter (permease) involved in siderophore uptake	-1.18	-2.26	2.0E-02
RBAM_001590	*rpmD* - ribosomal protein L30 (BL27)	-1.17	-2.26	3.7E-03
RBAM_021370	*ypuF* - conserved hypothetical protein	-1.17	-2.25	5.6E-03
RBAM_023700	*yqeY* - conserved hypothetical protein	-1.17	-2.25	1.6E-06
RBAM_015600	*prkC* - protein kinase involved in germination in response to muropeptides	-1.16	-2.23	3.3E-02
RBAM_009280	*yhbJ* - hypothetical protein	-1.15	-2.22	6.0E-03
RBAM_026020	*dnaI* - primosomal protein	-1.15	-2.22	2.2E-02
RBAM_004400	*ydaB* - putative acid-CoA ligase	-1.15	-2.22	4.1E-04
RBAM_025360	*ilvH* - acetolactate synthase (acetohydroxy-acid synthase) (small subunit)	-1.13	-2.19	3.7E-02
RBAM_009290	*yhcA* - hypothetical transport protein	-1.13	-2.19	1.9E-04
RBAM_006470	*groES* - class I heat-shock protein (chaperonin)	-1.13	-2.19	5.9E-06
RBAM_014220	*abh* - putative transition state regulator	-1.11	-2.17	5.4E-05
RBAM_025990	*thrS* - threonyl-tRNA synthetase	-1.11	-2.16	1.5E-03
RBAM_020170	*ypbS* - hypothetical protein	-1.11	-2.15	1.5E-02
RBAM_032610	*comFB* - competence protein FB (ComFB)	-1.10	-2.15	5.0E-02
RBAM_020010	*ilvD* - dihydroxy-acid dehydratase	-1.09	-2.13	4.3E-03
RBAM_002660	hypothetical protein	-1.09	-2.13	5.6E-05
RBAM_031200	hypothetical protein	-1.09	-2.13	3.7E-02
RBAM_007420	putative ABC transporter (ATP-binding protein)	-1.08	-2.12	2.8E-02
RBAM_010830	*yisB* - hypothetical protein	-1.08	-2.11	3.3E-02
RBAM_010500	*aprE* - serine alkaline protease (subtilisin E)	-1.07	-2.11	1.6E-04
RBAM_027120	*ytzE* - hypothetical protein	-1.07	-2.11	4.7E-04
RBAM_023770	*dnaK* - class I heat-shock protein (molecular chaperone)	-1.07	-2.10	1.2E-05
RBAM_011120	hypothetical protein	-1.07	-2.10	2.8E-02
RBAM_037780	*ydeO* - conserved hypothetical protein	-1.07	-2.10	1.0E-02
RBAM_015680	*sdaAB* - L-serine dehydratase (beta chain)	-1.07	-2.10	9.3E-04
RBAM_001630	*map* - methionine aminopeptidase	-1.06	-2.09	1.4E-02
RBAM_000850	*pabB* - para-aminobenzoate synthase chain A involved in the biosynthesis of folate	-1.06	-2.09	3.2E-05
RBAM_018820	*yobK* - hypothetical protein	-1.06	-2.09	4.0E-02
RBAM_016470	*infB* - initiation factor (IF-2)	-1.06	-2.08	2.2E-05
RBAM_005070	*sigB* - RNA polymerase sigma-B factor (Sigma-37) (General stress protein 84)	-1.06	-2.08	5.4E-03
RBAM_019970	*thyB* - thymidylate synthase B (ThyB)	-1.06	-2.08	2.0E-02
RBAM_015770	*smc* - chromosome partition protein	-1.05	-2.07	4.6E-02

RBAM_023960	*aroD* - shikimate 5-dehydrogenase	-1.05	-2.07	4.3E-02
RBAM_004730	*gsiB* - general stress protein	-1.05	-2.07	1.3E-02
RBAM_007940	*cotP* - spore coat protein	-1.04	-2.06	1.6E-02
RBAM_013700	*spIB* - spore photoproduct lyase	-1.04	-2.06	3.9E-02
RBAM_015840	*ylqD* - hypothetical protein	-1.04	-2.06	5.0E-02
RBAM_001080	*ctsR* - transcriptional regulator.	-1.04	-2.06	2.5E-04
RBAM_010230	*hpr* - protease production regulatory protein	-1.04	-2.06	1.2E-02
RBAM_003540	*nasA* - nitrate transporter	-1.04	-2.05	1.9E-02
RBAM_023710	*rpsU* - ribosomal protein S21	-1.04	-2.05	1.5E-04
RBAM_024610	*trmU* - tRNA (5-methylaminomethyl-2-thiouridylate) methyltransferase	-1.03	-2.05	1.3E-02
RBAM_016330	*rpsB* - ribosomal protein S2	-1.03	-2.04	1.6E-03
RBAM_023240	*yqzC* - hypothetical protein	-1.02	-2.03	1.4E-03
RBAM_020080	*degR* - regulatory protein	-1.02	-2.03	3.6E-06
RBAM_001100	*mcsB* - modulation of CtsR repression protein	-1.02	-2.02	1.1E-04
RBAM_003610	*nucA* - membrane-associated nuclease	-1.01	-2.02	6.5E-04
RBAM_015530	*ylol* - putative pantothenate metabolism flavoprotein involved in biosynthesis of coenzyme A	-1.00	-2.00	1.3E-03
RBAM_017160	*ymzA* - hypothetical protein	-1.00	-2.00	6.8E-04
RBAM_018960	*yocH* - putative cell-wall binding protein	-0.99	-1.98	8.6E-06
RBAM_005410	*ydeB* - conserved hypothetical protein	-0.99	-1.98	2.2E-04
RBAM_007870	*yflI* - hypothetical protein	-0.98	-1.98	4.5E-04
RBAM_007660	*yfmM* - putative ABC transporter (ATP-binding protein)	-0.97	-1.96	7.2E-04
RBAM_019240	hypothetical protein	-0.96	-1.94	2.4E-05
RBAM_016540	*ylxY* - putative deacetylase	-0.95	-1.94	3.5E-02
RBAM_006690	*ydjO* - hypothetical protein	-0.94	-1.92	4.5E-05
RBAM_025650	*mutSB* - DNA mismatch repair protein	-0.94	-1.92	2.2E-04
RBAM_015210	*ylmE* - conserved hypothetical protein	-0.94	-1.92	9.4E-03
RBAM_001490	*rpmC* - ribosomal protein L29	-0.93	-1.90	8.3E-04
RBAM_037340	hypothetical protein	-0.92	-1.90	4.0E-03
RBAM_025930	*infC* - initiation factor IF-3	-0.92	-1.90	7.8E-04
RBAM_006610	*ydjI* - conserved hypothetical protein	-0.92	-1.90	2.3E-02
RBAM_011990	hypothetical protein	-0.92	-1.89	8.9E-03
RBAM_016340	*tsf* - translation elongation factor ef-ts	-0.91	-1.88	2.8E-03
RBAM_001550	*rpsH* - ribosomal protein S8 (BS8)	-0.91	-1.87	9.3E-03
RBAM_017710	*yneF* - conserved hypothetical protein	-0.90	-1.87	1.3E-05
RBAM_000160	*serS* - seryl-tRNA synthetase	-0.89	-1.85	4.4E-03

RBAM_002440	*yxaC* - conserved hypothetical protein	-0.89	-1.85	5.4E-02
RBAM_004260	*ycsD* - conserved hypothetical protein	-0.89	-1.85	5.7E-03
RBAM_020060	*ugtP* - putative glycosyl transferase	-0.88	-1.85	3.0E-02
RBAM_010570	*yhfW* - putative Rieske [2Fe-2S] iron-sulfur protein	-0.88	-1.84	2.7E-02
RBAM_012360	*yjlC* - conserved hypothetical protein	-0.88	-1.84	9.7E-03
RBAM_001560	*rplF* - ribosomal protein L6 (BL8)	-0.88	-1.84	1.2E-02
B_amylo_FZB42_3947	predicted ncRNA	-0.88	-1.84	1.3E-08
RBAM_027060	*ytlP* - putative 2'-5' RNA-ligase involved in RNA metabolism	-0.87	-1.83	2.2E-02
RBAM_000690	*yabP* - hypothetical protein involved in sporulation	-0.87	-1.83	1.3E-03
RBAM_004010	*rapC* - response regulator aspartate phosphatase involved in the control of sporulation initiation	-0.87	-1.83	4.6E-04
RBAM_025890	*ysdB* - hypothetical protein involved in the survival of heat stress	-0.87	-1.83	1.9E-03
RBAM_024980	*spo0B* - sporulation initiation phosphotransferase B (Spo0B)	-0.87	-1.82	1.5E-02
RBAM_022580	*ahrC* - arginine transcriptional repressor	-0.86	-1.82	1.9E-02
RBAM_001300	*rplL* - ribosomal protein L12 (BL9)	-0.86	-1.81	1.7E-03
RBAM_025970	*lytS* - two-component sensor histidine kinase involved in the regulation of the rate of autolysis	-0.86	-1.81	4.6E-02
RBAM_021300	*rluB* - ribosomal large subunit pseudouridine synthase B(RluB)	-0.85	-1.81	3.3E-04
RBAM_023300	hypothetical protein - putative transposase	-0.85	-1.80	4.2E-02
RBAM_009580	*yhcY* - putative two-component sensor histidine kinase	-0.85	-1.80	3.9E-02
RBAM_014000	*rok* - comK repressor Rok involved in the regulation of genetic competence	-0.85	-1.80	2.9E-05
RBAM_004380	*ycsN* - putative aryl-alcohol dehydrogenase	-0.84	-1.80	1.4E-04
RBAM_016700	conserved hypothetical protein	-0.84	-1.79	1.0E-02
RBAM_018420	*fenE* - fengycin synthetase involved in antibiotics production	-0.84	-1.79	1.4E-03
RBAM_013150	*ykoL* - stress response protein	-0.84	-1.79	3.9E-02
RBAM_025910	*rplT* - 50S ribosomal protein L20	-0.83	-1.78	3.6E-03
RBAM_015380	*pyrF* - orotidine 5'-phosphate decarboxylase involved in pyrimidine biosynthesis	-0.83	-1.78	8.3E-07
RBAM_001280	*rplA* - ribosomal protein L1 (BL1)	-0.83	-1.78	8.6E-04
RBAM_010700	*yhjE* - conserved hypothetical protein	-0.83	-1.78	5.1E-02
RBAM_015830	*ylqC* - conserved hypothetical protein	-0.83	-1.78	1.0E-02
RBAM_016310	*sigD* - RNA polymerase sigma-28 factor involved in regulation of flagella, motility, chemotaxis and autolysis	-0.83	-1.78	1.7E-02
RBAM_018710	*yoxD* - hypothetical oxidoreductase	-0.82	-1.77	1.8E-03
RBAM_022850	*yqhM* - conserved hypothetical protein	-0.82	-1.77	8.6E-03
RBAM_019910	*ypmP* - conserved hypothetical protein	-0.82	-1.77	3.8E-03

RBAM_029020	*dhbB* - isochorismatase involved in siderophere synthesis	-0.82	-1.77	8.8E-08
RBAM_001640	*infA* - translation initiation factor I	-0.81	-1.76	1.6E-02
RBAM_021990	*difH* - modular polyketide synthase of type I involved in dificidine biosynthesis	-0.81	-1.75	1.5E-02
RBAM_001370	*fusA* - elongation factor G	-0.81	-1.75	1.5E-03
RBAM_025540	*lysC* - aspartokinase II alpha subunit and beta subunit involved in biosynthesis of lysine	-0.81	-1.75	5.6E-03
RBAM_014190	*ampS* - aminopeptidase required for biofilm formation	-0.81	-1.75	6.0E-04
RBAM_015920	*sucC* - succinyl-CoA synthetase (beta subunit) involved in TCA cycle	-0.81	-1.75	4.4E-04
RBAM_023550	*ccpN* - transcriptional regulator involved in repression of genes from gluconeogenesis	-0.81	-1.75	2.7E-04
RBAM_012370	*yjlD* - NADH dehydrogenase-like protein	-0.81	-1.75	3.8E-04
RBAM_013670	*ptsH* - phosphocarrier protein involved in PTS-dependent sugar transport and carbon catabolite repression	-0.81	-1.75	2.5E-04
RBAM_010560	*hemAT* - haem-based aerotactic transducer	-0.80	-1.74	1.0E-02
RBAM_014580	*ylaB* - hypothetical protein	-0.80	-1.74	3.7E-02
RBAM_010460	*yhfK* - hypothetical protein	-0.80	-1.74	3.5E-05
B_amylo_FZB42_3931	predicted ncRNA	-0.80	-1.74	5.5E-03
RBAM_023940	*nadD* - nicotinamide-nucleotide adenylyltransferase involved in NAD biosynthesis	-0.80	-1.74	8.4E-04

APPENDIX III: Bacterial up-regulated genes in the transitional phase by seed exudates

Gene ID	Gene and function	M		p_value
RBAM_026080	*ytbD* - putative arabinose efflux permease	2.56	5.88	9E-06
RBAM_013340	*mtnK* - methylthioribose kinase involved in methionine salvage	2.20	4.60	9.4E-05
B_amylo_FZB42_3957	predicted ncRNA	2.15	4.44	4.4E-03
RBAM_029050	*dhbA* - 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase involved in siderophore biosynthesis	2.06	4.18	1.3E-06
B_amylo_FZB42_3810	predicted ncRNA	2.03	4.10	1.6E-02
RBAM_011650	*yjbQ* - putative Na+/H+ antiporter involved in the pH and Na+ cellular homeostasis	2.02	4.07	4.5E-03
RBAM_029060	*besA* - trilactone hydrolase involved in iron aquisition	1.99	3.98	2.2E-06
B_amylo_FZB42_3943	predicted ncRNA	1.74	3.35	9.2E-04
B_amylo_FZB42_3965	predicted ncRNA	1.73	3.32	1.2E-04
RBAM_036310	hypothetical protein	1.72	3.30	1.4E-02
RBAM_029040	*dhbC* - isochorismate synthase DhbC involved in siderophore biosynthesis	1.68	3.20	3.4E-09
RBAM_034950	putative drug resistance transporter	1.59	3.01	5.0E-04
RBAM_010880	*gerPB* - spore germination protein	1.55	2.92	7.1E-03
RBAM_004880	*ydbO2* - hypothetical protein	1.55	2.92	2.1E-03
B_amylo_FZB42_3892	predicted ncRNA	1.51	2.86	2.7E-02
B_amylo_FZB42_3827	predicted ncRNA	1.49	2.82	3.9E-04
RBAM_022730	*spoIIIAD* - stage III sporulation protein AD (SpoIIIAD) involved in the activation of SigG	1.44	2.71	4.4E-04
RBAM_004220	hypothetical protein	1.44	2.71	4.8E-02
RBAM_036140	*yxjA* - purine nucleoside transporter involved in purine uptake	1.42	2.68	2.0E-03
RBAM_002100	*feuC* - iron-uptake system permease protein	1.42	2.68	5.7E-10
RBAM_035960	*yxlA* - putative purine-cytosine permease	1.41	2.65	6.8E-04
RBAM_029020	*dhbB* - isochorismatase involved in siderophore biosynthesis	1.40	2.64	6.1E-05
RBAM_028310	*tlpA* - methyl-accepting chemotaxis protein involved in control of chemotaxis	1.40	2.63	4.4E-02
RBAM_038010	*engD* - GTP-dependent nucleic acid-binding protein .	1.36	2.56	1.4E-03
RBAM_030420	*fhuG* - ferrichrome ABC transporter (permease) involved in siderophore uptake	1.35	2.55	6.9E-08
RBAM_009460	*yhcS* - conserved hypothetical protein	1.32	2.50	1.5E-02

RBAM_025450	*gerM* - germination protein	1.32	2.49	3.6E-04
RBAM_033520	*glcR* - transcriptional regulator (DeoR family)	1.31	2.49	3.2E-04
RBAM_034210	*maeA* - NAD-dependent malate dehydrogenase involved in malate utilization	1.25	2.38	9.6E-03
RBAM_019710	*yoqN* - hypothetical protein	1.23	2.34	2.6E-02
RBAM_024270	*fatR* - transcriptional repressor	1.23	2.34	1.9E-03
RBAM_013980	*ykuT* - mechanosensitive channel, similar to MscS involved in resistance to osmotic downshock	1.22	2.34	2.2E-03
B_amylo_FZB42_3978	predicted ncRNA	1.19	2.29	5.3E-03
RBAM_026780	hypothetical protein	1.18	2.26	3.3E-02
RBAM_034790	hypothetical protein	1.17	2.26	1.8E-02
RBAM_037990	*ssb* - single-strand DNA-binding protein (Helix-destabilizing protein)	1.17	2.24	7.2E-06
RBAM_019930	*yplP* - putative sigma L dependent transcriptional regulator required for survival at low temperatures	1.17	2.24	8.1E-03
B_amylo_FZB42_3828	predicted ncRNA	1.17	2.24	3.6E-03
RBAM_035530	*ywbN* - elemental iron uptake system (binding protein) involved in iron uptake	1.16	2.24	2.6E-02
B_amylo_FZB42_3946	predicted ncRNA	1.16	2.24	2.0E-02
RBAM_013930	*ykuO* - conserved hypothetical protein	1.15	2.23	4.8E-03
RBAM_011730	*fabI* - enoyl-[acyl-carrier-protein] reductase involved in fatty acids biosynthesis	1.15	2.22	5.0E-02
RBAM_024320	*yrhE* - putative formate dehydrogenase	1.14	2.21	7.4E-05
RBAM_011090	*yitT* - conserved hypothetical protein	1.12	2.17	3.6E-02
RBAM_010850	*gerPE* - spore germination protein	1.11	2.17	1.4E-04
RBAM_025000	*ysxB* - conserved hypothetical protein	1.11	2.16	5.4E-07
RBAM_014660	*ylaJ* - hypothetical protein	1.11	2.16	2.7E-02
RBAM_001320	*rpoB* - RNA polymerase (beta subunit)	1.10	2.15	4.0E-05
RBAM_036160	*yxiS* - hypothetical protein involved in the survival to ethanol and salt stresses	1.09	2.13	3.0E-04
B_amylo_FZB42_3973	predicted ncRNA	1.09	2.13	1.1E-02
B_amylo_FZB42_3871	predicted ncRNA	1.09	2.13	7.7E-03
RBAM_034040	*atpI* - ATP synthase (subunit I)	1.08	2.12	1.5E-06
RBAM_019580	*yosT* - conserved hypothetical protein	1.08	2.12	1.3E-02
RBAM_014520	*ykzI* - hypothetical protein	1.08	2.11	3.3E-02
RBAM_033710	*bcrC* - undecaprenyl pyrophosphate phosphatase involved in resistance to bacitracin and oxidative stress	1.06	2.08	1.4E-07
RBAM_026130	*polA* - DNA polymerase I	1.05	2.07	5.1E-02
RBAM_002410	putative ABC-type transport system,permease involved in detoxification	1.05	2.07	1.3E-03
RBAM_012990	hypothetical protein	1.05	2.07	2.3E-02

RBAM_019280	*yodA* - conserved hypothetical protein	1.05	2.07	2.6E-02
RBAM_034110	*ywlC* - conserved hypothetical protein	1.04	2.05	5.4E-04
RBAM_026600	*ywdH* - putative aldehyde dehydrogenase	1.04	2.05	4.3E-02
RBAM_033610	*mbl* - mreB-like protein involved in cell-shape determination	1.03	2.05	2.0E-06
RBAM_035830	putative ferrichrome ABC transporter (permease) involved in iron acquisition	1.03	2.05	5.4E-10
RBAM_030670	*cadA* - cadmium transporting ATPase involved in cadmium export	1.02	2.03	3.9E-02
RBAM_009970	*yheF* - hypothetical protein	1.02	2.03	5.0E-02
RBAM_011180	*yitZ* - putative multidrug resistance protein	1.02	2.02	3.1E-02
RBAM_002590	hypothetical protein	1.01	2.01	3.6E-02
RBAM_012690	*pit* - putative low-affinity inorganic phosphate transporter	1.00	2.00	4.5E-04
RBAM_038150	*rnpA* - ribonuclease P protein component (RNaseP)	0.99	1.98	4.8E-03
RBAM_033510	*ywpJ* - conserved hypothetical protein	0.99	1.98	2.6E-06
RBAM_037750	conserved ypothetical protein	0.98	1.97	1.9E-02
RBAM_021090	*prsW* - protease involved in the control of SigW activity	0.98	1.97	3.1E-03
RBAM_004060	*yclO* - putative ferrichrome ABC transporter (permease) involved in iron acquisition	0.97	1.96	1.9E-04
RBAM_031860	*yraN* - putative LysR-family transcription regulator	0.95	1.94	7.3E-03
RBAM_029090	*yuiF* - conserved hypothetical protein	0.95	1.93	3.1E-04
RBAM_027140	*ytgP* - putative enzyme involved in polysaccharide biosynthesis	0.95	1.93	3.1E-06
RBAM_011700	*thiG* - hydroxyethylthiazole phosphate biosynthesis involved in the biosynthesis of thiamine	0.94	1.92	2.2E-02
RBAM_009340	*yhcG* - putative ABC transporter ATP-binding protein	0.94	1.92	4.2E-03
B_amylo_FZB42_3937	predicted ncRNA	0.94	1.92	6.1E-03
RBAM_033990	*atpA* - ATP synthase (subunit alpha)	0.93	1.90	5.8E-06
RBAM_036150	hypothetical protein	0.92	1.90	3.0E-08
RBAM_020420	*yppE* - hypothetical protein	0.92	1.89	4.6E-02
RBAM_023970	*yqeH* - GTPase involved in the assembly/ stability of the 30S subunit of the ribosome, assembly of the 70S ribosome	0.91	1.88	3.9E-03
RBAM_002120	*feuA* - iron-binding protein	0.91	1.88	4.8E-08
RBAM_035470	*gtaC* - teichoic acid glycosylation protein involved in the biosynthesis of teichoic acids	0.9	1.87	0.00164
RBAM_037720	hypothetical protein	0.90	1.87	2.1E-02
RBAM_036560	*yxeB* - hydroxamate siderophore ABC transporter (only ferrioxamine) (binding protein) involved in siderophore uptake	0.90	1.86	4.2E-06
RBAM_037910	*yyaK* - conserved hypothetical protein	0.89	1.86	8.5E-05
RBAM_002110	*feuB* - iron-uptake system permease protein	0.89	1.85	5.4E-07
RBAM_033750	*ywnG* - hypothetical protein	0.89	1.85	5.0E-12

RBAM_001260	*nusG* - transcription antitermination factor	0.89	1.85	3.2E-04
RBAM_028770	*comP* - two-component sensor histidine kinase involved in regulation of genetic competence and quorum sensing	0.88	1.85	3.6E-05
RBAM_030400	*yvrL* - anti-Sig(YvrI-YvrHa) involved in control of Sig(YvrI-YvrHa) activity	0.88	1.84	3.3E-06
RBAM_022630	*xseA* - putative exodeoxyribonuclease VII (large subunit)	0.88	1.84	1.8E-04
RBAM_034150	*ywkF* - hypothetical protein	0.87	1.82	1.1E-02
B_amylo_FZB42_3926	predicted ncRNA	0.86	1.81	1.2E-02
RBAM_029010	*dhbF* - dimodular nonribosomal peptide synthetase involved in siderophore biosynthesis	0.86	1.81	4.6E-05
B_amylo_FZB42_3972	predicted ncRNA	0.85	1.81	4.1E-02
RBAM_030230	*liaF* - negative effector of LiaR which regulates the operon responsive to bacitracin	0.85	1.80	2.9E-04
RBAM_033470	*ptkA* - protein tyrosine kinase involved in protein phosphorylation	0.85	1.80	2.4E-04
RBAM_028520	*yugF* - conserved hypothetical protein	0.84	1.78	2.2E-02
RBAM_037710	conserved hypothetical protein with homology to LysR family transcriptional regulator	0.83	1.78	5.5E-05
RBAM_038000	*rpsF* - 30S ribosomal protein S6 (BS9)	0.82	1.77	1.4E-05
RBAM_028680	*mrpA* - Na+/H+ antiporter subunit A (MrpA) involved in sodium export	0.82	1.76	9.4E-03
RBAM_030060	*yusV* - ABC-transporter for the siderophores enterobactin and bacillibactin (ATPase)	0.82	1.76	5.1E-04
B_amylo_FZB42_3843	predicted ncRNA	0.82	1.76	9.9E-07
RBAM_026100	*ytaG* - dephospho-CoA kinase involved in synthesis of coenzyme A	0.82	1.76	5.9E-04
RBAM_032130	*hisG* - ATP phosphoribosyltransferase involved in the biosynthesis of histidine	0.82	1.76	1.5E-04
RBAM_037590	*yybT* - conserved hypothetical protein	0.81	1.76	3.7E-02
RBAM_030280	*yvqK* - conserved hypothetical protein	0.81	1.76	7.8E-07
RBAM_001410	*rplC* - ribosomal protein L3 (BL3)	0.81	1.75	6.8E-05
RBAM_024620	*yrvO* - conserved hypothetical protein	0.81	1.75	8.8E-04
RBAM_038140	*spoIIIJ* - membrane protein translocase involved in membrane insertion of proteins and protein secretion	0.81	1.75	8.3E-08
RBAM_030570	*yvgN* - putative dehydrogenase	0.80	1.75	8.7E-08
RBAM_016380	*cdsA* - phosphatidate cytidylyltransferase (CDP-diglyceride synthase) involved in the biosynthesis of phospholipids	0.80	1.74	7.3E-04
RBAM_003310	*ycgE* - conserved hypothetical protein	0.80	1.74	1.0E-02
RBAM_023740	*yqeU* - conserved hypothetical protein	0.80	1.74	1.3E-02
B_amylo_FZB42_3982	predicted ncRNA	0.80	1.74	2.3E-06
RBAM_038110	*gidA* - glucose inhibited division protein A	0.80	1.74	3.3E-05

APPENDIX IV: Bacterial down-regulated genes in the transitional phase by seed exudates

Gene ID	Gene and function	M	Fold-change	p_value
RBAM_035760	*licH* - 6-phospho-beta-glucosidase involved in lichenan utilization	-4.61	-24.41	0.00
RBAM_007980	*treA* - trehalose-6-phosphate hydrolase involved in trehalose utilization	-3.31	-9.94	0.00
RBAM_013450	*motA* - motility protein	-3.14	-8.81	0.00
RBAM_012150	*galK1* - galactokinase	-3.04	-8.23	0.00
RBAM_016830	*tdh* - L-threonine 3-dehydrogenase involved in theonine utilization	-2.84	-7.14	0.00
RBAM_035790	*licB* - phosphotransferase system (PTS) lichenan specific enzyme IIB component involved in lichenan utilization	-2.70	-6.48	0.00
RBAM_035770	*licA* - phosphotransferase system (PTS) lichenan specific enzyme IIA component involved in lichenan utilization	-2.64	-6.25	0.00
RBAM_036710	*iolH* - inositol utilization protein H	-2.63	-6.17	0.00
RBAM_003370	*lci* - putative antimicrobial peptide	-2.53	-5.79	0.00
RBAM_035780	*licC* - phosphotransferase system (PTS) lichenan specific enzyme IIC component involved in lichenan uptake and phosphorylation	-2.46	-5.48	0.00
RBAM_025130	*folC* - folyl-polyglutamate synthetase involved in the biosynthesis of folate	-2.43	-5.41	0.00
RBAM_014130	*fruK* - fructose 1-phosphate kinase involved in fructose utilization	-2.40	-5.29	0.00
RBAM_012170	*lacF* - phosphotransferase system cellobiose-specific component	-2.38	-5.22	0.00
RBAM_033130	*rbsB* - ribose ABC transporter (ribose-binding protein)	-2.35	-5.09	0.00
RBAM_036770	*iolB* - inositol utilization protein B (IolB) involved in myo-inositol catabolism	-2.34	-5.06	0.00
RBAM_022090	putative transcription antiterminator	-2.32	-4.99	0.00
RBAM_018030	*yndH* - hypothetical protein	-2.31	-4.95	0.00
RBAM_033120	*rbsC* - ribose ABC transporter (permease)	-2.28	-4.86	0.01
RBAM_006170	*gmuR* - transcriptional repressor (GntR family) involved in the regulation of glucomannan utilization	-2.27	-4.83	0.00
RBAM_005520	*ybfA* - conserved hypothetical protein	-2.25	-4.76	0.00
RBAM_025950	*ysbA* - conserved hypothetical protein	-2.23	-4.70	0.01
RBAM_012180	*lacG* - 6-phospho-beta-galactosidase	-2.23	-4.70	0.00
RBAM_020080	*degR* - positive effector of DegU-phosphate stability involved in the control of DegU activity	-2.22	-4.64	0.00
RBAM_024190	*manA* - mannose-6-phosphate isomerase involved in mannose utilization	-2.17	-4.50	0.00
RBAM_009090	*katA* - vegetative catalase involved in the detoxification of hydrogen peroxide	-2.13	-4.38	0.00
RBAM_025820	*araM* - glycerol-1-phosphate dehydrogenase involved in the biosynthesis of	-2.09	-4.26	0.00

	phosphoglycerolipids			
RBAM_009700	*citA* - citrate synthase I	-2.06	-4.18	0.00
RBAM_036760	*iolC* - inositol utilization protein C	-2.02	-4.05	0.00
RBAM_004040	*yclM* - putative homoserine dehydrogenase	-2.01	-4.02	0.01
RBAM_036780	*iolA* - methylmalonate-semialdehyde dehydrogenase I	-1.95	-3.85	0.00
RBAM_026150	*phoP* - two-component response regulator involved in the regulation of phosphate metabolism	-1.94	-3.85	0.00
RBAM_021750	*yqkF* - conserved hypothetical protein	-1.89	-3.72	0.00
RBAM_015600	*prkC* - protein kinase involved in germination in response to muropeptides	-1.89	-3.70	0.04
RBAM_027580	*pckA* - phosphoenolpyruvate carboxykinase involved in the synthesis of phosphoenolpyruvate	-1.83	-3.55	0.00
RBAM_005560	conserved hypothetical protein	-1.81	-3.51	0.01
RBAM_004120	*ycnE* - conserved hypothetical protein	-1.80	-3.47	0.00
RBAM_011360	*appD* - oligopeptide transport ATP-binding protein	-1.77	-3.41	0.00
RBAM_036740	*iolE* - inositol utilization protein E	-1.77	-3.40	0.00
RBAM_003540	*nasA* - nitrate transporter	-1.74	-3.35	0.01
RBAM_019360	*bglA* - 6-phospho-beta-glucosidase involved in beta-glucoside utilization	-1.71	-3.28	0.00
RBAM_016840	*kbl* - 2-amino-3-ketobutyrate CoA ligase involved in threonine utilization	-1.71	-3.27	0.00
RBAM_019060	*dhaS* - aldehyde dehydrogenase	-1.68	-3.21	0.00
RBAM_018170	*bmyB* - bacillomycin D synthetase B involved in antibiotics production	-1.67	-3.19	0.00
RBAM_026650	hypothetical protein	-1.66	-3.15	0.01
RBAM_003510	*nasD* - assimilatory nitrite reductase (subunit) involved in the utilization of nitrate as a nitrogen source	-1.65	-3.15	0.02
RBAM_018540	*ggt* - gamma-glutamyltranspeptidase involved in the degradation of poly-glutamate capsules	-1.65	-3.14	0.00
RBAM_025940	*ysbB* - antiholin-like protein	-1.64	-3.11	0.01
RBAM_010180	*yhaL* - involved in sporulation	-1.63	-3.10	0.00
RBAM_014250	*ktrC* - low affinity potassium transporter KtrCD, peripheric membrane component (proton symport) involved in potassium uptake	-1.62	-3.07	0.01
RBAM_014220	*abh* - putative transition state regulator	-1.60	-3.03	0.00
RBAM_021580	*spoIIAA* - anti-sigma F factor antagonist	-1.60	-3.02	0.00
RBAM_002170	*murP* - N-acetyl muramic acid-specific phosphotransferase system, EIIBC component involved in N-acetyl muramic acid uptake and phosphorylation	-1.59	-3.01	0.01
RBAM_012430	*yjnA* - conserved hypothetical protein	-1.57	-2.96	0.02
RBAM_006700	*cotA* - spore coat protein (outer) involved in resistance of the spore	-1.56	-2.94	0.02
RBAM_018240	*bioI* - cytochrome P450 enzyme involved in the biosynthesis of biotin	-1.55	-2.92	0.01
RBAM_012200	*pgm1* - predicted phosphatase/phosphohexomutase	-1.53	-2.89	0.00

RBAM_018930	*yocC* - hypothetical protein	-1.52	-2.86	0.01
RBAM_021050	*ypfA* - hypothetical protein	-1.50	-2.82	0.00
RBAM_037220	conserved hypothetical protein	-1.49	-2.81	0.01
RBAM_017930	*ynfC* - hypothetical protein	-1.49	-2.80	0.01
RBAM_019600	*cgeD* - spore maturation protein	-1.48	-2.80	0.02
RBAM_033040	*capB* - poly-gamma-glutamate synthetase involved in capsule synthesis	-1.47	-2.78	0.04
RBAM_027670	*ytkA* - hypothetical protein	-1.46	-2.75	0.01
RBAM_027120	*ytzE* - hypothetical protein	-1.45	-2.73	0.00
RBAM_004230	*mtlA* - phosphotransferase system (PTS) mannitol-specific enzyme IIABC component involved in mannitol uptake and phosphorylation, control of MtlR activity	-1.44	-2.72	0.00
RBAM_009900	*dat* - D-alanine aminotransferase involved in peptidoglycan precursor biosynthesis	-1.44	-2.72	0.00
RBAM_014480	hypothetical protein	-1.42	-2.68	0.00
RBAM_020930	*folE* - GTP cyclohydrolase IA - involved in the biosynthesis of folate	-1.42	-2.68	0.01
RBAM_011380	*appA* - oligopeptide-binding protein	-1.40	-2.64	0.00
RBAM_017910	*parC* - DNA topoisomerase IV subunit A involved in chromosome segregation and compaction	-1.38	-2.61	0.00
RBAM_008570	*yfhE* - involved in survival of salt and ethanol stresses and low temperatures	-1.38	-2.61	0.01
RBAM_026680	hypothetical protein	-1.38	-2.60	0.01
RBAM_019990	*ypiP* - conserved hypothetical protein	-1.37	-2.59	0.01
RBAM_026040	*ytcG* - putative regulator protein	-1.35	-2.56	0.01
B_amylo_FZB42_3947	predicted ncRNA	-1.34	-2.54	0.00
RBAM_004800	*dctP* - C4-dicarboxylate transport protein involved in the uptake of fumarate, succinate and malate	-1.34	-2.54	0.00
RBAM_006400	*ydiF* - putative ABC transporter ATP-binding	-1.33	-2.52	0.01
RBAM_012340	*yjlA* - hypothetical protein	-1.30	-2.46	0.01
RBAM_033080	*rbsR* - transcriptional repressor (LacI family) involved in regulation of ribose utilization	-1.30	-2.46	0.00
RBAM_033140	*ywsB* - involved in survival to ethanol and salt stresses	-1.29	-2.45	0.00
RBAM_036350	*bglH* - beta-glucosidase involved in salicin utilization	-1.29	-2.45	0.02
RBAM_005420	*yrkD* - conserved hypothetical protein	-1.28	-2.44	0.01
RBAM_013890	*abbA* - anti-repressor involved in the inhibition of AbrB	-1.28	-2.42	0.00
RBAM_004110	*ycnD* - NADPH-FMN oxidoreductase involved in the delivery of FMN to enzymes	-1.27	-2.41	0.00
RBAM_014570	*ylaA2* - hypothetical protein	-1.24	-2.37	0.01
RBAM_031210	hypothetical protein	-1.24	-2.36	0.00
RBAM_003610	*nucA* - membrane-associated nuclease involved in genetic transformation, DNA uptake	-1.23	-2.34	0.00
RBAM_009550	*glpK* - glycerol kinase (ATP:glycerol 3-phosphotransferase) (Glycerokinase) involved in	-1.22	-2.34	0.00

	glycerol utilization			
RBAM_032460	*yvyD* - required for survival at low temperatures		-1	0.00
RBAM_019170	*yojL* - D,L-endopeptidase, peptidoglycan hydrolase involved in cell wall metabolism	-1.21	-2.32	0.00
B_amylo_FZB42_4014	predicted ncRNA	-1.19	-2.29	0.01
B_amylo_FZB42_3849	predicted ncRNA	-1.18	-2.26	0.00
RBAM_021990	*difH* - modular polyketide synthase of type I	-1.18	-2.26	0.00
RBAM_013160	*ykoM* - putative transcriptional regulator (MarR family)	-1.17	-2.25	0.00
RBAM_010660	conserved hypothetical protein	-1.16	-2.23	0.00
RBAM_011580	*yjbJ* - putative lytic transglycosylase involved in cell wall turnover	-1.15	-2.22	0.00
RBAM_016750	*rodZ* - morphogenic protein required for cell shape determination	-1.15	-2.22	0.00
RBAM_013150	*ykoL* - stress response protein	-1.14	-2.20	0.01
RBAM_018500	*iseA* - inhibitor of autolysins involved in the protection against cell envelope stress	-1.12	-2.17	0.00
RBAM_004410	*ydaD* - putative alcohol dehydrogenase	-1.12	-2.17	0.00
RBAM_003710	hypothetical protein	-1.12	-2.17	0.00
RBAM_003380	*tmrB* - tunicamycin resistance protein involved in resistance to tunicamycin	-1.11	-2.16	0.01
RBAM_003200	*yceH* - putative toxic anion resistance protein	-1.11	-2.15	0.00
RBAM_031430	*gntK* - gluconate kinase involved in gluconate utilization	-1.10	-2.14	0.00
RBAM_009620	*yhdC* - hypothetical protein	-1.08	-2.12	0.01
RBAM_027050	*ytlQ* - conserved hypothetical protein	-1.08	-2.12	0.00
RBAM_017160	*ymzA* - hypothetical protein	-1.08	-2.12	0.01
RBAM_033090	*rbsK* - ribokinase involved in ribose utilization	-1.07	-2.10	0.01
RBAM_017650	*lexA* - negative transcriptional regulator of the SOS regulon involved in regulation of DNA damage repair	-1.06	-2.09	0.03
RBAM_007880	*yflH* - hypothetical protein	-1.05	-2.08	0.00
RBAM_007600	*yfmS* - putative methyl-accepting chemotaxis protein	-1.05	-2.07	0.00
RBAM_022750	*spoIIIAB* - stage III sporulation protein involved in the activation of SigG	-1.05	-2.07	0.04
RBAM_021250	*resE* - two-component sensor histidine kinase involved in the regulation of aerobic and anaerobic respiration	-1.05	-2.07	0.03
RBAM_012920	*ykkE* - formyltetrahydrofolate deformylase involved in purine nucleotide synthesis	-1.04	-2.06	0.03
RBAM_012750	*htrA* - serine protease Do (heat-shock protein) probably involved in processing, maturation, or secretion of extracellular enzymes	-1.03	-2.04	0.00
RBAM_005760	putative Na+/H+ antiporter	-1.03	-2.04	0.03
RBAM_001100	*mcsB* - protein arginine kinase involved in the control of CtsR activity	-1.03	-2.04	0.00
RBAM_012130	*galT* - galactose-1-phosphate uridyltransferase involved in galactose utilization	-1.02	-2.03	0.02
RBAM_004260	*ycsD* - conserved hypothetical protein	-1.02	-2.03	0.00

RBAM_002540	*yolA* - hypothetical protein	-1.02	-2.03	0.00
RBAM_002370	hypothetical protein RBAM00	-1.01	-2.02	0.00
RBAM_001080	*ctsR* - transcriptional regulator of protein degradation	-1.01	-2.01	0.00
RBAM_001110	*clpC* - class III stress response-related ATPase	-1.01	-2.01	0.00
RBAM_021030	*cmk* - cytidylate kinase involved in the synthesis of CTP and dCTP	-1.01	-2.01	0.00
RBAM_015530	*ylol* - putative pantothenate metabolism flavoprotein involved in the biosynthesis of Coenzyme A	-0.99	-1.99	0.01
RBAM_000580	*spoVG* - negative effector of asymetric septation involved in cell division, control of sporulation initiation	-0.99	-1.99	0.00
RBAM_029810	*metQ* - methionine ABC transporter	-0.99	-1.99	0.05
RBAM_031440	*gntP* - gluconate permease	-0.99	-1.98	0.00
RBAM_012000	hypothetical protein	-0.98	-1.97	0.00
RBAM_016860	*ymcA* - antagonist of biofilm repression by SinR involved in regulation of biofilm formation	-0.97	-1.95	0.00
B_amylo_FZB42_3952	predicted ncRNA	-0.96	-1.94	0.00
RBAM_009560	*glpD* - glycerol-3-phosphate dehydrogenase involved in glycerol utilization	-0.96	-1.94	0.02
RBAM_004730	*gsiB* - general stress protein	-0.96	-1.94	0.02
RBAM_026900	*ytxG* - putative general stress protein	-0.96	-1.94	0.00
RBAM_009470	*yhcT* - hypothetical pseudouridine synthase (Uracil hydrolyase)	-0.96	-1.94	0.02
RBAM_026070	*ytcD* - conserved hypothetical protein	-0.95	-1.94	0.00
RBAM_012140	*galE* - UDP-glucose 4-epimerase involved in galactose utilization	-0.95	-1.93	0.02
RBAM_004180	*gdh* - glucose 1-dehydrogenase involved in germination	-0.95	-1.93	0.03
RBAM_033100	*rbsD* - ribose ABC transporter (membrane protein) involved in ribose uptake	-0.95	-1.93	0.00
RBAM_013710	*mcpC* - methyl-accepting chemotaxis protein involved in the control of chemotaxis	-0.94	-1.92	0.00
RBAM_018720	*yoxC* - conserved hypothetical protein	-0.94	-1.92	0.01
RBAM_013310	*kinE* - two-component sensor histidine kinase involved in initiation of sporulation	-0.94	-1.92	0.02
RBAM_028350	*yrpB* - putative 2-nitropropane dioxygenase	-0.93	-1.91	0.02
RBAM_023540	*yqfL* - modulator of CcpN activity involved in the inhibition of CcpN activity	-0.93	-1.91	0.04
RBAM_005450	*yrkF* - conserved hypothetical protein	-0.93	-1.90	0.00
RBAM_014000	*rok* - comK repressor involved in regulation of genetic competence	-0.93	-1.90	0.00
RBAM_029430	*lipA* - trigger enzyme: lipoic acid synthase involved in lipid metabolism, required for the synthesis of branched-chain amino acids	-0.92	-1.89	0.03
RBAM_017510	hypothetical protein	-0.92	-1.89	0.00
RBAM_010910	*yisK* - putative 5-oxo-1,2,5-tricarboxilic-3-penten aciddecarboxylase	-0.91	-1.88	0.00
RBAM_002990	*lmrA* - transcriptional regulator involved in control of quercetin utilization	-0.91	-1.88	0.05
RBAM_007990	*treR* - trehalose operon transcriptional repressor	-0.91	-1.88	0.00

RBAM_011430	*oppA* - oligopeptide ABC transporter (binding protein) involved in initiation of sporulation, competence development	-0.91	-1.88	0.01
RBAM_016310	*sigD* - RNA polymerase sigma-28 factor involved in regulation of flagella, motility, chemotaxis and autolysis	-0.91	-1.87	0.00
RBAM_037250	*rocD* - ornithine aminotransferase involved in arginine, ornithine and citrulline utilization	-0.90	-1.87	0.00
RBAM_011210	*argB* - acetylglutamate kinase involved in the biosynthesis of arginine	-0.90	-1.87	0.00
RBAM_022070	*difY* - hypothetical protein involved in dificidine	-0.90	-1.86	0.03
RBAM_005410	*ydeB* - conserved hypothetical protein	-0.89	-1.85	0.00
RBAM_022570	*recN* - DNA repair protein	-0.89	-1.85	0.01
RBAM_035440	*ydaS* - hypothetical protein	-0.89	-1.85	0.00
RBAM_026120	*mutM* - formamidopyrimidine-DNA glycosidase involved in DNA repair	-0.89	-1.85	0.00
RBAM_029340	*yuxL* - putative peptidase	-0.89	-1.85	0.04
RBAM_005840	*ydeS* - conserved hypothetical protein	-0.88	-1.85	0.01
RBAM_007700	*yfmB* - hypothetical protein	-0.88	-1.85	0.00
RBAM_004490	*ydaJ* - hypothetical protein	-0.88	-1.84	0.00
RBAM_008760	*ygaB* - hypothetical protein	-0.88	-1.84	0.01
RBAM_014510	*yktB* - hypothetical protein	-0.88	-1.84	0.01
RBAM_018410	*yngL* - conserved hypothetical protein	-0.88	-1.84	0.00
RBAM_000390	*yaaR* - conserved hypothetical protein	-0.87	-1.83	0.00
RBAM_024850	*ruvA* - holliday junction DNA helicase involved in recombination	-0.87	-1.83	0.03
RBAM_031200	hypothetical protein	-0.87	-1.83	0.05
RBAM_013640	*ykvZ* - putative HTH-type transcriptional regulator	-0.87	-1.83	0.04
RBAM_037340	hypothetical protein	-0.86	-1.82	0.02
RBAM_008580	*yfhF* - conserved hypothetical protein involved in survival of ethonol stress and at low temperatures	-0.86	-1.82	0.00
RBAM_006190	*yycB* - conserved hypothetical protein	-0.86	-1.81	0.00
RBAM_010590	*yhzC* - hypothetical protein	-0.85	-1.81	0.00
RBAM_019830	*yokA* - site-specific recombinase [Bacteriophage SPBc2]	-0.85	-1.81	0.00
RBAM_024390	*yrzA* - hypothetical protein	-0.85	-1.81	0.04
RBAM_006560	*ydjE* - fructokinase homolog involved in utilization of sucrose and glucitol	-0.85	-1.80	0.00
RBAM_008500	*yfiT* - hypothetical protein	-0.85	-1.80	0.00
RBAM_006270	putative sugar transporter	-0.85	-1.80	0.01
RBAM_018420	*fenE* - fengycin synthetase involved in antibiotic production	-0.85	-1.80	0.00
RBAM_027550	*ytnA* - putative amino acid permease	-0.84	-1.79	0.00
RBAM_029600	*bsn* - putative extracellular ribonuclease precursor	-0.84	-1.79	0.00

RBAM_004680	hypothetical protein	-0.84	-1.79	0.00
RBAM_013560	*ykvR* - hypothetical protein	-0.83	-1.78	0.02
RBAM_037680	*dinB* - nuclease inhibitor involved in response to DNA damage	-0.83	-1.77	0.02
RBAM_020650	*ypjD* - putative pyrophosphatase	-0.82	-1.77	0.02
RBAM_000850	*pabB* - para-aminobenzoate synthase chain A involved in the biosynthesis of folate	-0.82	-1.77	0.00
RBAM_009800	*yhdR* - putative aspartate aminotransferase	-0.82	-1.76	0.00
RBAM_010820	*yirY* - putative DNA exonuclease involved in DNA inter-strand cross-link repair	-0.81	-1.76	0.00
RBAM_022130	*yqjL* - putative hydrolase involved in resistence against paraquat	-0.81	-1.75	0.00
RBAM_020720	*qcrA* - menaquinol-cytochrome c reductase iron-sulfur subunit (Rieske iron-sulfur protein) involved in respiration	-0.81	-1.75	0.01
RBAM_017670	*yneB* - conserved hypothetical protein	-0.81	-1.75	0.01
RBAM_016030	*fliE* - flagellar hook-basal body protein involved in movement and chemotaxis	-0.80	-1.75	0.00
RBAM_018870	*yobT* - conserved hypothetical protein	-0.80	-1.75	0.03
RBAM_012070	hypothetical protein	-0.80	-1.74	0.00
RBAM_009960	*yheG* - conserved hypothetical protein	-0.80	-1.74	0.04
RBAM_014140	*fruA* - phosphotransferase system (PTS) fructose-specific enzyme IIABC component involved in fructose uptake and phosphorylation	-0.80	-1.74	0.00
RBAM_032580	*flgM* - anti-SigD involved in control of SigD activity	-0.80	-1.74	0.02

APPENDIX V: Bacterial up-regulated genes in the logarithmic phase by root exudates

Gene ID	Gene and function	M	Fold-change	p-value
RBAM_009310	*yhcC* - hypothetical protein.	1.72	3.30	0.00
RBAM_002540	*yolA1* - hypothetical protein	1.39	2.63	0.00
RBAM_036860	*yocF* - putative two-component sensor histidine kinase	1.37	2.58	0.00
RBAM_003620	*hxlB* - 6-phospho-3-hexuloisomerase (PHI) involved in ribulose monophosphate pathway for formaldehyde fixation	1.36	2.57	0.00
RBAM_017400	hypothetical protein	1.34	2.53	0.00
RBAM_017030	*baeS* - putative cytochrome P450 107K1	1.31	2.48	0.00
RBAM_036620	*yxdK* - putative two-component sensor histidine kinase	1.28	2.43	0.00
RBAM_026420	*tpx* - thiol peroxidase	1.28	2.42	0.01
RBAM_034450	*ywiC* - hypothetical protein	1.18	2.26	0.01
B_amylo_FZB42_3878	predicted ncRNA	1.16	2.23	0.00
RBAM_015590	*prpC* - protein phosphatase	1.14	2.20	0.02
B_amylo_FZB42_3971	predicted ncRNA	1.12	2.17	0.02
RBAM_009090	*katA* - vegetative catalase involved in detoxification (degradation) of hydrogen peroxide	1.12	2.17	0.03
RBAM_032290	*yvkN* - hypothetical protein	1.10	2.14	0.04
RBAM_027140	*ytgP* - putative spore cortex protein	1.09	2.13	0.00
RBAM_033820	*ureB* - urease (beta subunit) involved in the utilization of urea	1.08	2.11	0.00
RBAM_031690	*pnbA* - Para-nitrobenzyl esterase (intracellular esterase B) involved in lipid degradation	1.07	2.11	0.00
RBAM_018730	*yoxB* - hypothetical protein	1.05	2.07	0.00
RBAM_035360	*nfrA* - FMN-containing NADPH-linked nitro/flavin reductase	1.03	2.05	0.00
RBAM_025560	*trxA* - thioredoxin involved in protection against oxidative damage	1.02	2.03	0.00
B_amylo_FZB42_3962	predicted ncRNA	1.01	2.01	0.05
RBAM_036140	*nupG* - purine nucleoside transporter	0.99	1.99	0.00
B_amylo_FZB42_3981	predicted ncRNA	0.99	1.99	0.02
RBAM_020280	*ypvA* - probable ATP-dependent helicase	0.98	1.98	0.02
RBAM_032930	*gerBB* - spore germination nutrient receptor protein BB (GerBB)	0.97	1.96	0.01
RBAM_000580	*spoVG* - stage V sporulation protein involved in the control of sporulation initiation	0.97	1.96	0.00
RBAM_026960	*ytoQ* - conserved hypothetical protein	0.97	1.96	0.00
RBAM_000780	*tilS* - tRNAIle-lysidine synthetase	0.95	1.93	0.00
B_amylo_FZB42_3853	predicted ncRNA	0.94	1.92	0.00

RBAM_032900	*lytD* - beta-N-acetylglucosaminidase (major autolysin)	0.94	1.92	0.01
RBAM_008790	*ygaE* - conserved hypothetical protein.	0.94	1.92	0.04
RBAM_023340	*sodA* - superoxide dismutase [Mn] involved in detoxification of oxygen radicals	0.92	1.89	0.00
RBAM_004640	hypothetical protein	0.92	1.89	0.00
RBAM_034140	*ywlA* - conserved hypothetical protein	0.90	1.87	0.00
RBAM_036020	*cimH* - putative citrate/malate transporter	0.89	1.86	0.05
B_amylo_FZB42_3979	predicted ncRNA	0.86	1.81	0.02
RBAM_017090	*ymaD* - conserved hypothetical protein	0.85	1.80	0.00
RBAM_011910	*yjcG* - putative 2'-5' RNA-ligase	0.85	1.80	0.00
RBAM_036800	*iolS* - inositol utilization protein S	0.84	1.80	0.00
RBAM_027370	*ytrC* - acetoin ABC transporter	0.84	1.79	0.00
B_amylo_FZB42_3921	predicted ncRNA	0.84	1.79	0.04
RBAM_016860	*ymcA* - antagonist of biofilm repression by SinR/regulation of biofilm formation	0.84	1.79	0.04
RBAM_033480	*tkmA* - modulator of PtkA activity/control of protein tyrosine phosphorylation	0.84	1.79	0.00
RBAM_026690	hypothetical protein	0.82	1.76	0.00
RBAM_033150	*ywrO* - putaitve NAD(P)H oxidoreductase	0.82	1.76	0.02
RBAM_003170	*yceE* - protein required for survival of ethanol stress and at low temperatures	0.81	1.76	0.00
RBAM_036440	*hutG* - formiminoglutamate hydrolase involved in histidine utilization	0.81	1.75	0.01

APPENDIX VI: Bacterial down-regulated genes in the logarithmic phase by root exudates

Gene ID	Gene and function	M	Fold-change	p-value
RBAM_027750	hypothetical protein	-2.76	-6.78	0.00
RBAM_025990	*thrS* - threonyl-tRNA synthetase involved in translation	-2.60	-6.07	0.01
RBAM_012360	*yjlC* - conserved hypothetical protein	-2.55	-5.84	0.00
RBAM_002120	*feuA* - iron-binding protein	-2.07	-4.20	0.00
RBAM_010180	*yhaL* - protein involved in sporulation	-1.85	-3.60	0.00
RBAM_023610	*dgkA* - diacylglycerol kinase involved in the biosynthesis of phospholipids	-1.76	-3.39	0.02
RBAM_019470	hypothetical protein	-1.65	-3.13	0.00
RBAM_019210	*rsbRC*- RsbR paralog/control of SigB activity	-1.61	-3.04	0.02
RBAM_019010	*yozN* - hypothetical protein	-1.56	-2.94	0.00
RBAM_036630	*yxdJ* - two-component response regulator involved in regulation of the ABC transporter YxdL-YxdM	-1.51	-2.84	0.02
RBAM_035720	*dltB* - D-alanine export protein	-1.49	-2.81	0.00
RBAM_001640	*infA* - translation initiation factor IF-I	-1.49	-2.80	0.00
RBAM_021880	*yqjT* - conserved hypothetical protein	-1.43	-2.69	0.00
RBAM_012680	*spoIISA* - toxin involved in programmed cell death	-1.41	-2.66	0.00
RBAM_021840	*yqjX* - hypothetical protein	-1.40	-2.64	0.01
RBAM_010490	*yhfN* - conserved hypothetical protein	-1.33	-2.52	0.02
RBAM_023970	*yqeH* - involved in assembly/ stability of the 30S subunit of the ribosome, assembly of the 70S ribosome	-1.32	-2.50	0.03
RBAM_016530	*pnpA* - polynucleotide phosphorylase (PNPase) necessary for competence development	-1.31	-2.48	0.01
RBAM_034640	hypothetical protein	-1.29	-2.44	0.02
RBAM_018300	*yngA* - conserved hypothetical protein	-1.25	-2.37	0.02
RBAM_010340	*pbpF* - penicillin-binding protein 2C	-1.19	-2.28	0.01
RBAM_008240	*yfjP* - putative DNA-3-methyladenine glycosidase II	-1.17	-2.26	0.00
RBAM_008270	hypothetical protein	-1.15	-2.22	0.00
RBAM_016470	*infB* - translation initiation factor (IF-2)	-1.14	-2.21	0.02
RBAM_035600	*ywbE* - conserved hypothetical protein	-1.14	-2.20	0.00
RBAM_002360	hypothetical protein	-1.13	-2.18	0.00
RBAM_012500	*xkdA* - phage-like element	-1.12	-2.17	0.04
RBAM_010710	*sipV* - type I signal peptidase involved in protein secretion	-1.11	-2.16	0.00
RBAM_009620	*yhdC* - hypothetical protein	-1.07	-2.10	0.00

RBAM_012280	*yjgD* - involved in survival to ethanol stress	-1.06	-2.09	0.00
RBAM_032780	*lytB* - amidase enhancer precursor (Modifier protein of major autolysin)	-1.04	-2.05	0.00
RBAM_020080	*degR* - regulatory protein involved in the control of DegU activity	-1.01	-2.01	0.00
RBAM_024490	*yrrK* - putative Holliday junction resolvase	-0.98	-1.98	0.09
RBAM_021850	*yqzH* - hypothetical protein	-0.98	-1.98	0.03
RBAM_008570	*yfhE* - hypothetical protein involved in survival of salt and ethanol stresses/and low temperatures	-0.97	-1.96	0.00
RBAM_013150	*ykoL* - stress response protein	-0.97	-1.96	0.00
RBAM_002400	putative ABC-type antimicrobial peptide transport system, ATPase	-0.96	-1.95	0.02
RBAM_023810	*lepA* - GTP-binding protein	-0.95	-1.93	0.00
RBAM_026020	*dnaI* - primosomal protein involved in DNA replication	-0.94	-1.92	0.02
RBAM_012370	*ndh* - NADH dehydrogenase-like protein involved in respiration	-0.94	-1.92	0.00
RBAM_005840	*ydeS* - conserved hypothetical protein	-0.93	-1.90	0.00
RBAM_036590	*yxeA* - hypothetical protein	-0.92	-1.90	0.00
RBAM_015100	*divIB* - cell-division initiation protein	-0.92	-1.90	0.00
RBAM_002810	*ycbA* - putative two-component sensor histidine kinase involved in the regulation of the glsA-glnT operon	-0.92	-1.89	0.04
RBAM_023150	*yqgU* - hypothetical protein	-0.90	-1.87	0.00
RBAM_032690	*tuaH* - putative teichuronic acid biosynthesis glycosyl transferase	-0.90	-1.86	0.04
B_amylo_FZB42_3908	predicted ncRNA	-0.90	-1.86	0.01
RBAM_007940	*cotP* - probable spore coat protein which confers resistance to the spore	-0.89	-1.86	0.00
RBAM_018520	site-specific recombinase (phage integrase family)	-0.89	-1.86	0.00
RBAM_007440	hypothetical protein	-0.87	-1.82	0.03
RBAM_002410	putative ABC-type transport system,permease	-0.86	-1.82	0.00
RBAM_010010	*yheC* - hypothetical protein	-0.86	-1.81	0.00
RBAM_011460	*oppD* - oligopeptide ABC transporter (ATP-binding protein)	-0.85	-1.80	0.00
RBAM_024570	hypothetical protein	-0.84	-1.80	0.00
RBAM_012350	*yjlB* - conserved hypothetical protein	-0.84	-1.79	0.00
RBAM_017520	*ynaE* - hypothetical protein	-0.83	-1.78	0.02
RBAM_008150	*chaA* - calcium transport in/out via proton antiporter/calcium uptake/ export	-0.82	-1.77	0.00
RBAM_019730	*rapA1* - response regulator aspartate phosphatase A (RapA1)	-0.82	-1.77	0.02
RBAM_008350	*sspH* - small, acid-soluble spore protein involved in the protection of spore DNA	-0.82	-1.76	0.00
RBAM_007380	*yetN* - hypothetical protein	-0.81	-1.75	0.03
RBAM_015180	*ylmB* - N-formyl-4-amino-5-aminomethyl-2-methylpyrimidine deformylase/thiamine salvage	-0.80	-1.74	0.01

APPENDIX VII: Bacterial up-regulated genes in the transitional phase by root exudates

Gene ID	Gene and function	M	Fold-change	p-value
RBAM_018930	*yocC* - hypothetical protein	2.31	4.95	0.00
RBAM_019000	*yocM* - hypothetical protein	2.27	4.84	0.03
RBAM_006930	*purN* - phosphoribosylglycinamide formyltransferase - involved in purine biosynthesis	2.19	4.56	0.03
RBAM_029040	*dhbC* - isochorismate synthase involved in siderophore biosynthesis	2.11	4.33	0.00
RBAM_011020	*yitJ* - conserved hypothetical protein	2.11	4.33	0.00
RBAM_011300	*med* - positive regulator of comK involved in regulation of competence	2.09	4.27	0.02
RBAM_025130	*folC* - folyl-polyglutamate synthetase involved in biosynthesis of folate	2.02	4.05	0.03
RBAM_019320	*mhqD* - hypothetical protein which may be involved in protection against methyl-hydroquinone	2.01	4.03	0.03
RBAM_013190	*ykoV* - hypothetical protein which confers dry-heat resistance to dormant spores	2.01	4.02	0.01
RBAM_015630	*thiN* - thiamin pyrophosphokinase involved in thiamine salvage	1.93	3.80	0.02
B_amylo_FZB42_3873	predicted ncRNA	1.91	3.76	0.02
RBAM_018620	*gltA* - glutamate synthase [NADPH] large subunit involved in glutamate biosynthesis	1.85	3.60	0.00
RBAM_020920	*mtrB* - tryptophan operon RNA-binding attenuation protein (TRAP)	1.81	3.51	0.01
B_amylo_FZB42_3910	predicted ncRNA	1.78	3.43	0.01
RBAM_031310	*cggR* - central glycolytic genes regulator	1.75	3.37	0.01
RBAM_019190	*yojJ* - conserved hypothetical protein	1.75	3.37	0.04
B_amylo_FZB42_3937	predicted ncRNA	1.74	3.34	0.01
RBAM_014910	*ylbL* - conserved hypothetical protein	1.70	3.24	0.00
RBAM_013790	hypothetical protein	1.68	3.20	0.01
RBAM_024980	*spo0B* - sporulation initiation phosphotransferase B involved in initiation of sporulation	1.66	3.16	0.01
RBAM_006170	*gmuR* - transcriptional repressor (GntR family) involved in regulation of glucomannan utilization	1.65	3.14	0.01
B_amylo_FZB42_3830	predicted ncRNA	1.65	3.14	0.03
RBAM_006910	*purF* - glutamine phosphoribosylpyrophosphate amidotransferase involved in purine biosynthesis	1.63	3.10	0.00
RBAM_023450	*cshB* - DEAD-box RNA helicase involved in RNA helicase	1.63	3.09	0.01
RBAM_023170	*glcK* - glucose kinase involved in phosphorylation of the free glucose moiety of di-and oligosaccharides	1.62	3.08	0.00
RBAM_002420	*ybdO* - hypothetical protein	1.54	2.90	0.01
RBAM_003400	*ycbE* - galactarate/glucarate transporter in (proton symport) involved in glucarate uptake	1.53	2.90	0.02
RBAM_019830	*yokA* - site-specific recombinase [Bacteriophage SPBc2]	1.53	2.90	0.03

RBAM_011340	*fabF* - beta-ketoacyl-acyl carrier protein synthase II involved in fatty acid biosynthesis	1.43	2.69	0.04
RBAM_033170	*alsS* - acetolactate synthase involved in overflow metabolism	1.42	2.68	0.01
RBAM_020480	*ypoC* - hypothetical protein	1.41	2.65	0.01
RBAM_025370	*ilvB* - acetolactate synthase (acetohydroxy-acid synthase) (large subunit) involved in biosynthesis of branched-chain amino acids	1.38	2.60	0.01
RBAM_025300	*ysoA* - conserved hypothetical protein	1.38	2.59	0.05
RBAM_002720	*psd* - phosphatidylserine decarboxylase involved in biosynthesis of phospholipids	1.36	2.58	0.01
B_amylo_FZB42_3911	predicted ncRNA	1.36	2.56	0.02
RBAM_018350	*yngG* - hydroxymethylglutaryl-CoA lyase homolog involved in biosynthesis of ketone bodies	1.36	2.56	0.03
RBAM_021220	*serA* - D-3-phosphoglycerate dehydrogenase involved in the biosynthesis of serine	1.34	2.53	0.02
RBAM_026650	hypothetical protein	1.34	2.53	0.02
RBAM_018840	*csaA* - molecular chaperone involved in protein secretion	1.32	2.51	0.01
RBAM_031580	*espJ* - conserved hypothetical protein protein	1.28	2.43	0.02
RBAM_020080	*degR* - positive effector of DegU-phosphate stability involved in control of DegU activity	1.27	2.40	0.04
RBAM_033330	hypothetical protein	1.25	2.38	0.01
RBAM_006180	putative transcriptional regulator (gntr family)	1.23	2.35	0.01
RBAM_003500	*nasE* - assimilatory nitrite reductase (subunit) involved in utilization of nitrite as nitrogen source	1.23	2.35	0.04
RBAM_021850	*yqzH* - hypothetical protein	1.22	2.33	0.01
RBAM_002170	*murP* - N-acetyl muramic acid-specific phosphotransferase system, involved in N-acetyl muramic acid uptake and phosphorylation	1.21	2.31	0.00
RBAM_021720	*ansR* - HTH-type transcriptional regulator AnsR (Ans operon repressor) involved in negative regulation of the ansA-ansB operon	1.20	2.30	0.01
RBAM_000010	*dnaA* - chromosomal replication initiatior protein	1.20	2.30	0.01
RBAM_000850	*pabB* - para-aminobenzoate synthase chain A involved in biosynthesis of folate	1.20	2.30	0.03
RBAM_016530	*pnpA* - polynucleotide phosphorylase (PNPase) necessary for competence development	1.19	2.28	0.03
RBAM_025630	*ywbB* - hypothetical protein	1.18	2.27	0.03
B_amylo_FZB42_3799	predicted ncRNA	1.17	2.26	0.05
RBAM_036590	*yxeA* - hypothetical protein	1.17	2.25	0.05
RBAM_022180	*polY1* - translesion synthesis (TLS-) DNA polymerase Y1 involved in generation of mutations in stationary phase	1.17	2.24	0.04
B_amylo_FZB42_3884	predicted ncRNA	1.15	2.22	0.03
RBAM_019300	*yodB* - transcriptional repressor (MarR-type) involved in regulation of quinone detoxification	1.14	2.21	0.03
RBAM_032700	*tuaG* - sugar transferase involved in teichuronic acid biosynthesis	1.14	2.21	0.03
RBAM_020440	*sspM* - small, acid-soluble spore protein involved in protection of spore DNA	1.13	2.19	0.01
B_amylo_FZB42_3909	predicted ncRNA	1.12	2.17	0.03

RBAM_026370	*arg* - argininosuccinate lyase involved in biosynthesis of arginine	1.12	2.17	0.02
RBAM_004140	*gabR* - transcriptional regulator (GntR/MocR family) involved in the regulation of gamma-amino butyric acid utilization	1.11	2.16	0.04
RBAM_004490	*ydaJ* - hypothetical protein	1.11	2.16	0.00
RBAM_027050	*ytlQ* - conserved hypothetical protein	1.10	2.14	0.02
RBAM_028210	*hmp1* - flavohemoglobin	1.10	2.14	0.04
RBAM_026630	hypothetical protein	1.10	2.14	0.03
RBAM_017980	*uxuA* - D-mannonate hydrolase involved in hexuronate utilization	1.09	2.14	0.04
RBAM_003140	*yceB* - conserved hypothetical protein	1.09	2.13	0.02
RBAM_012470	*xlyB* - N-acetylmuramoyl-L-alanine amidase involved in PBSX prophage-mediated lysis	1.09	2.13	0.05
RBAM_024130	*yyaR* - hypothetical protein	1.06	2.09	0.02
RBAM_006400	*ydiF* - putative ABC transporter ATP-binding	1.06	2.08	0.03
RBAM_022810	*yqhQ* - conserved hypothetical protein involved in survival of stress conditions	1.05	2.07	0.04
RBAM_017390	hypothetical protein	1.05	2.07	0.03
RBAM_025360	*ilvH* - acetolactate synthase (acetohydroxy-acid synthase) (small subunit) involved in biosynthesis of branched-chain amino acids	1.05	2.07	0.01
RBAM_033520	*glcR* - transcriptional regulator (DeoR family)	1.04	2.06	0.04
RBAM_006890	*purQ* - phosphoribosylformylglycinamidine synthetase involved in purine biosynthesis	1.03	2.05	0.00
RBAM_018520	site-specific recombinase (phage integrase family)	1.03	2.04	0.04
RBAM_026550	*hisJ* - histidinol phosphatase involved in biosynthesis of histidine	1.03	2.04	0.02
RBAM_004870	*ydbO* - hypothetical protein	1.03	2.04	0.04
B_amylo_FZB42_3942	predicted ncRNA	1.02	2.03	0.03
RBAM_022450	*mmgA* - degradative acetoacetyl-CoA thiolase involved in mother cell metabolism	1.02	2.02	0.01
RBAM_022240	*yqjB* - conserved hypothetical protein	1.00	2.00	0.05
RBAM_019760	putative oxidoreductase	0.99	1.98	0.04
RBAM_008750	*sspE* - small acid-soluble spore protein (major gamma-type SASP) involved in protection of spore DNA	0.99	1.98	0.00
RBAM_021800	*yqkA* - conserved hypothetical protein	0.98	1.98	0.01
RBAM_007610	*yhxD* .- putative dehydrogenase involved in survival of salt and ethanol stresses	0.98	1.97	0.02
RBAM_008210	*yfjR* - hypothetical oxidoreductase	0.97	1.96	0.02
RBAM_015190	*ylmC* - conserved hypothetical protein	0.96	1.95	0.02
RBAM_029460	*yunD* - conserved hypothetical protein	0.96	1.94	0.01
RBAM_026920	*sftA* - DNA translocase involved in resolution of chromosome dimers	0.96	1.94	0.01
RBAM_017970	*kdgA* - 2-keto-3-deoxygluconate-6-phosphate aldolase involved in utilization of galacturonic acid	0.95	1.93	0.02
RBAM_033840	*csbD* - stress response protein	0.94	1.92	0.02

RBAM_021550	*spoVAA* - stage V sporulation protein AA (SpoVAA) involved in spore maturation	0.94	1.92	0.04
RBAM_013760	*ykuA* - penicillin-binding protein H involved in formation of a rod-shaped peptidoglycan cell wall	0.94	1.91	0.03
RBAM_001320	*rpoB* - RNA polymerase beta subunit involved in transcription RNA polymerase (beta subunit)	0.94	1.91	0.04
RBAM_003790	*yclA* - putative transcription regulator LysR family involved in regulation of resistance to salicylic acid	0.94	1.91	0.04
RBAM_027400	*ytrA* - transcriptional regulator (GntR family) involved in regulation of acetoine uptake	0.93	1.91	0.03
RBAM_025760	*ysfD* - putative glycolate oxidase subunit	0.92	1.89	0.01
RBAM_019920	*ilvA* - threonine dehydratase involved in biosynthesis of branched-chain amino acids	0.92	1.89	0.01
RBAM_029110	*yuiD* - conserved hypothetical protein	0.91	1.88	0.05
RBAM_004730	*gsiB* - general stress protein	0.91	1.88	0.00
RBAM_021650	*spoIIM* - stage II sporulation protein M (SpoIIM) involved in dissolution of the septal cell wall	0.91	1.88	0.03
RBAM_013510	*ykvL* - conserved hypothetical protein involved in tRNA modification	0.91	1.87	0.05
RBAM_008700	*fhO* - hypothetical protein	0.90	1.87	0.01
RBAM_026590	*rpsD* - 30S ribosomal protein S4	0.90	1.87	0.03
RBAM_010530	*yhfS* - putative acetyl-CoA C-acetyltransferase	0.90	1.87	0.02
RBAM_026710	*hmp* - flavohemoglobin (Nitric oxide dioxygenase) involved in resistance to NO	0.90	1.87	0.04
RBAM_006880	*purS* - phosphoribosylformylglycinamidine synthetase involved in purine biosynthesis	0.90	1.86	0.00
RBAM_024220	conserved hypothetical protein	0.89	1.86	0.01
RBAM_003830	*yclD2* - hypothetical protein	0.89	1.86	0.02
RBAM_013180	*ykoU* - conserved hypothetical protein which confers dry-heat resistance to dormant spores	0.89	1.85	0.02
RBAM_021200	*ribU* - riboflavin transporter involved in riboflavin uptake	0.89	1.85	0.04
RBAM_017280	hypothetical protein	0.88	1.84	0.03
RBAM_023650	*yqfD* - conserved hypothetical protein involved in sporulation	0.87	1.83	0.03
RBAM_008190	*yfjT* - hypothetical protein	0.87	1.83	0.02
RBAM_026990	*malS* - malate dehydrogenase (decarboxylating) involved in malate utilization	0.85	1.80	0.00
RBAM_016560	*ymxH* - conserved hypothetical protein	0.85	1.80	0.01
RBAM_023750	*yqeT* - putative Ribosomal protein L11 methyltransferase(L11 Mtase)	0.85	1.80	0.03
RBAM_024540	*glnH* - glutamine ABC transporter (glutamine-binding protein) involved in glutamine uptake	0.85	1.80	0.04
RBAM_008620	*ybfJ* - hypothetical protein	0.84	1.79	0.02
RBAM_002510	*yfiM* - putative ABC transporter, permease component	0.83	1.78	0.04
RBAM_029070	*yuiH* - putative sulfite oxidase	0.83	1.78	0.02
RBAM_004550	*mutT* - antimutator protein involved in DNA repair, protection against oxidative stress	0.83	1.77	0.02
RBAM_021250	*resE* - two-component sensor histidine kinase	0.82	1.77	0.03
RBAM_020120	*ypdP* - conserved hypothetical protein	0.82	1.77	0.00
RBAM_001830	*ybaN* - polysaccharide deacetylase involved in spore cortex formation	0.82	1.76	0.02

RBAM_026530	*braB* - branched-chain amino acid transporter involved in the uptake of branched-chain amino acids	0.81	1.76	0.03
RBAM_012790	*dppC* - dipeptide transport system permease protein	0.81	1.75	0.00
RBAM_017670	*yneB* - conserved hypothetical protein	0.81	1.75	0.02
RBAM_011290	*yjaV* - hypothetical protein involved in sporulation	0.80	1.74	0.03
RBAM_029180	*guaC* - GMP reductase involved in purine salvage and interconversion	0.80	1.74	0.02
RBAM_006870	*purC* -.phosphoribosylaminoimidazole succinocarboxamide synthase involved in purine biosynthesis	0.80	1.74	0.00
RBAM_008040	*yfkM* - conserved hypothetical protein involved in survival of salt and ethanol stresses	0.80	1.74	0.01

APPENDIX VIII: Bacterial down-regulated genes in the transitional phase by root exudates

Gene ID	Gene and function	M	Fold-change	p-value
RBAM_032970	*ywtF* - putative transcriptional regulator	-1.69	-3.24	0.00
RBAM_028730	*mrpF* - Na(+)/H(+) antiporter subunit F involved in sodium export	-1.47	-2.77	0.00
RBAM_013570	hypothetical protein	-1.44	-2.71	0.05
RBAM_024270	*yrhI* - transcriptional repressor of the fatR yrhJ operon (TetR family)	-1.34	-2.53	0.00
RBAM_000700	*yabQ* - hypothetical protein involved in sporulation	-1.29	-2.45	0.00
RBAM_018900	*yocA* - conserved hypothetical protein	-1.29	-2.45	0.04
RBAM_007480	hypothetical protein	-1.27	-2.42	0.05
RBAM_010610	conserved hypothetical protein	-1.23	-2.34	0.01
RBAM_011120	hypothetical protein	-1.21	-2.32	0.00
RBAM_004880	*ydbO* - hypothetical protein	-1.20	-2.30	0.01
RBAM_021420	*ribD* - 5-amino-6-(5-phosphoribosylamino)uracil reductase involved in riboflavin biosynthesis	-1.20	-2.29	0.05
B_amylo_FZB42_4040	predicted ncRNA	-1.18	-2.26	0.03
RBAM_036510	*yxeG* - hypothetical protein	-1.17	-2.25	0.00
RBAM_017400	hypothetical protein	-1.14	-2.20	0.02
B_amylo_FZB42_3963	predicted ncRNA	-1.13	-2.19	0.05
RBAM_010470	*lcfB* - long-chain fatty-acid-CoA ligase involved in fatty acid degradation	-1.12	-2.18	0.05
RBAM_009560	*glpD* - glycerol-3-phosphate dehydrogenase involved in glycerol utilization	-1.11	-2.16	0.00
RBAM_036140	*yxjA* - purine nucleoside transporter involved in purine uptake	-1.10	-2.15	0.01
RBAM_025230	*hemA* - glutamyl-tRNA reductase involved in porphyrin biosynthesis	-1.05	-2.08	0.01
B_amylo_FZB42_3875	predicted ncRNA	-1.03	-2.05	0.03
RBAM_037870	hypothetical protein	-1.02	-2.03	0.03
RBAM_034580	hypothetical protein	-0.97	-1.96	0.00
RBAM_003590	*yckE* - aryl-β-glucosidase involved in utilization of aryl-β-glucosides	-0.97	-1.95	0.00
RBAM_036890	*aldX* - aldehyde dehydrogenase	-0.96	-1.95	0.01
RBAM_003840	*yclF* - putative di-tripeptide ABC transporter, permease	-0.95	-1.93	0.05
RBAM_023630	*yqfF* - conserved hypothetical protein	-0.93	-1.91	0.01
RBAM_007400	hypothetical protein	-0.93	-1.90	0.01
RBAM_035860	*ydfA* - As(III) efflux pump confers resistance to arsenite	-0.92	-1.89	0.00
RBAM_037880	*ynaF* - conserved hypothetical protein	-0.90	-1.87	0.00

RBAM_032900	*lytD* - glucosaminidase major autolysin, cell separation	-0.90	-1.87	0.01
RBAM_034800	hypothetical protein	-0.89	-1.85	0.00
RBAM_035700	hypothetical protein	-0.88	-1.84	0.02
RBAM_035150	*spsB* - spore coat polysaccharide synthesis protein	-0.87	-1.82	0.01
RBAM_035400	*qoxD* - quinol oxidase subunit IV involved in respiration	-0.86	-1.81	0.00
RBAM_008400	*yfiB* - putative ABC transporter (ATP binding protein)	-0.84	-1.79	0.03
RBAM_032580	*flgM* - anti-SigD involved in the control of SigD activity	-0.82	-1.77	0.00
RBAM_037380	*yycH* - negative effector of WalK involved in the control of cell wall metabolism	-0.79	-1.73	0.00

APPENDIX IX: Functional groups of differentially expressed bacterial genes by seed and root exudates

Functional groups	Seed exudates				Root exudates			
	OD 1.0		OD 3.0		OD 1.0		OD 3.0	
	up	Down	up	down	up	down	up	down
Cell wall	1	0	2	3	1	4	2	1
Transport/binding proteins and lipoproteins	12	17	18	21	2	3	8	5
Sensors (signal transduction)	1	3	1	2	2	2	1	0
Membrane bioenergetics (electron transport chain and ATP synthase)	2	5	3	2	3	1	2	1
Mobility and chemotaxis	0	2	1	5	0	0	0	1
Protein secretion	0	2	0	0	0	1	1	0
Cell division	2	1	1	0	1	1	0	0
Sporulation	5	9	3	6	2	5	7	1
Germination	1	0	3	0	1	0	0	0
Transformation/competence	0	2	0	1	0	0	1	0
Metabolism of carbohydrates and related molecules	9	7	4	26	3	0	8	2
Metabolism of amino acids and related molecules	1	11	1	10	2	1	8	0
Metabolism of nucleotides and nucleic acids	2	6	1	5	0	1	7	0
Metabolism of lipids	0	6	2	1	0	1	6	1
Metabolism of coenzymes and prosthetic groups	0	8	4	8	0	0	5	2
Metabolism of phosphate	0	0	0	0	0	0	0	0
Metabolism of sulfur	1	0	0	0	0	0	1	0
DNA replication	0	1	2	0	0	1	2	0
DNA restriction/modification and repair	0	1	0	2	1	1	2	0
DNA recombination	0	2	0	3	0	0	0	0
DNA packaging and segregation	0	0	0	0	0	0	0	0
RNA synthesis	6	18	7	17	1	1	10	2
RNA modification	0	2	1	0	0	0	1	0
Protein synthesis	1	19	4	0	0	5	1	0
Protein modification	0	3	0	1	1	0	1	0
Protein folding	0	2	0	0	0	0	0	0
Adaptation to atypical conditions	1	7	2	11	1	5	2	0

Detoxification	2	3	1	4	4	2	1	1
Antibiotic production	1	3	0	5	2	0	0	0
Phage-related functions	2	0	1	1	0	2	4	0
Transposon and IS	0	1	0	1	0	0	1	1
Miscellaneous	0	0	1	1	0	0	0	0
From *B. subtilis*	21	54	31	45	10	16	33	8
From other organisms	6	7	5	5	1	1	2	2
No similarity	6	11	4	9	2	6	5	5
ncRNA	6	3	16	4	7	1	3	3
Sum of genes	89	216	119	199	47	61	125	36

APPENDIX X: Bacterial up-regulated genes in the logarithmic phase by N-deficient maize root exudates

Gene ID	Gene and Function	M	Fold-change	p-value
RBAM_006170	*gmuR* - transcriptional repressor involved in the regulation of glucomannan utilization	2.22	4.67	0.00
RBAM_018700	hypothetical protein	1.54	2.91	0.01
RBAM_018760	*yoaD* - putative phosphoglycerate dehydrogenase	1.45	2.73	0.01
B_amylo_FZB42_4018	predicted ncRNA	1.37	2.58	0.04
RBAM_017570	hypothetical protein	1.36	2.57	0.03
RBAM_021880	*yqjT* - conserved hypothetical protein	1.33	2.51	0.00
RBAM_034640	hypothetical protein	1.24	2.35	0.00
RBAM_013810	*ykyB* - hypothetical protein	1.23	2.35	0.01
RBAM_027830	*ytaB* - conserved hypothetical protein involved in survival of ethanol and salt stresses	1.18	2.27	0.00
RBAM_016100	*fliK* - flagellar hook-length control protein involved in motility and chemotaxis	1.17	2.25	0.00
RBAM_019990	*ypiP* - conserved hypothetical protein	1.16	2.23	0.03
B_amylo_FZB42_3830	predicted ncRNA	1.14	2.20	0.00
RBAM_025030	*spoIVFA* - stage IV sporulation protein FA involved in the control of SigK activation	1.11	2.16	0.01
RBAM_035600	*ywbE* - conserved hypothetical protein	1.09	2.13	0.00
RBAM_019350	hypothetical protein	1.07	2.10	0.00
RBAM_011810	hypothetical protein	1.06	2.09	0.00
B_amylo_FZB42_3940	predicted ncRNA	1.06	2.08	0.01
RBAM_007430	putative ABC transporter permease	1.05	2.08	0.01
RBAM_010490	*yhfN* - conserved hypothetical protein	1.05	2.06	0.01
RBAM_018520	site-specific recombinase (phage integrase family)	1.03	2.04	0.00
RBAM_008260	hypothetical protein	1.03	2.04	0.02
B_amylo_FZB42_3984	predicted ncRNA	1.00	2.01	0.01
RBAM_019580	*yosT* - conserved hypothetical protein	0.99	1.99	0.02
RBAM_016540	*ylxY* - putative deacetylase	0.96	1.95	0.01
RBAM_008170	*yfkC* - mechanosensitive channel, involved in resistance to osmotic downshock	0.96	1.94	0.01
RBAM_012350	*yjlB* - conserved hypothetical protein	0.95	1.93	0.00
RBAM_027750	hypothetical protein	0.94	1.92	0.01
RBAM_009080	*senN* - transcriptional regulatory protein	0.94	1.92	0.02
RBAM_021720	*ansR* - HTH-type transcriptional regulator involved in negative regulation of the ansA-ansB operon	0.94	1.92	0.02
RBAM_022750	*spoIIIAB* - stage III sporulation protein involved in the activation of SigG	0.94	1.92	0.00

RBAM_022720	*spoIIIAE* - stage III sporulation protein involved in the activation of SigG	0.94	1.91	0.00
RBAM_037680	*dinB* - nuclease inhibitor involved in response to DNA damage	0.93	1.91	0.04
RBAM_020440	*sspM* - small, acid-soluble spore protein involved in protection of spore DNA	0.93	1.91	0.00
RBAM_004870	*ydbO1* - hypothetical protein with 85% similarity with a putative cation efflux transporter from *B. subtilis*	0.93	1.91	0.00
RBAM_019010	*yozN* - hypothetical protein	0.93	1.91	0.00
RBAM_029460	*yunD* - conserved hypothetical protein	0.93	1.90	0.00
RBAM_013150	*ykoL* - stress response protein	0.91	1.87	0.00
RBAM_018110	hypothetical protein	0.91	1.87	0.01
RBAM_012200	*pgm1* - encodes for an enzyme involved in glycolysis/gluconeogenesis	0.91	1.87	0.00
B_amylo_FZB42_3992	predicted ncRNA	0.89	1.85	0.00
RBAM_006570	*yjdJ* - hypothetical protein	0.88	1.84	0.00
RBAM_019460	*yozD* - hypothetical protein	0.87	1.83	0.00
RBAM_023400	*yqfW* - conserved hypothetical protein	0.87	1.83	0.00
RBAM_035610	putative transcriptional regulator (MerR family)	0.87	1.82	0.00
RBAM_034440	*arfM* - putative transcriptional regulator involved in regulation of anaerobic genes	0.85	1.80	0.00
RBAM_010480	*yhfM* - hypothetical protein	0.85	1.80	0.00
RBAM_023210	*yqgN* - conserved hypothetical protein	0.85	1.80	0.00
B_amylo_FZB42_3855	predicted ncRNA	0.84	1.79	0.00
RBAM_016690	hypothetical protein	0.84	1.79	0.00
RBAM_002350	hypothetical protein	0.83	1.78	0.01
RBAM_033310	*ywqL* - putative endonuclease	0.83	1.78	0.01
RBAM_028410	*yugN* - hypothetical protein	0.83	1.77	0.01
B_amylo_FZB42_3900	predicted ncRNA	0.82	1.76	0.00
RBAM_024570	hypothetical protein	0.81	1.76	0.00
RBAM_003860	*yczF* - hypothetical protein	0.81	1.75	0.01
RBAM_035880	*gmuA* - glucomannan-specific phosphotransferase system enzyme involved in glucomannan uptake and phosphorylation	0.81	1.75	0.01
RBAM_033660	*ywoD* - conserved hypothetical protein with 80% similarity with a putative efflux transporter from *B. subtilis*	0.81	1.75	0.00
RBAM_005220	*lrpA* - transcriptional regulator involved in repression of glyA transcription and KinB-dependent sporulation	0.80	1.74	0.00
RBAM_020600	*cca* - tRNA nucleotidyltransferase involved in tRNA modification	0.80	1.74	0.00
RBAM_021850	*yqzH* - hypothetical protein	0.80	1.74	0.01
B_amylo_FZB42_3834	predicted ncRNA	0.80	1.74	0.00

RBAM_034560	hypothetical protein	0.80	1.74	0.01
RBAM_008730	*yfhS* - hypothetical protein	0.80	1.74	0.00
RBAM_008760	*ygaB* - hypothetical protein	0.79	1.73	0.00

APPENDIX XI: Bacterial down-regulated genes in the logarithmic phase by N-deficient maize root exudates

Gene ID	Gene and Function	M	Fold-change	p-value
RBAM_003170	*yceE* - putative tellurium resistance protein required for survival of ethanol stress and at low temperatures	-1.87	-3.66	0.00
RBAM_001550	*rpsH* - ribosomal protein S8 (BS8) involved in translation	-1.86	-3.64	0.00
RBAM_001360	*rpsG* - ribosomal protein S7 (BS7) involved in translation	-1.73	-3.31	0.00
RBAM_016860	*ymcA* - conserved hypothetical protein involved in regulation of biofilm formation	-1.61	-3.04	0.03
RBAM_009370	*cspB* - major cold-shock protein (RNA chaperone)	-1.59	-3.01	0.00
RBAM_001520	*rplX* - ribosomal protein L24 (BL23) (histone-like protein HPB12) involved in translation	-1.59	-3.01	0.00
RBAM_001650	*rpmJ* - ribosomal protein L36 (ribosomal protein B) involved in translation	-1.56	-2.94	0.01
RBAM_001380	*tufA* - elongation factor Tu involved in translation	-1.52	-2.87	0.00
B_amylo_FZB42_4030	predicted ncRNA	-1.43	-2.69	0.00
RBAM_001460	*rplV* - ribosomal protein L22 (BL17) in translation	-1.42	-2.67	0.00
RBAM_001500	*rpsQ* - ribosomal protein S17 (BS16) in translation	-1.41	-2.67	0.00
RBAM_000580	*spoVG* - stage V sporulation protein involved in cell division, control of sporulation initiation	-1.40	-2.63	0.00
RBAM_001510	*rplN* - ribosomal protein L14 in translation	-1.38	-2.60	0.00
RBAM_037980	*rpsR* - ribosomal protein S18 in translation	-1.37	-2.58	0.00
RBAM_001480	*rplP* - ribosomal protein L16 involved in translation	-1.36	-2.57	0.00
RBAM_001440	*rplB* - ribosomal protein L2 (BL2) involved in translation	-1.34	-2.54	0.00
RBAM_015650	*rpmB* - 50S ribosomal protein L28 involved in translation	-1.33	-2.52	0.00
RBAM_031300	*gapA* - glyceraldehyde-3-phosphate dehydrogenase involved in glycolysis	-1.32	-2.49	0.00
RBAM_034230	*rpmE* - 50S ribosomal protein L31 involved in translation	-1.31	-2.49	0.00
RBAM_001530	*rplE* - ribosomal protein L5 (BL6) involved in translation	-1.29	-2.45	0.00
RBAM_002270	*rsiW* - control of SigW activity	-1.29	-2.44	0.00
B_amylo_FZB42_3843	predicted ncRNA	-1.27	-2.42	0.00
RBAM_011500	*spx* - transcriptional regulator Spx	-1.27	-2.41	0.00
RBAM_010030	*yheA* - conserved hypothetical protein	-1.25	-2.38	0.00
RBAM_001410	*rplC* - ribosomal protein L3 (BL3) involved in translation	-1.24	-2.36	0.00
RBAM_032510	*hag* - flagellin protein involved in motility and chemotaxis	-1.23	-2.34	0.00
RBAM_003160	*yceD* - putative tellurium resistance protein required for survival to ethanol stress	-1.21	-2.32	0.00
B_amylo_FZB42_4026	predicted ncRNA	-1.21	-2.31	0.00
RBAM_020310	*yqgA* - hypothetical protein	-1.21	-2.31	0.00

RBAM_025920	*rpmI* - 50S ribosomal protein involved in translation	-1.20	-2.30	0.00
RBAM_001640	*infA* - translation initiation factor IF-I	-1.20	-2.29	0.05
RBAM_001560	*rplF* - ribosomal protein L6 (BL8) involved in translation	-1.18	-2.26	0.00
RBAM_017920	hypothetical protein	-1.17	-2.26	0.00
RBAM_020330	*ypsB* - hypothetical protein involved in cell division, cell elongation	-1.15	-2.22	0.01
RBAM_006610	*ydjI* - conserved hypothetical protein	-1.14	-2.21	0.02
RBAM_011910	*yjcG* - conserved hypothetical protein involved in RNA metabolism	-1.13	-2.19	0.00
RBAM_035430	*qoxA* - quinol oxidase subunit II precursor involved in respiration	-1.13	-2.19	0.00
RBAM_001670	*rpsK* - ribosomal protein S11 (BS11) involved in translation	-1.13	-2.19	0.00
RBAM_036020	*cimH* - citrate/malate transporter	-1.12	-2.17	0.00
RBAM_001610	*secY* - preprotein translocase subunit involved in protein secretion	-1.10	-2.15	0.00
RBAM_008820	*perR* - peroxide operon regulator involved in regulation of the response to peroxide	-1.10	-2.15	0.00
RBAM_001400	*rpsJ* - ribosomal protein S10 (BS13) involved in translation	-1.10	-2.14	0.00
RBAM_031290	*pgk* - phosphoglycerate kinase involved in glycolysis/gluconeogenesis	-1.10	-2.14	0.00
RBAM_023220	*rpmGA* - 50S ribosomal protein L33 type I involved in translation	-1.10	-2.14	0.00
RBAM_033420	*ywqI* - hypothetical protein	-1.09	-2.13	0.00
RBAM_011430	*oppA* - oligopeptide ABC transporter (binding protein) involved in initiation of sporulation, competence development	-1.09	-2.12	0.00
RBAM_018500	*iseA* - inhibitor of autolysins involved in protection against cell envelope stress	-1.08	-2.12	0.00
RBAM_001290	*rplJ* - ribosomal proteinL10 (BL5) involved in translation	-1.08	-2.11	0.01
B_amylo_FZB42_3878	predicted ncRNA	-1.07	-2.10	0.00
RBAM_033960	*atpC* - ATP synthase (subunit epsilon)	-1.07	-2.10	0.00
RBAM_001590	*rpmD* - ribosomal protein L30 (BL27) involved in translation	-1.06	-2.09	0.00
RBAM_015870	*rplS* - ribosomal protein L19 involved in translation	-1.06	-2.09	0.00
RBAM_004640	hypothetical protein	-1.06	-2.08	0.00
RBAM_027680	*luxS* - s-ribosylhomocysteine lyase involved in methionine salvage	-1.06	-2.08	0.00
B_amylo_FZB42_3840	predicted ncRNA	-1.05	-2.07	0.00
B_amylo_FZB42_3941	predicted ncRNA	-1.05	-2.06	0.00
RBAM_025560	*trxA* - thioredoxin involved in protection of proteins against oxidative damage	-1.04	-2.06	0.00
B_amylo_FZB42_3982	predicted ncRNA	-1.04	-2.06	0.00
RBAM_001570	*rplR* - ribosomal protein L18 involved in translation	-1.04	-2.06	0.00
RBAM_016970	*baeI* - enoyl-CoA-hydratase involved in antibiotics production	-1.02	-2.03	0.02
RBAM_001660	*rpsM* - ribosomal protein S13 involved in translation	-1.01	-2.02	0.01
RBAM_014080	*yknW* - hypothetical protein involved in resistence against SdpC toxin	-1.01	-2.01	0.00
RBAM_033160	*alsD* - alpha-acetolactate decarboxylase involved in overflow metabolism	-1.00	-2.01	0.00

RBAM_001370	*fusA* - elongation factor G involved in translation	-1.00	-2.00	0.00
RBAM_008680	*yfhM* - conserved hypothetical protein involved in survival to ethanol stress	-0.99	-1.99	0.00
RBAM_028490	*yugI* - putative polyribonucleotide nucleotidyl transferase	-0.99	-1.98	0.00
RBAM_015840	*ylqD* - hypothetical protein	-0.99	-1.98	0.00
RBAM_035790	*licB* - phosphotransferase system (PTS) lichenan specific enzyme IIB component involved in lichenan uptake and phosphorylation, control of LicR activity	-0.99	-1.98	0.00
RBAM_006140	*pbpE* - penicillin-binding protein (endopeptidase)	-0.98	-1.97	0.00
RBAM_001680	*rpoA* - RNA polymerase (alpha subunit) involved in transcription	-0.98	-1.97	0.02
RBAM_024500	*yrzL* - conserved hypothetical protein	-0.98	-1.97	0.00
RBAM_015750	*acpA* - acyl carrier protein involved in fatty acid biosynthesis	-0.98	-1.97	0.00
RBAM_025910	*rplT* - 50S ribosomal protein L20 involved in translation	-0.97	-1.96	0.00
RBAM_015830	*ylqC* - conserved hypothetical protein	-0.97	-1.95	0.00
RBAM_013670	*ptsH* - phosphocarrier protein HPr component involved in PTS-dependent sugar transport and carbon catabolite repression	-0.96	-1.95	0.00
RBAM_002260	*sigW* - RNA polymerase ECF-type sigma factor involved in resistance against SdpC that functions in detoxification and/or production of antimicrobial compounds	-0.96	-1.94	0.00
RBAM_030260	*yvqI* - hypothetical protein	-0.96	-1.94	0.04
RBAM_001450	*rpsS* - ribosomal protein S19 (BS19) involved in translation	-0.94	-1.92	0.00
RBAM_028800	*degQ* - degradation enzyme regulation protein involved in regulation of exoenzyme synthesis	-0.94	-1.92	0.00
RBAM_017100	*ebrB* - multidrug resistance protein	-0.94	-1.92	0.00
RBAM_001280	*rplA* - ribosomal protein L1 (BL1) involved in translation	-0.94	-1.92	0.01
RBAM_028960	*yukE* - conserved hypothetical protein	-0.94	-1.91	0.00
RBAM_003670	*comS* - competence protein S involved in control of ComK degradation	-0.93	-1.91	0.00
RBAM_015800	*ylxM* - conserved hypothetical protein	-0.92	-1.90	0.00
RBAM_001250	*secE* - preprotein translocase subunit involved in protein secretion	-0.92	-1.89	0.00
RBAM_013550	hypothetical protein	-0.92	-1.89	0.00
RBAM_006940	*purH* - inosine-monophosphate cyclohydrolase involved in purine biosynthesis	-0.92	-1.89	0.00
RBAM_019690	*yorC* - hypothetical protein	-0.91	-1.88	0.03
RBAM_034020	*atpE* - ATP synthase (subunit C) involved in ATP synthesis	-0.91	-1.88	0.00
RBAM_016520	*rpsO* - ribosomal protein S15 (BS18) involved in translation	-0.91	-1.88	0.00
RBAM_000530	*veg* - conserved hypothetical protein	-0.91	-1.87	0.00
B_amylo_FZB42_4007	predicted ncRNA	-0.90	-1.87	0.02
RBAM_032250	*yvlD* - conserved hypothetical protein	-0.90	-1.87	0.00
RBAM_001330	*rpoC* - RNA polymerase (beta subunit) involved in transcription	-0.90	-1.87	0.00

RBAM_023690	*yqeZ* - conserved hypothetical protein involved in resistance against sublancin	-0.90	-1.86	0.02
B_amylo_FZB42_3905	predicted ncRNA	-0.90	-1.86	0.02
RBAM_025540	*lysC* - aspartokinase II alpha subunit and beta subunit involved in biosynthesis of lysine	-0.90	-1.86	0.00
RBAM_009090	*katA* - vegetative catalase involved in detoxification (degradation) of hydrogen peroxide	-0.89	-1.85	0.02
RBAM_026870	*aroA* – chorismate mutase involved in biosynthesis of aromatic amino acids	-0.88	-1.85	0.00
RBAM_030250	*yvqH* – conserved hypothetical protein involved in protection against daptamycin	-0.88	-1.84	0.05
RBAM_009900	*dat* - D-alanine aminotransferase involved in peptidoglycan precursor biosynthesis	-0.88	-1.84	0.00
RBAM_013880	*ykuK* - conserved hypothetical protein	-0.87	-1.83	0.00
RBAM_001430	*rplW* - ribosomal protein L23 involved in translation	-0.87	-1.83	0.03
RBAM_022930	*sinR* - HTH-type transcriptional regulator	-0.87	-1.82	0.00
RBAM_004660	*mntH* - manganese transport protein involved in manganese uptake	-0.86	-1.82	0.00
B_amylo_FZB42_3931	predicted ncRNA	-0.86	-1.81	0.00
RBAM_023140	*yqgV* – conserved hypothetical protein	-0.85	-1.80	0.00
RBAM_002660	hypothetical protein	-0.85	-1.80	0.00

APPENDIX XII: Bacterial up-regulated genes in the transitional phase by N-deficient maize root exudates

Gene ID	Gene and Function	M	Fold-change	p-value
RBAM_020820	*trpC* - indol-3-glycerol phosphate synthase involved in biosynthesis of tryptophan	1.68	3.21	0.00
B_amylo_FZB42_3976	predicted ncRNA	1.37	2.58	0.00
RBAM_037980	*rpsR* - ribosomal protein S18 involved in translation	1.16	2.23	0.00
RBAM_002320	*glmS* - l-glutamine-D-fructose-6-phosphate amidotransferase involved in cell wall synthesis	1.14	2.20	0.00
RBAM_001560	*rplF* - ribosomal protein L6 (BL8) involved in translation	1.13	2.19	0.00
RBAM_024250	*yybF1* - hypothetical transport protein	1.12	2.18	0.00
RBAM_029920	*yusL* - 3-hydroxyacyl-CoA dehydrogenase involved in fatty acid degradation	1.07	2.11	0.02
RBAM_036680	*mrsE* - putative ABC-transporter integral membrane protein	1.03	2.04	0.02
RBAM_006700	*cotA* - spore coat protein (outer) involved in resistence to the spore	1.03	2.04	0.00
RBAM_015370	pyrD - dihydroorotate dehydrogenase (catalytic subunit) involved in pyrimidine biosynthesis	1.02	2.02	0.00
RBAM_027300	*bceB* - bacitracin export permease protein involved in bacitracin export	1.01	2.01	0.00
RBAM_002040	*gabT1* - putative 4-aminobutyrate aminotransferase	1.01	2.01	0.00
RBAM_001510	*rplN* - ribosomal protein L14 involved in translation	0.97	1.96	0.00
RBAM_001530	*rplE* - ribosomal protein L5 (BL6) involved in translation	0.96	1.95	0.00
RBAM_015380	*pyrF* - orotidine 5'-phosphate decarboxylase involved in pyrimidine biosynthesis	0.95	1.93	0.00
RBAM_032900	*lytD* - beta-N-acetylglucosaminidase (major autolysin) involved in cell separation	0.95	1.93	0.00
RBAM_035400	*qoxD* - quinol oxidase subunit IV involved in respiration	0.94	1.92	0.00
RBAM_001590	*rpmD* - ribosomal protein L30 (BL27) involved in translation	0.94	1.91	0.01
RBAM_016520	*rpsO* - ribosomal protein S15 (BS18) involved in translation	0.92	1.89	0.01
RBAM_001550	*rpsH* - ribosomal protein S8 (BS8) involved in translation	0.91	1.89	0.00
RBAM_015830	*ylqC* - conserved hypothetical protein	0.91	1.88	0.00
RBAM_001500	*rpsQ* - ribosomal protein S17 (BS16) involved in translation	0.90	1.87	0.00
RBAM_014940	*rpmF* - ribosomal protein L32involved in translation	0.89	1.85	0.02
RBAM_015820	*rpsP* - 30S ribosomal protein S16 involved in translation	0.87	1.83	0.00
RBAM_033960	*atpC* - ATP synthase (subunit epsilon)	0.87	1.82	0.00
RBAM_030200	*gerAC* - nutrient receptor involved in germination response to L-alanine	0.86	1.81	0.00
RBAM_001450	*rpsS* - ribosomal protein S19 (BS19) involved in translation	0.85	1.80	0.00
RBAM_025140	*valS* - valyl-tRNA synthetase involved in translation	0.85	1.80	0.00

APPENDIX XIII: Bacterial down-regulated genes in the transitional phase by N-deficient maize root exudates

Gene ID	Gene and Function	M	Fold-change	p-value
RBAM_016860	*ymcA* – antagonist of biofilm repression by SinR involved in regulation of biofilm formation	-0.85	-1.80	0.00
RBAM_020410	*yppF* - hypothetical protein	-0.85	-1.81	0.00
RBAM_033070	*gerKC1* - nutrient receptor involved in germination	-0.86	-1.81	0.03
RBAM_021760	*yqkE* - hypothetical protein	-0.86	-1.82	0.00
RBAM_004360	*yczJ* - conserved hypothetical protein	-0.87	-1.82	0.01
RBAM_001080	*ctsR* - transcriptional regulator involved in regulation of protein degradation	-0.87	-1.83	0.00
RBAM_003080	*rapJ* - response regulator aspartate phosphatase	-0.87	-1.83	0.04
RBAM_004090	*ycnB* - putative multidrug resistance protein	-0.87	-1.83	0.02
RBAM_017650	*lexA* - negative transcriptional regulator of the SOS regulon involved in regulation of DNA damage repair	-0.88	-1.84	0.01
RBAM_017570	hypothetical protein	-0.88	-1.84	0.01
RBAM_022360	*bcd* - leucine dehydrogenase involved in utilization of branched-chain keto acids	-0.88	-1.84	0.03
RBAM_016150	*fliM* - flagellar motor switch protein involved in movement and chemotaxis	-0.89	-1.85	0.00
RBAM_021160	*ypbD* – conserved hypothetical protein	-0.89	-1.85	0.04
RBAM_021220	*serA* - D-3-phosphoglycerate dehydrogenase involved in biosynthesis of serine	-0.91	-1.88	0.02
RBAM_006010	putative beta-ketoacyl-acyl carrier protein synthase II	-0.91	-1.88	0.02
RBAM_019200	*yojI* - putative multidrug resistance protein	-0.92	-1.89	0.02
B_amylo_FZB42_3873	predicted ncRNA	-0.92	-1.89	0.01
RBAM_016750	*rodZ* - morphogenic protein required for cell shape determination	-0.93	-1.90	0.00
B_amylo_FZB42_3909	predicted ncRNA	-0.93	-1.91	0.02
RBAM_016840	*kbl* - 2-amino-3-ketobutyrate coenzyme A ligase involved in threonine utilization	-0.93	-1.91	0.00
RBAM_027240	*ytwF* - conserved hypothetical protein	-0.93	-1.91	0.03
RBAM_016830	*tdh* - L-threonine 3-dehydrogenase involved in threonine utilization	-0.93	-1.91	0.00
B_amylo_FZB42_4040	predicted ncRNA	-0.94	-1.91	0.02
RBAM_024200	*manP* - mannose-specific enzyme IIBCA component involved in mannose uptake and phosphorylation	-0.94	-1.92	0.00
RBAM_005410	*ydeB* – conserved hypothetical protein	-0.94	-1.92	0.00
RBAM_026870	*aroA* – chorismate mutase AroA involved in biosynthesis of aromatic amino acids	-0.95	-1.93	0.00
RBAM_014710	*ftsW* - cell division protein	-0.95	-1.93	0.03

RBAM_007980	*treA* - trehalose-6-phosphate hydrolase involved in trehalose utilization	-0.95	-1.94	0.00
B_amylo_FZB42_3849	predicted ncRNA	-0.96	-1.95	0.00
RBAM_005370	putative transcriptional regulator	-0.96	-1.95	0.02
RBAM_012280	*yjgD* - conserved hypothetical protein involved in survival to ethanol stress	-0.96	-1.95	0.01
RBAM_016030	*fliE* - flagellar hook-basal body protein involved in motility and chemotaxis	-0.97	-1.96	0.04
RBAM_036760	*iolC* - inositol utilization protein C involved in myo-inositol catabolism	-0.97	-1.96	0.00
RBAM_022350	*buk* - butyrate kinase involved in utilization of branched-chain keto acids	-0.97	-1.97	0.00
RBAM_016720	*ymfI* - conserved hypothetical protein	-0.98	-1.98	0.00
RBAM_012700	*ykaA* - conserved hypothetical protein	-0.99	-1.98	0.00
RBAM_015520	*rpoZ* - DNA-directed RNA polymerase omega chain	-0.99	-1.98	0.03
RBAM_002150	*ybbD* - putative Beta-hexosaminidase	-0.99	-1.99	0.01
RBAM_013130	*tnrA* - HTH-type transcriptional regulator involved in regulation of nitrogen assimilation	-1.00	-1.99	0.03
RBAM_018960	*yocH* - putative cell-wall binding protein	-1.00	-2.00	0.00
RBAM_013430	*mhqR* - transcription regulator involved in the regulation of resistence to methyl-hydroxyquinone	-1.00	-2.00	0.02
RBAM_018470	*dacC* - penicillin-binding carboxypeptidase	-1.00	-2.00	0.00
RBAM_013310	*kinE* -. two-component sensor histidine kinase homolog involved in initiation of sporulation	-1.00	-2.01	0.00
RBAM_036720	*iolG* - myo-inositol 2-dehydrogenase involved in myo-inositol catabolism	-1.01	-2.01	0.00
RBAM_013160	*ykoM* - putative transcriptional regulator (MarR family)	-1.01	-2.01	0.00
RBAM_006370	*ydiC* - glycoprotein endopeptidase homolog	-1.02	-2.02	0.01
RBAM_001110	*clpC* - class III stress response-related ATPase involved in protein degradation	-1.02	-2.03	0.00
RBAM_009750	*yhdL* - anti-sigma M factor involved in control of SigM activity	-1.02	-2.03	0.05
RBAM_023170	*glcK* - glucose kinase involved in phosphorylation of the free glucose moiety of di- and oligosaccharides	-1.03	-2.04	0.02
RBAM_036590	*yxeA* - hypothetical protein	-1.03	-2.04	0.03
RBAM_020280	*ypvA* - probable ATP-dependent helicase	-1.03	-2.04	0.02
RBAM_023450	*cshB* - putative ATP-dependent RNA helicase	-1.04	-2.05	0.03
RBAM_014530	*suhB* - inositol-1-monophosphatase	-1.04	-2.05	0.00
B_amylo_FZB42_3895	predicted ncRNA	-1.04	-2.05	0.05
B_amylo_FZB42_3832	predicted ncRNA	-1.04	-2.06	0.00
RBAM_030140	*yirB* - hypothetical protein	-1.04	-2.06	0.02
RBAM_019380	*ctpA* - carboxy-terminal processing protease	-1.05	-2.07	0.04
RBAM_001100	*mcsB* - modulation of CtsR repression protein involved in regulation of protein degradation	-1.05	-2.07	0.00
RBAM_026760	putative transcriptional regulator	-1.05	-2.07	0.00
RBAM_017500	*yoaO* - hypothetical protein	-1.06	-2.09	0.04
RBAM_016120	*flgE* - flagellar basal-body rod protein involved in motility and chemotaxis	-1.07	-2.09	0.00

RBAM_020730	*ypiF* - hypothetical protein	-1.07	-2.10	0.00
RBAM_026670	hypothetical protein	-1.08	-2.11	0.01
RBAM_035780	*licC* - phosphotransferase system (PTS) lichenan specific enzyme involved in lichenan uptake and phosphorylation	-1.08	-2.12	0.00
RBAM_006180	putative transcriptional regulator (gntr family)	-1.08	-2.12	0.00
RBAM_014000	*rok* - comK repressor involved in regulation of genetic competence	-1.09	-2.12	0.00
RBAM_030260	*lial* - hypothetical protein	-1.09	-2.13	0.00
RBAM_013900	*ykuL* - conserved hypothetical protein	-1.09	-2.13	0.01
RBAM_008710	*yfhP* - conserved hypothetical protein	-1.10	-2.14	0.03
RBAM_005560	conserved hypothetical protein	-1.11	-2.15	0.00
RBAM_017890	*yneT* - conserved hypothetical protein	-1.12	-2.17	0.02
RBAM_026150	*phoP* - two-component response regulator involved in regulation of phosphate metabolism	-1.12	-2.17	0.00
RBAM_036740	*iolE* - inositol utilization protein E involved in myo-inositol catabolism	-1.13	-2.18	0.01
RBAM_036350	*bglH* - beta-glucosidase involved in salicin utilization	-1.15	-2.22	0.00
RBAM_004120	*ycnE* - conserved hypothetical protein	-1.16	-2.24	0.00
RBAM_005550	hypothetical protein	-1.17	-2.25	0.00
RBAM_026550	*hisJ* - histidinol phosphatase involved in biosynthesis of histidine	-1.19	-2.28	0.03
RBAM_019060	*dhaS* - aldehyde dehydrogenase	-1.19	-2.28	0.02
RBAM_013640	*ykvZ* - putative HTH-type transcriptional regulator	-1.19	-2.28	0.01
RBAM_035790	*licB* - phosphotransferase system (PTS) lichenan specific enzyme IIB component lichenan uptake and phosphorylation, control of LicR activity	-1.19	-2.29	0.00
RBAM_021820	*yqjY* - conserved hypothetical protein	-1.20	-2.30	0.00
RBAM_025670	*yshB* - hypothetical protein	-1.21	-2.31	0.00
RBAM_033130	*rbsB* - ribose ABC transporter (ribose-binding protein) involved in ribose uptake	-1.22	-2.32	0.05
RBAM_037130	hypothetical protein	-1.23	-2.35	0.03
RBAM_011340	*fabF* - beta-ketoacyl-acyl carrier protein synthase II involved in fatty acid biosynthesis	-1.23	-2.35	0.01
RBAM_018540	*ggt* - gamma-glutamyltranspeptidase involved in degradation of poly-glutamate capsules	-1.24	-2.36	0.00
RBAM_004040	*yclM* - aspartokinase III with unknown function	-1.24	-2.36	0.01
RBAM_019040	hypothetical protein	-1.26	-2.39	0.00
RBAM_021850	*yqzH* - hypothetical protein	-1.27	-2.41	0.02
RBAM_022920	*sinI* - sinR antagonist	-1.27	-2.41	0.00
RBAM_027450	*nrsC* - non-ribosomal peptide synthetase	-1.27	-2.42	0.00
RBAM_019970	*thyB* - thymidylate synthase B involved in biosynthesis of thymidine nucleotides	-1.28	-2.42	0.02
RBAM_016100	*fliK* - flagellar hook-length control protein involved in motility and chemotaxis	-1.28	-2.43	0.00
RBAM_021570	*spoIIAB* - anti-sigma F factor involved in control of sporulation	-1.28	-2.43	0.00

RBAM_030250	*liaH* - conserved hypothetical protein involved in protection against daptomycin	-1.28	-2.44	0.00
RBAM_016060	*fliH* - flagellar assembly protein involved in motility and chemotaxis	-1.29	-2.45	0.00
RBAM_008100	*yfkK* - hypothetical protein	-1.29	-2.45	0.00
RBAM_021290	*resA* - thiol-disulfide oxidoreductase involved in cytochrome c biogenesis	-1.29	-2.45	0.02
RBAM_022530	hypothetical protein	-1.30	-2.46	0.03
RBAM_034930	*bacA* - bacilysin synthetase A	-1.31	-2.48	0.00
RBAM_009700	*citA* - citrate synthase I	-1.32	-2.49	0.00
RBAM_005340	putative oxidoreductase (short-chain dehydrogenase/reductase family)	-1.33	-2.52	0.01
RBAM_034550	putative transcriptional regulator	-1.36	-2.57	0.01
RBAM_014220	*abh* - transition state regulator	-1.36	-2.57	0.00
RBAM_020040	*bsaA* - putative glutathione peroxidase	-1.37	-2.58	0.01
RBAM_017910	*parC* - DNA topoisomerase IV subunit A involved in chromosome segregation and compaction	-1.37	-2.59	0.00
B_amylo_FZB42_3911	predicted ncRNA	-1.39	-2.63	0.01
RBAM_011990	hypothetical protein	-1.40	-2.64	0.03
RBAM_016900	*baeB* - hydroxyacylglutathione hydrolase involved in antibiotics production	-1.42	-2.67	0.03
RBAM_032460	*yvyD* - conserved hypothetical protein required for survival at low temperatures	-1.42	-2.68	0.00
RBAM_020470	*ponA* - bifunctional glucosyl transferase/ transpeptidase penicillin-binding proteins IA/IB	-1.42	-2.69	0.01
RBAM_011860	*blm* - beta-lactamase II precursor (Penicillinase) (Cephalosporinase)	-1.43	-2.69	0.00
RBAM_021700	*ansB* - aspartate ammonia-lyase involved in aspartate degradation	-1.44	-2.71	0.00
RBAM_023810	*lepA* - GTP-binding protein	-1.45	-2.73	0.01
RBAM_022690	*spoIIIAH* - stage III sporulation protein AH	-1.45	-2.73	0.02
RBAM_024190	*manA* - mannose-6-phosphate isomerase involved in mannose utilization	-1.46	-2.75	0.00
RBAM_020080	degR - positive effector of DegU-phosphate stability involved in control of DegU activity	-1.48	-2.78	0.02
RBAM_019360	*bglA* - 6-phospho-beta-glucosidase involved in beta-glucoside utilization	-1.48	-2.78	0.02
RBAM_012150	*galK1* - galactokinase involved in galactose utilization	-1.52	-2.87	0.02
RBAM_019730	*rapA1* - response regulator aspartate phosphatase A involved in control of sporulation initiation	-1.53	-2.88	0.00
RBAM_015710	*fapR* - transcription factor (Fatty acid and phospholipid biosynthesis regulator)	-1.55	-2.92	0.00
RBAM_004730	*gsiB* - general stress protein	-1.60	-3.03	0.00
RBAM_012010	hypothetical protein	-1.60	-3.03	0.01
B_amylo_FZB42_3925	predicted ncRNA	-1.63	-3.10	0.01
RBAM_011590	*yjbK* - conserved hypothetical protein	-1.67	-3.18	0.01
RBAM_020640	*dapB* - dihydrodipicolinate reductase involved in biosynthesis of lysine and peptidoglycan	-1.70	-3.25	0.00
RBAM_012000	hypothetical protein	-1.71	-3.27	0.01
RBAM_019170	*cwlS* - D,L-endopeptidase, peptidoglycan hydrolase involved in cell wall metabolism	-1.72	-3.29	0.00
RBAM_000430	*yabB* – conserved hypothetical protein	-1.72	-3.30	0.01

RBAM_035760	*licH* - 6-phospho-beta-glucosidase involved in lichenan utilization	-1.74	-3.35	0.00
RBAM_021430	*ypuD* - hypothetical protein	-1.77	-3.41	0.01
RBAM_020920	*mtrB* - transcription attenuation protein regulation of tryptophan biosynthesis (and translation) attenuation in the trp operon; repression of the folate operon	-1.81	-3.50	0.00
RBAM_020170	*ypbS* - hypothetical protein	-1.81	-3.51	0.00
RBAM_012080	*penP* - beta-lactamase precursor involved in resistance to beta-lactam antibiotics	-1.84	-3.59	0.01
B_amylo_FZB42_3910	predicted ncRNA	-1.84	-3.59	0.00
RBAM_035770	*licA* - phosphotransferase system (PTS) lichenan specific enzyme involved in lichenan uptake and phosphorylation	-1.86	-3.62	0.00
RBAM_022090	putative transcription antiterminator	-1.87	-3.66	0.03
RBAM_015190	*ylmC* – conserved hypothetical protein	-1.89	-3.72	0.00
RBAM_018180	*bmyA* - bacillomycin D synthetase A involved in antibiotics production	-1.90	-3.74	0.01
RBAM_009900	*dat* - D-alanine aminotransferase involved in peptidoglycan precursor biosynthesis	-1.95	-3.86	0.00
RBAM_019440	*yodL* - hypothetical protein	-1.98	-3.94	0.00
B_amylo_FZB42_3908	predicted ncRNA	-2.03	-4.09	0.02
RBAM_000010	*dnaA* - chromosomal replication initiatior protein	-2.15	-4.42	0.00
RBAM_025130	*folC* - folyl-polyglutamate synthetase involved in biosynthesis of folate	-2.16	-4.48	0.00
RBAM_009090	*katA* - vegetative catalase involved in detoxification (degradation) of hydrogen peroxide	-2.19	-4.57	0.00
RBAM_034560	hypothetical protein	-2.20	-4.59	0.01
RBAM_013050	*ispA* - major intracellular serine protease precursor (ISP-1) involved in protein degradation	-2.22	-4.65	0.00
RBAM_013890	*abbA* - anti-repressor involved in inhibition of AbrB	-2.22	-4.66	0.00
RBAM_036710	*iolH* - inositol utilization protein H involved in myo-inositol catabolism	-2.23	-4.68	0.00
RBAM_027670	*ytkA* - hypothetical protein	-2.25	-4.76	0.00
RBAM_006610	*ydjI* - conserved hypothetical protein	-2.30	-4.92	0.00
RBAM_011020	*yitJ* - conserved hypothetical protein	-2.46	-5.50	0.01
RBAM_036870	*des* - fatty acid desaturase involved in adaptation of membrane fluidity at low temperatures	-2.46	-5.50	0.00
RBAM_018030	*yndH* - hypothetical protein	-2.64	-6.23	0.00
B_amylo_FZB42_4014	predicted ncRNA	-2.85	-7.20	0.00

APPENDIX XIV: Bacterial up-regulated genes in the logarithmic phase by P-deficient maize root exudates

Gene ID	Gene and function	M	Fold-change	p-value
B_amylo_FZB42_3805	ncRNA	1.52	2.88	0.02
RBAM_021880	*yqjT* - conserved hypothetical protein	1.42	2.68	0.00
RBAM_034640	hypothetical protein	1.32	2.50	0.04
RBAM_027670	*ytkA* - hypothetical protein	1.31	2.48	0.00
RBAM_013450	*motA* - motility protein	1.25	2.38	0.03
RBAM_007430	putative ABC transporter permease	1.20	2.29	0.02
RBAM_034440	*arfM* - putative transcriptional regulator involved in regulation of anaerobic genes	1.13	2.20	0.00
RBAM_007740	hypothetical protein	1.13	2.19	0.03
RBAM_017570	hypothetical protein	1.12	2.17	0.00
RBAM_018880	*yobW* - hypothetical protein involved in sporulation	1.07	2.10	0.03
RBAM_034560	hypothetical protein	1.07	2.10	0.00
RBAM_000510	*ksgA* - dimethyladenosine transferase involved in resistance to kasugamycin	1.06	2.09	0.04
RBAM_000010	*dnaA* - chromosomal replication initiatior protein	1.05	2.07	0.00
B_amylo_FZB42_3992	ncRNA	1.04	2.06	0.00
RBAM_025990	*thrS* - threonyl-tRNA synthetase	0.98	1.97	0.04
RBAM_023810	*lepA* - GTP-binding protein	0.97	1.96	0.00
RBAM_008260	hypothetical protein	0.96	1.94	0.01
RBAM_019470	hypothetical protein	0.93	1.90	0.03
RBAM_006400	*ydiF* - putative ABC transporter ATP-binding	0.92	1.89	0.00
RBAM_016580	*spoVFB* - dipicolinate synthase subunit B involved in dipicolic acid production	0.92	1.89	0.04
RBAM_027830	*ytaB* - conserved hypothetical protein involved in survival of ethanol and salt stresses	0.85	1.80	0.01
RBAM_024930	*nifS* - cysteine desulfurase involved in NAD biosynthesis	0.84	1.80	0.05
RBAM_023370	*yqfZ* - conserved hypothetical protein	0.82	1.76	0.05
RBAM_019600	*cgeD* - spore maturation protein	0.82	1.76	0.01
RBAM_035720	*dltB* - D-alanine export protein involved in biosynthesis of teichoic acid	0.81	1.76	0.02
RBAM_037880	*ynaF* - conserved hypothetical protein	0.81	1.75	0.02
RBAM_026630	hypothetical protein	0.80	1.74	0.02

APPENDIX XV: Bacterial down-regulated genes in the logarithmic phase by P-deficient maize root exudates

Gene ID	Gene and function	M	Fold-change	p-value
RBAM_034710	*ywfO* - conserved hypothetical protein	-1.05	2.07	0.02

APPENDIX XVI: Bacterial up-regulated genes in the transitional phase by P-deficient maize root exudates

Gene ID	Gene and function	M	Fold-change	p-value
RBAM_031360	*yvbV* - conserved hypothetical protein	2.98	7.91	0.00
RBAM_009310	*yhcC* - hypothetical protein	1.75	3.37	0.03
RBAM_011580	*yjbJ* - putative lytic transglycosylase involved in cell wall turnover	1.75	3.37	0.01
RBAM_014130	*fruK* - fructose 1-phosphate kinase involved in fructose utilization	1.73	3.31	0.00
RBAM_014120	*fruR* - transcription repressor of fructose operon	1.67	3.19	0.00
RBAM_010560	*hemAT* - haem-based aerotactic transducer involved in movement towards oxygen	1.58	2.99	0.00
RBAM_032580	*flgM* - negative regulator of flagellin synthesis (Anti-sigma-D factor)	1.48	2.79	0.00
RBAM_033080	*rbsR* - ribose operon transcriptional repressor involved in the regulation of ribose utilization	1.45	2.73	0.00
RBAM_015890	*rnhB* - ribonuclease HI involved in endonucleolytic cleavage of RNA in RNA-DNA hybrid molecules	1.42	2.67	0.00
RBAM_032970	*ywtF* - putative transcriptional regulator	1.37	2.58	0.01
RBAM_029060	*besA* - trilactone hydrolase involved in iron acquisition	1.34	2.54	0.02
RBAM_000700	*yabQ* - hypothetical protein involved in sporulation	1.33	2.51	0.00
RBAM_036620	*yxdK* - two-component sensor histidine kinase involved in the regulation of the ABC transporter YxdL-YxdM	1.33	2.51	0.02
RBAM_032490	*fliS* - flagellar protein involved in motility and chemotaxis	1.31	2.48	0.00
RBAM_032550	*flgL* - flagellar hook-associated protein III (HAPIII) involved in motility and chemotaxis	1.23	2.35	0.00
RBAM_001560	*rplF* - ribosomal protein L6 (BL8) involved in translation	1.23	2.35	0.00
RBAM_017920	hypothetical protein	1.23	2.34	0.00
RBAM_033000	*pgdS* - gamma-DL-glutamyl hydrolase precursor involved in polyglutamic acid degradation	1.20	2.29	0.00
RBAM_006940	*purH* - inosine-monophosphate cyclohydrolase involved in purine biosynthesis	1.19	2.29	0.00
RBAM_013440	*motB* - motility protein	1.19	2.29	0.00
RBAM_002320	*glmS* - l-glutamine-D-fructose-6-phosphate amidotransferase involved in cell wall synthesis	1.19	2.28	0.00
RBAM_033100	*rbsD* - ribose ABC transporter (membrane protein) involved in ribose uptake	1.17	2.25	0.00

RBAM_004240	PTS mannitol-specific enzyme IIA component	1.14	2.20	0.00
RBAM_015680	*sdaAB* - L-serine dehydratase (beta chain) involved in serine utilization	1.10	2.15	0.01
RBAM_033120	*rbsC* - ribose ABC transporter (permease) involved in ribose uptake	1.08	2.12	0.00
RBAM_033090	*rbsK* - ribokinase involved in ribose utilization	1.07	2.10	0.00
RBAM_032560	*flgK* - flagellar hook-associated protein I (HAPI) involved in motility and chemotaxis	1.06	2.08	0.00
RBAM_017930	*ynfC* - hypothetical protein	1.03	2.05	0.00
RBAM_032570	*yvyG* - conserved hypothetical protein	1.03	2.04	0.00
RBAM_032480	*fliT* - flagellar protein involved in motility and chemotaxis	1.03	2.04	0.00
RBAM_024640	*yrvN* - conserved hypothetical protein	1.02	2.02	0.00
RBAM_025310	*leuD* - isopropylmalate isomerase small subunit involved in biosynthesis of leucine	1.01	2.01	0.00
RBAM_001530	*rplE* - ribosomal protein L5 (BL6) involved in translation	1.00	2.00	0.00
RBAM_032500	*fliD* - flagellar hook-associated protein II (HAPII) involved in motility and chemotaxis	1.00	2.00	0.00
RBAM_011880	ABC (ATP-binding cassette) transporter nucleotide-binding domain	0.99	1.99	0.00
RBAM_002110	*feuB* - iron-uptake system permease protein involved in iron acquisition	0.99	1.99	0.00
RBAM_001550	*rpsH* - ribosomal protein S8 (BS8) involved in translation	0.99	1.98	0.01
RBAM_001570	*rplR* - ribosomal protein L18 involved in translation	0.97	1.96	0.00
RBAM_001680	*rpoA* - RNA polymerase (alpha subunit) involved in transcription	0.96	1.94	0.00
RBAM_029080	*yuiG* - conserved hypothetical protein	0.95	1.94	0.02
RBAM_037980	*rpsR* - ribosomal protein S18 involved in translation	0.94	1.92	0.03
RBAM_001610	*secY* - preprotein translocase subunit involved in protein secretion	0.93	1.91	0.00
RBAM_011870	putative ABC-type multidrug transport system, permease	0.93	1.90	0.00
RBAM_001640	*infA* - translation initiation factor IF-I involved in translation	0.93	1.90	0.00
RBAM_025950	*ysbA* - conserved hypothetical protein	0.91	1.88	0.00
RBAM_025940	*ysbB* - antiholin-like protein	0.91	1.87	0.00
RBAM_031440	*gntP* - gluconate permease involved in gluconate uptake	0.88	1.85	0.00
RBAM_015370	*pyrD* - dihydroorotate dehydrogenase (catalytic subunit)involved in pyrimidine biosynthesis	0.88	1.84	0.01
RBAM_015820	*rpsP* - 30S ribosomal protein S16 involved in translation	0.87	1.83	0.01
RBAM_015380	*pyrF* - orotidine 5'-phosphate decarboxylase involved in pyrimidine biosynthesis	0.87	1.83	0.00
RBAM_016010	*flgB* - flagellar basal-body rod protein involved in motility and chemotaxis	0.87	1.82	0.00
RBAM_001520	*rplX* - ribosomal protein L24 (BL23) (histone-like protein HPB12) involved in translation	0.86	1.82	0.00
RBAM_036830	*yxcA* - hypothetical protein	0.86	1.82	0.04
RBAM_001510	*rplN* - ribosomal protein L14 involved in translation	0.86	1.82	0.00
RBAM_037500	*yycC* - hypothetical protein	0.85	1.81	0.01

APPENDIX XVII: Bacterial down-regulated genes in the transitional phase by P-deficient maize root exudates

Gene ID	Gene and function	M	Fold-change	p-value
RBAM_015730	*fabD* - malonyl CoA-acyl carrier protein transacylase	-0.86	-1.81	0.00
B_amylo_FZB42_3909	predicted ncRNA	-0.86	-1.81	0.02
RBAM_012790	*dppC* - dipeptide transport system permease protein involved in uptake of dipeptides	-0.86	-1.82	0.00
RBAM_019740	*uvrX* - UV-damage repair protein involved in DNA repair after UV damage	-0.87	-1.83	0.05
RBAM_036720	*iolG* - myo-inositol 2-dehydrogenase involved in myo-inositol catabolism	-0.87	-1.83	0.00
RBAM_022130	*yqjL* - putative hydrolase involved in resistence against paraquat	-0.88	-1.84	0.00
RBAM_016540	*ylxY* - putative deacetylase	-0.89	-1.86	0.01
RBAM_018540	*ggt* - gamma-glutamyltranspeptidase involved in degradation of poly-glutamate capsules	-0.90	-1.86	0.05
RBAM_011810	hypothetical protein	-0.90	-1.86	0.04
RBAM_022570	*recN* - DNA repair protein	-0.92	-1.89	0.00
RBAM_022330	*bkdAA* - branched-chain alpha-keto acid dehydrogenase E1 subunit (2-oxoisovalerate dehydrogenase alpha) involved in utilization of branched-chain keto acids	-0.92	-1.89	0.00
RBAM_004730	*gsiB* - general stress protein	-0.92	-1.90	0.00
RBAM_031200	hypothetical protein	-0.93	-1.90	0.03
RBAM_020440	*sspM* - small, acid-soluble spore protein involved in protection of spore DNA	-0.93	-1.90	0.00
RBAM_021700	*ansB* - aspartate ammonia-lyase involved in aspartate degradation	-0.94	-1.91	0.02
RBAM_013790	hypothetical protein	-0.94	-1.91	0.00
RBAM_000430	*yabB* - conserved hypothetical protein	-0.94	-1.92	0.04
B_amylo_FZB42_3884	predicted ncRNA	-0.94	-1.92	0.03
RBAM_009760	*sigM* - RNA polymerase ECF(extracytoplasmic function)-type sigma factor involved in resistance against cell envelope stress, oxidative stress and salt stress	-0.95	-1.93	0.03
B_amylo_FZB42_3895	predicted ncRNA	-0.96	-1.94	0.00
RBAM_025670	*yshB* - hypothetical protein	-0.97	-1.96	0.02
RBAM_003780	*tcyA* - cystine ABC transporter binding protein involved in cystine uptake	-0.98	-1.97	0.04
RBAM_015090	*murB* - UDP-N-acetylenolpyruvoylglucosamine reductase involved in peptidoglycan precursor biosynthesis	-0.98	-1.97	0.00
RBAM_009090	*katA* - vegetative catalase involved in detoxification (degradation) of hydrogen peroxide	-1.02	-2.03	0.00
RBAM_032010	*trxB* - thioredoxin reductase	-1.03	-2.04	0.01
RBAM_025290	*tig* - trigger factor (prolyl isomerase) involved in protein folding	-1.04	-2.06	0.00

RBAM_008570	*yfhE* - hypothetical protein involved in survival of salt and ethanol stresses and low temperatures	-1.06	-2.08	0.02
RBAM_019240	hypothetical protein	-1.06	-2.09	0.02
RBAM_025650	*mutSB* - DNA mismatch repair protein involved in DNA repair	-1.07	-2.10	0.01
RBAM_012470	*xlyB* - N-acetylmuramoyl-L-alanine amidase involved in PBSX prophage-mediated lysis	-1.07	-2.10	0.00
RBAM_013750	*ykwD* - conserved hypothetical protein	-1.08	-2.11	0.02
RBAM_021160	*ypbD* - conserved hypothetical protein	-1.08	-2.12	0.03
RBAM_012700	*ykaA* - conserved hypothetical protein	-1.09	-2.13	0.00
RBAM_029480	*yunF* - conserved hypothetical protein	-1.09	-2.13	0.04
RBAM_014630	*ylaG* - putative GTP-binding elongation factor	-1.10	-2.15	0.01
RBAM_029210	putative ABC-transporter ATP-binding protein	-1.12	-2.17	0.00
RBAM_020640	*dapB* - dihydrodipicolinate reductase involved in biosynthesis of lysine and peptidoglycan	-1.12	-2.18	0.01
RBAM_012410	*uxaB* - tagaturonate reductase (altronate oxidoreductase) involved in hexuronate utilization	-1.13	-2.19	0.04
RBAM_018620	*gltA* - glutamate synthase [NADPH] large subunit	-1.14	-2.21	0.01
B_amylo_FZB42_4038	predicted ncRNA	-1.17	-2.26	0.03
RBAM_020470	*ponA* - bifunctional glucosyl transferase/ transpeptidase penicillin-binding proteins IA/IB	-1.18	-2.27	0.01
RBAM_020630	*mgsA* - methylglyoxal synthase involved in bypass of glycolysis	-1.19	-2.28	0.05
RBAM_019440	*yodL* - hypothetical protein	-1.22	-2.34	0.03
RBAM_027250	*leuS* - leucyl-tRNA synthetase involved in translation	-1.24	-2.36	0.05
RBAM_036710	*iolH* - inositol utilization protein H involved in myo-inositol catabolism	-1.30	-2.47	0.04
RBAM_015960	*trmFO* - tRNA:m(5)U-54 methyltransferase involved in tRNA modification	-1.34	-2.54	0.04
RBAM_017340	*xylR* - xylose operon repressor protein involved in regulation of xylan and xylose utilization	-1.35	-2.55	0.04
RBAM_006180	putative transcriptional regulator (gntr family)	-1.36	-2.56	0.00
B_amylo_FZB42_3910	predicted ncRNA	-1.37	-2.58	0.00
RBAM_026380	*argG* - argininosuccinate synthase involved in biosynthesis of arginine	-1.39	-2.62	0.00
RBAM_018180	*bmyA* - bacillomycin D synthetase A involved in antibiotics production	-1.45	-2.73	0.03
RBAM_027410	*ytzC* - hypothetical protein	-1.45	-2.74	0.01
RBAM_023040	*comGB* - DNA transport machinery protein involved in competence, DNA uptake	-1.48	-2.78	0.04
RBAM_023450	*cshB* -.putative ATP-dependent RNA helicase	-1.48	-2.79	0.01
RBAM_019360	*bglA* - 6-phospho-beta-glucosidase involved in beta-glucoside utilization	-1.50	-2.83	0.00
RBAM_011340	*fabF* - beta-ketoacyl-acyl carrier protein synthase II involved in fatty acid biosynthesis	-1.58	-3.00	0.00
RBAM_008360	*malA* - maltose-6'-phosphate glucosid involved in maltose utilization	-1.59	-3.01	0.00
RBAM_004120	*ycnE* - conserved hypothetical protein	-1.60	-3.03	0.02
RBAM_012080	*penP* - beta-lactamase precursor involved in resistance to beta-lactam antibiotics	-1.64	-3.13	0.01
RBAM_018960	*yocH* - putative cell-wall binding protein	-1.66	-3.16	0.00

RBAM_021430	*ypuD* - hypothetical protein	-1.66	-3.16	0.02
RBAM_011020	*yitJ* - conserved hypothetical protein	-1.67	-3.18	0.01
RBAM_036870	*des* - fatty acid desaturase involved in adaptation of membrane fluidity at low temperatures	-1.67	-3.18	0.01
RBAM_017910	*parC* - DNA topoisomerase IV subunit A involved in chromosome segregation and compaction	-1.74	-3.33	0.01
RBAM_013910	*ccpC* - transcriptional repressor involved in regulation of tricarboxylic acid branch of the TCA cycle	-1.79	-3.45	0.00
RBAM_025130	*folC* - folyl-polyglutamate synthetase involved in biosynthesis of folate	-1.80	-3.49	0.05
RBAM_012010	hypothetical protein	-1.80	-3.49	0.01
RBAM_013050	*ispA* - major intracellular serine protease precursor involved in protein degradation	-1.91	-3.76	0.00
RBAM_017190	*nrdF* - ribonucleoside-diphosphate reductase beta subunit involved in synthesis of deoxyribonucleoside triphosphates	-1.94	-3.85	0.01
RBAM_036590	*yxeA* - hypothetical protein	-2.05	-4.14	0.00
B_amylo_FZB42_3830	predicted ncRNA	-2.29	-4.89	0.00
B_amylo_FZB42_3908	predicted ncRNA	-3.22	-9.29	0.00

APPENDIX XVIII: Bacterial up-regulated genes in the logarithmic phase by Fe-deficient maize root exudates

Gene ID	Gene and Function	M	Fold-change	p_value
RBAM_004030	hypothetical protein	1.83	3.55	0.01
RBAM_000330	*csfB* - hypothetical protein involved in the control of SigG activity	1.03	2.05	0.00
RBAM_012730	*ykcB* - conserved hypothetical protein	0.98	1.98	0.01
RBAM_018880	*yobW* - hypothetical protein involved in sporulation	0.94	1.91	0.01
RBAM_002460	*ybxG* - putative proline-specific permease	0.88	1.84	0.02
B_amylo_FZB42_3940	predicted ncRNA	0.82	1.77	0.03
RBAM_003840	*yclF* - putative di-tripeptide ABC transporter, permease	0.81	1.76	0.00

APPENDIX XIX: Bacterial up-regulated genes in the logarithmic phase by Fe-deficient maize root exudates

Gene ID	Gene and Function	M	Fold-change	p_value
RBAM_002540	*yolA1* - hypothetical protein	-0.98	1.97	0.03
B_amylo_FZB42_3947	predicted ncRNA	-0.88	1.84	0.04

APPENDIX XX: Bacterial up-regulated genes in the transitional phase by Fe-deficient maize root exudates

Gene ID	Gene and Function	M	Fold-change	p_value
RBAM_009310	*yhcC* - hypothetical protein	2.18	4.54	0.05
B_amylo_FZB42_3976	predicted ncRNA	2.09	4.26	0.01
RBAM_020820	*trpC* - indol-3-glycerol phosphate synthase involved in biosynthesis of tryptophan	1.73	3.31	0.00
RBAM_032970	*ywtF* - putative transcriptional regulator	1.59	3.01	0.00
RBAM_034790	hypothetical protein	1.40	2.65	0.05
RBAM_037980	*rpsR* - ribosomal protein S18 involved in translation	1.35	2.55	0.04
RBAM_006700	*cotA* - spore coat protein (outer) involved in resistance to the spore	1.28	2.43	0.00
RBAM_003930	*yxeN* - putative amino acid ABC transporter (permease)	1.26	2.39	0.02
RBAM_036390	*abn2* - endo-1,5-alpha-L-arabinosidase involved in arabinan degradation	1.25	2.38	0.00
RBAM_030200	*gerAC* - nutrient receptor involved in germination response to L-alanine	1.24	2.36	0.00
RBAM_019100	*yocS* - putative sodium-dependent transporter	1.22	2.34	0.05
RBAM_007100	*yerO* - putative transcription regulator (tetr/acrr family)	1.21	2.32	0.04
RBAM_016810	*ymdB* - conserved hypothetical protein	1.16	2.23	0.03
RBAM_002040	*gabT1* - 4-aminobutyrate aminotransferase	1.09	2.12	0.00
RBAM_025310	*leuD* - isopropylmalate isomerase small subunit involved in biosynthesis of leucine	1.07	2.10	0.00
RBAM_022900	*yqhH* - putative SNF2 helicase	1.05	2.08	0.00
RBAM_024250	*yybF1* - hypothetical transport protein	1.03	2.05	0.00
RBAM_002110	*feuB* - iron-uptake system permease protein involved in iron acquisition	1.01	2.02	0.00
RBAM_018780	*yoaF* - hypothetical protein YoaF	0.97	1.95	0.00
RBAM_027300	*bceB* - bacitracin export permease protein involved in bacitracin export	0.94	1.92	0.00
RBAM_023100	*mgsR* - transcriptional regulator which controls a subset of general stress genes	0.93	1.90	0.00
RBAM_015560	*fmt* - methionyl-tRNA formyltransferase involved in formylation of Met-tRNA(fMet)	0.93	1.90	0.00
RBAM_006520	hypothetical protein	0.92	1.90	0.01
RBAM_006840	*purE* - phosphoribosylaminoimidazole carboxylase involved in purine biosynthesis	0.92	1.90	0.00
RBAM_005430	*yrkE* - conserved hypothetical protein	0.92	1.89	0.04
RBAM_030670	*cadA* - cadmium transporting ATPase involved in cadmium export	0.91	1.87	0.01
RBAM_035400	*qoxD* - quinol oxidase subunit IV involved in respiration	0.90	1.87	0.00
RBAM_033490	*ywqB* - conserved hypothetical protein	0.88	1.85	0.00
RBAM_031130	*yvbI* - hypothetical protein	0.87	1.83	0.00
B_amylo_FZB42_3891	predicted ncRNA	0.87	1.82	0.03

RBAM_024270	*yrhI* - conserved hypothetical protein	0.86	1.81	0.05
RBAM_009270	*yhbI* - putative transcriptional regulator (MarR family)	0.86	1.81	0.01
RBAM_025000	*ysxB* - conserved hypothetical protein	0.85	1.81	0.01
RBAM_012970	*ohrB* - organic hydroperoxide resistance protein (General stress protein 17o) (Gsp17o)	0.85	1.80	0.00
RBAM_021770	*yqkD* - hypothetical protein	0.85	1.80	0.00

APPENDIX XXI: Bacterial down-regulated genes in the transitional phase by Fe-deficient maize root exudates

Gene ID	Gene and Function	M	Fold-change	p_value
RBAM018030	*yndH* - hypothetical protein	-3.52	-11.46	0.02
RBAM008360	*malA* - maltose-6'-phosphate glucosid involved in maltose utilization	-3.30	-9.83	0.00
B_amylo_FZB42_4014	predicted ncRNA	-2.91	-7.53	0.01
RBAM035760	*licH* - 6-phospho-beta-glucosidase involved in lichenan utilization	-2.73	-6.62	0.04
RBAM012080	*penP* - beta-lactamase precursor involved in resistance to beta-lactam antibiotics	-2.66	-6.31	0.00
RBAM019440	*yodL* - hypothetical protein	-2.61	-6.10	0.00
RBAM012150	*galK1* - galactokinase involved in galactose utilization	-2.45	-5.48	0.01
RBAM004120	*ycnE* - conserved hypothetical protein	-2.39	-5.23	0.00
RBAM035770	*licA* - phosphotransferase system (PTS) lichenan specific enzyme IIA component involved in lichenan uptake and phosphorylation	-2.34	-5.06	0.03
RBAM036710	*iolH* - inositol utilization protein H involved in myo-inositol catabolism	-2.27	-4.83	0.02
RBAM018960	*yocH* - putative cell-wall binding protein	-2.20	-4.59	0.02
RBAM030250	*liaH* - conserved hypothetical protein involved in protection against daptamycin	-2.11	-4.31	0.00
RBAM019360	*bglA* - 6-phospho-beta-glucosidase involved in beta-glucoside utilization	-2.09	-4.27	0.01
RBAM015710	*fapR* - transcription factor (Fatty acid and phospholipid biosynthesis regulator)	-2.00	-4.01	0.03
RBAM027670	*ytkA* - hypothetical protein	-2.00	-3.99	0.00
RBAM006550	*gutA* - probable glucitol transport protein	-1.92	-3.80	0.03
RBAM022920	*sinI* - sinR antagonist	-1.92	-3.79	0.01
RBAM011340	*fabF* - beta-ketoacyl-acyl carrier protein synthase II involved in fatty acid biosynthesis	-1.89	-3.70	0.05
RBAM012010	hypothetical protein	-1.88	-3.68	0.01
RBAM006610	*ydjI* - conserved hypothetical protein	-1.81	-3.50	0.01
RBAM006180	putative transcriptional regulator (gntr family)	-1.81	-3.50	0.00
RBAM026370	*argH* - argininosuccinate lyase involved in biosynthesis of arginine	-1.77	-3.42	0.01
RBAM020080	*degR* - positive effector of DegU-phosphate stability involved in control of DegU activity	-1.75	-3.36	0.00
RBAM032460	*yvyD* - conserved hypothetical protein required for survival at low temperatures	-1.75	-3.35	0.03
RBAM016830	*tdh* - L-threonine 3-dehydrogenase involved in threonine utilization	-1.74	-3.35	0.04
RBAM026150	*phoP* - two-component response regulator involved in regulation of phosphate metabolism	-1.72	-3.30	0.00
RBAM019060	*dhaS* - aldehyde dehydrogenase	-1.66	-3.17	0.00
RBAM030260	*liaI* - hypothetical protein	-1.65	-3.13	0.00
RBAM011020	*yitJ* - conserved hypothetical protein	-1.58	-2.99	0.00

RBAM012130	*galT1* - galactose-1-phosphate uridyltransferase involved in galactose utilization	-1.57	-2.96	0.05
RBAM020730	*ypiF* - hypothetical protein	-1.53	-2.88	0.01
RBAM016840	*kbl* - 2-amino-3-ketobutyrate coenzyme A ligase involved in threonine utilization	-1.52	-2.86	0.02
RBAM015190	*ylmC* - conserved hypothetical protein	-1.50	-2.83	0.01
RBAM019730	*rapA1* - response regulator aspartate phosphatase A involved in control of sporulation initiation	-1.50	-2.82	0.00
RBAM009090	*katA* - vegetative catalase involved in detoxification (degradation) of hydrogen peroxide	-1.50	-2.82	0.02
RBAM014220	*abh* - transition state regulator	-1.50	-2.82	0.00
B_amylo_FZB42_3873	predicted ncRNA	-1.48	-2.78	0.00
RBAM031450	*lutC* - conserved hypothetical protein involved in utilization of lactate	-1.47	-2.78	0.00
RBAM027190	*msmE* - ABC transporter probably melibiose uptake	-1.47	-2.76	0.03
RBAM013890	*abbA* - anti-repressor involved in inhibition of AbrB	-1.46	-2.76	0.00
RBAM013790	hypothetical protein	-1.44	-2.71	0.00
RBAM020170	*ypbS* - hypothetical protein	-1.43	-2.69	0.04
RBAM010180	*yhaL* - hypothetical protein involved in sporulation	-1.42	-2.68	0.02
RBAM012470	*xlyB* - N-acetylmuramoyl-L-alanine amidase involved in PBSX prophage-mediated lysis	-1.42	-2.68	0.00
RBAM007980	*treA* - trehalose-6-phosphate hydrolase involved in trehalose utilization	-1.39	-2.61	0.02
RBAM018540	*ggt* - gamma-glutamyltranspeptidase involved in degradation of poly-glutamate capsules	-1.36	-2.58	0.01
RBAM036870	*des* - fatty acid desaturase involved in adaptation of membrane fluidity at low temperatures	-1.36	-2.57	0.02
RBAM028110	*yuaG* - conserved ypothetical protein involved in the early stages of sporulation	-1.35	-2.55	0.00
RBAM036760	*iolC* - inositol utilization protein C involved in myo-inositol catabolism	-1.35	-2.54	0.03
RBAM034560	hypothetical protein	-1.34	-2.54	0.02
RBAM021290	*resA* - thiol-disulfide oxidoreductase involved in cytochrome c biogenesis	-1.34	-2.53	0.03
RBAM029400	*yutF* - conserved hypothetical protein	-1.33	-2.51	0.04
RBAM019170	*cwlS* - D,L-endopeptidase, peptidoglycan hydrolase involved in cell wall metabolism	-1.33	-2.51	0.00
RBAM021570	*spoIIAB* - anti-sigma F factor involved in control of sporulation	-1.33	-2.51	0.00
RBAM020640	*dapB* - dihydrodipicolinate reductase involved in biosynthesis of lysine and peptidoglycan	-1.33	-2.51	0.04
RBAM005410	*ydeB* - conserved hypothetical protein	-1.32	-2.49	0.00
RBAM032010	*trxB* - thioredoxin reductase	-1.32	-2.49	0.00
RBAM012790	*dppC* - dipeptide transport system permease protein involved in uptake of dipeptides	-1.31	-2.47	0.02
RBAM026510	*thiI* - putative thiamine biosynthesis protein	-1.30	-2.45	0.01
RBAM023140	*yqgV* - conserved hypothetical protein	-1.29	-2.44	0.04
RBAM003400	*ycbE* - galactarate/glucarate transporter in (proton symport) involved in glucarate uptake	-1.28	-2.42	0.00
RBAM017790	*sspO* - small acid-soluble spore protein involved in protection of spore DNA	-1.28	-2.42	0.03
RBAM013630	*ykvY* - conserved hypothetical protein	-1.25	-2.39	0.03

RBAM012200	*pgm1* - predicted phosphatase/phosphohexomutase involved in enzyme in glycolysis/gluconeogenesis	-1.25	-2.37	0.01
RBAM022350	*buk* - butyrate kinase involved in utilization of branched-chain keto acids	-1.25	-2.37	0.00
RBAM017340	*xylR* - xylose operon repressor protein involved in regulation of xylan and xylose utilization	-1.24	-2.37	0.03
RBAM036720	*iolG* - myo-inositol 2-dehydrogenase involved in myo-inositol catabolism	-1.23	-2.35	0.01
RBAM026760	putative transcriptional regulator	-1.23	-2.34	0.02
RBAM031470	*lutA* - lactate oxidase involved in lactate utilization	-1.21	-2.31	0.01
RBAM002150	*ybbD* - putative Beta-hexosaminidase	-1.19	-2.28	0.00
RBAM036350	*bglH* - beta-glucosidase involved in salicin utilization	-1.18	-2.26	0.04
RBAM003380	*tmrB* - ATP-binding membrane protein involved in resistance to tunicamycin	-1.18	-2.26	0.03
RBAM013050	*ispA* - major intracellular serine protease precursor (ISP-1) involved in protein degradation	-1.18	-2.26	0.01
RBAM012410	*uxaB* - tagaturonate reductase (altronate oxidoreductase) involved in hexuronate utilization	-1.17	-2.25	0.03
RBAM006140	*pbpE* - penicillin-binding endopeptidase	-1.17	-2.25	0.00
RBAM011860	*blm* - beta-lactamase II precursor (Penicillinase) (Cephalosporinase)	-1.15	-2.22	0.00
RBAM018210	*scoB* - succinyl CoA:3-oxoacid CoA-transferase (subunit B) involved in lipid metabolism	-1.15	-2.22	0.01
RBAM012000	hypothetical protein	-1.14	-2.20	0.00
RBAM001100	*mcsB* - modulation of CtsR repression protein involved in regulation of protein degradation	-1.13	-2.20	0.00
RBAM022130	*yqjL* - putative hydrolase involved in resistence against paraquat	-1.13	-2.19	0.01
RBAM022360	*bcd* - leucine dehydrogenase involved in utilization of branched-chain keto acids	-1.13	-2.18	0.01
RBAM005550	hypothetical protein	-1.13	-2.18	0.00
RBAM016750	*rodZ* - morphogenic protein required for cell shape determination	-1.12	-2.17	0.05
RBAM018180	*bmyA* - bacillomycin D synthetase A involved in antibiotics production	-1.11	-2.16	0.01
RBAM018930	*yocC* - hypothetical protein	-1.10	-2.14	0.00
RBAM018470	*dacC* - penicillin-binding carboxypeptidase	-1.10	-2.14	0.00
RBAM003200	*yceH* - putative toxic anion resistance protein	-1.10	-2.14	0.03
RBAM022310	*bkdB* - branched-chain alpha-keto acid dehydrogenase E2 subunit (lipoamide acyltransferase) involved in utilization of branched-chain keto acids	-1.09	-2.12	0.00
RBAM022330	*bkdAA* - branched-chain alpha-keto acid dehydrogenase E1 subunit (2-oxoisovalerate dehydrogenase alpha) involved in utilization of branched-chain keto acids	-1.07	-2.11	0.00
RBAM020710	*qcrB* - menaquinol-cytochrome c reductase cytochrome b subunit involved in respiration	-1.05	-2.06	0.00
RBAM021850	*yqzH* - hypothetical protein	-1.04	-2.06	0.04
RBAM026650	hypothetical protein	-1.04	-2.06	0.03
RBAM016860	*ymcA* - antagonist of biofilm repression by SinR involved in regulation of biofilm formation	-1.03	-2.04	0.00
RBAM020470	*ponA* - bifunctional glucosyl transferase/ transpeptidase penicillin-binding proteins IA/IB	-1.02	-2.02	0.04
B_amylo_FZB42_3849	predicted ncRNA	-1.01	-2.02	0.00

RBAM006400	*ydiF* - putative ABC transporter ATP-binding	-1.01	-2.02	0.01
RBAM011300	*med* - positive regulator of comK involved in regulation of competence	-1.00	-2.00	0.00
RBAM021030	cmk - cytidylate kinase involved in the synthesis of CTP and dCTP	-0.99	-1.99	0.00
RBAM013160	*ykoM* - putative transcriptional regulator (MarR family)	-0.99	-1.99	0.01
RBAM005560	conserved hypothetical protein	-0.99	-1.99	0.00
RBAM027240	*ytwF* - conserved hypothetical protein	-0.99	-1.98	0.04
RBAM027580	*pckA* - phosphoenolpyruvate carboxykinase involved in synthesis of phosphoenolpyruvate	-0.97	-1.96	0.00
RBAM022970	*yqzG* - hypothetical protein	-0.96	-1.95	0.04
RBAM008350	*sspH* - small, acid-soluble spore protein involved in protection of spore DNA	-0.96	-1.95	0.04
RBAM024200	*manP* - phosphotransferase system (PTS) mannose-specificenzyme IIBCA component involved in mannose uptake and phosphorylation, control of ManR activity	-0.95	-1.94	0.00
RBAM031740	*yveG* - hypothetical protein	-0.95	-1.93	0.00
RBAM001110	*clpC* - class III stress response-related ATPase involved in protein degradation	-0.95	-1.93	0.00
RBAM008700	*fhO* - hypothetical protein	-0.94	-1.92	0.00
RBAM004730	*gsiB* - general stress protein	-0.92	-1.90	0.00
RBAM024210	*manR* - mannose operon Transcriptional antiterminator involved in regulation of mannose utilization	-0.92	-1.89	0.05
RBAM004110	*ycnD* - NADPH-flavin oxidoreductase involved in delivery of FMN to enzymes	-0.91	-1.89	0.00
RBAM002170	*murP* - N-acetyl muramic acid-specific phosphotransferase system, EIIBC component involved in N-acetyl muramic acid uptake and phosphorylation	-0.91	-1.88	0.00
RBAM004040	*yclM* - aspartokinase III with unknown function	-0.90	-1.87	0.00
RBAM013620	*zosA* - P-type zinc-transporting ATPase involved in zinc uptake	-0.90	-1.86	0.02
RBAM022370	*ptb* - phosphate butyryltransferase involved in utilization of branched-chain keto acids	-0.90	-1.86	0.00
B_amylo_FZB42_3895	predicted ncRNA	-0.89	-1.85	0.00
RBAM022960	*yqxM* - hypothetical protein involved in formation of biofilms and fruiting bodies	-0.87	-1.83	0.02
RBAM011540	*pepF* - oligoendopeptidase involved in protein degradation	-0.87	-1.83	0.00
RBAM020410	*yppF* - hypothetical protein	-0.87	-1.82	0.00
RBAM028990	*yukJ* - conserved hypothetical protein	-0.86	-1.82	0.02
RBAM036590	*yxeA* - hypothetical protein	-0.86	-1.82	0.04
RBAM017640	hypothetical protein	-0.85	-1.81	0.03
RBAM012310	*yjjA* - conserved hypothetical protein	-0.85	-1.80	0.01
RBAM010120	*yhaR* - putative enoyl CoA hydratase	-0.85	-1.80	0.04
RBAM003760	*tcyC* - putative ABC-type transport protein, ATPase component involved in cystine uptake	-0.85	-1.80	0.00

APPENDIX XXII: Bacterial up-regulated genes in the logarithmic phase by K-deficient maize root exudates

Gene ID	Gene and Function	M	Fold-change	p-value
RBAM_034640	hypothetical protein	1.40	2.64	0.02
RBAM_019990	*ypiP* - conserved hypothetical protein	1.35	2.54	0.03
RBAM_005370	putative transcriptional regulator	1.01	2.02	0.00
B_amylo_FZB42_3984	predicted ncRNA	0.99	1.99	0.01
B_amylo_FZB42_3912	predicted ncRNA	0.93	1.90	0.00
RBAM_005560	conserved hypothetical protein	0.93	1.90	0.04
B_amylo_FZB42_3943	predicted ncRNA	0.92	1.89	0.01
RBAM_003290	*ldh* - l-lactate dehydrogenase involved in overflow metabolism, fermentation	0.90	1.86	0.00
RBAM_012350	*yjlB* - conserved hypothetical protein	0.87	1.83	0.00
RBAM_008370	*glvR* - HTH-type transcriptional regulator involved in regulation of maltose utilization	0.86	1.82	0.04
RBAM_003860	*yczF* - hypothetical protein	0.86	1.81	0.00
B_amylo_FZB42_4034	predicted ncRNA	0.85	1.81	0.02
RBAM_006780	hypothetical protein	0.83	1.77	0.00
RBAM_019010	*yozN* - hypothetical protein	0.82	1.77	0.00
RBAM_012280	*yjgD* - conserved hypothetical protein involved in survival to ethanol stress	0.82	1.77	0.02
RBAM_037880	*ynaF* - conserved hypothetical protein	0.82	1.76	0.01
RBAM_008260	hypothetical protein	0.81	1.76	0.01
RBAM_005290	*ydeM* - conserved hypothetical protein	0.80	1.74	0.03
RBAM_005630	*ykkA* - hypothetical protein	0.80	1.74	0.02

APPENDIX XXIII: Bacterial up-regulated genes in the transitional phase by K-deficient maize root exudates

Gene ID	Gene and Function	M	Fold-change	p-value
B_amylo_FZB42_3875	predicted ncRNA	2.62	6.16	0.02
RBAM_009310	*yhcC* - hypothetical protein	1.97	3.92	0.02
RBAM_020820	*trpC* - indol-3-glycerol phosphate synthase involved in biosynthesis of tryptophan	1.69	3.24	0.00
RBAM_037980	*rpsR* - ribosomal protein S18 involved in translation	1.41	2.66	0.00
RBAM_024270	*fatR* - transcriptional repressor	1.19	2.28	0.02
RBAM_006700	*cotA* - spore coat protein (outer) involved in resistance of the spore	1.07	2.10	0.01
RBAM_024250	*yybF1* - hypothetical transport protein	0.92	1.89	0.01
RBAM_025310	*leuD* - isopropylmalate isomerase small subunit involved in biosynthesis of leucine	0.89	1.85	0.00
RBAM_034350	*uvsE* - UV DNA damage endonuclease	0.88	1.84	0.01
RBAM_000700	*yabQ* - hypothetical protein involved in sporulation	0.87	1.83	0.04
RBAM_030200	*gerAC* - nutrient receptor involved in germination response to L-alanine	0.87	1.83	0.00
RBAM_002040	*gabT1* - 4-aminobutyrate aminotransferase involved in utilization of gamma-amino butyric acid	0.86	1.82	0.00

APPENDIX XXIV: Bacterial down-regulated genes in the transitional phase by K-deficient maize root exudates

Gene ID	Gene and Function	M	Fold-change	p-value
RBAM_028990	*yukJ* - conserved hypothetical protein	-0.87	-1.83	0.02
RBAM_019730	*rapA1* - response regulator aspartate phosphatase A involved in control of sporulation initiation	-0.87	-1.83	0.04
RBAM_036720	*iolG* - myo-inositol 2-dehydrogenase involved in myo-inositol catabolism	-0.87	-1.83	0.02
RBAM_013620	*zosA* - P-type zinc-transporting ATPase involved in zinc uptake	-0.88	-1.85	0.04
RBAM_002420	*ybdO1* - hypothetical protein	-0.89	-1.86	0.03
RBAM_008700	*fhO* - hypothetical protein	-0.89	-1.86	0.00
RBAM_028980	*ald* - alanine dehydrogenase involved in alanine utilization	-0.91	-1.87	0.05
RBAM_031740	*yveG* - hypothetical protein	-0.91	-1.87	0.00
RBAM_034560	hypothetical protein	-0.93	-1.90	0.02
RBAM_022310	*bkdB* - branched-chain alpha-keto acid dehydrogenase E2 subunit (lipoamide acyltransferase) involved in utilization of branched-chain keto acids	-0.95	-1.93	0.04
RBAM_012280	*yjgD* - conserved hypothetical protein involved in survival to ethanol stress	-0.95	-1.93	0.04
RBAM_011300	*med* - positive regulator of comK involved in regulation of competence	-0.95	-1.94	0.00
RBAM_032010	*trxB* - thioredoxin reductase	-0.96	-1.95	0.00
RBAM_026370	*argH* - argininosuccinate lyase involved in biosynthesis of arginine	-0.96	-1.95	0.05
RBAM_022330	*bkdAA* - branched-chain alpha-keto acid dehydrogenase E1 subunit (2-oxoisovalerate dehydrogenase alpha) involved in utilization of branched-chain keto acids	-0.97	-1.95	0.04
RBAM_019970	*thyB* - thymidylate synthase B involved in biosynthesis of thymidine nucleotides	-0.98	-1.98	0.02
RBAM_016060	*fliH* - flagellar assembly protein involved in motility and chemotaxis	-0.99	-1.99	0.02
RBAM_017910	*parC* - DNA topoisomerase IV subunit A involved in chromosome segregation and compaction	-1.00	-1.99	0.05
RBAM_007870	*yflI* - hypothetical protein	-1.01	-2.01	0.04
RBAM_031450	*lutC* - conserved hypothetical protein involved in utilization of lactate	-1.01	-2.01	0.04
RBAM_024200	*manP* - phosphotransferase system (PTS) mannose-specificenzyme IIBCA component involved in mannose uptake and phosphorylation, control of ManR activity	-1.03	-2.04	0.03
RBAM_016840	*kbl* - 2-amino-3-ketobutyrate coenzyme A ligase involved in threonine utilization	-1.05	-2.07	0.05
RBAM_013310	*kinE* - two-component sensor histidine kinase homolog involved in initiation of sporulation	-1.06	-2.08	0.05
RBAM_018210	*scoB* - succinyl CoA:3-oxoacid CoA-transferase (subunit B) involved in lipid metabolism	-1.06	-2.09	0.03
RBAM_017720	*ynzD* - Spo0A-P phosphatase involved in control of sporulation initiation	-1.07	-2.10	0.02
RBAM_020170	*ypbS* - hypothetical protein	-1.07	-2.10	0.02

RBAM_019060	*dhaS* - aldehyde dehydrogenase	-1.08	-2.12	0.04
RBAM_036760	*iolC* - inositol utilization protein C involved in myo-inositol catabolism	-1.09	-2.13	0.04
RBAM_022350	*buk* - butyrate kinase involved in utilization of branched-chain keto acids	-1.13	-2.18	0.01
RBAM_021930	*proI* - pyrroline-5-carboxylate reductase II involved in biosynthesis of proline	-1.13	-2.18	0.04
RBAM_019240	hypothetical protein	-1.14	-2.21	0.02
RBAM_020470	*ponA* - bifunctional glucosyl transferase/ transpeptidase penicillin-binding proteins IA/IB	-1.14	-2.21	0.01
RBAM_013230	*rsgI* - anti-sigma factor involved in control of SigI activity	-1.16	-2.23	0.02
RBAM_013910	*ccpC* - transcriptional repressor involved in regulation of tricarboxylic acid branch of the TCA cycle	-1.17	-2.25	0.02
RBAM_012470	*xlyB* - N-acetylmuramoyl-L-alanine amidase involved in PBSX prophage-mediated lysis	-1.17	-2.25	0.02
RBAM_011340	*fabF* - beta-ketoacyl-acyl carrier protein synthase II involved in fatty acid biosynthesis	-1.19	-2.29	0.01
RBAM_036590	*yxeA* - hypothetical protein	-1.22	-2.32	0.03
RBAM_019170	*cwlS* - D,L-endopeptidase, peptidoglycan hydrolase involved in cell wall metabolism	-1.24	-2.36	0.00
RBAM_036640	*mrsK2* - putative sensor histidine kinase	-1.25	-2.37	0.03
RBAM_012790	*dppC* - dipeptide transport system permease protein involved in uptake of dipeptides	-1.27	-2.41	0.01
RBAM_023170	*glcK* - glucose kinase involved in phosphorylation of the free glucose moiety of di-and oligosaccharides	-1.28	-2.44	0.04
RBAM_006180	putative transcriptional regulator (gntr family)	-1.32	-2.49	0.00
RBAM_017650	*lexA* - negative transcriptional regulator of the SOS regulon involved in regulation of DNA damage repair	-1.34	-2.53	0.04
RBAM_004040	*yclM* - aspartokinase III with unknown function	-1.34	-2.53	0.04
RBAM_018930	*yocC* - hypothetical protein	-1.36	-2.56	0.02
RBAM_004120	*ycnE* - conserved hypothetical protein	-1.41	-2.66	0.04
RBAM_027410	*ytzC* - hypothetical protein	-1.44	-2.71	0.02
RBAM_013790	hypothetical protein	-1.44	-2.72	0.00
RBAM_018960	*yocH* - putative cell-wall binding protein	-1.45	-2.73	0.00
RBAM_004730	*gsiB* - general stress protein	-1.47	-2.78	0.03
RBAM_012410	*uxaB* - tagaturonate reductase (altronate oxidoreductase) involved in hexuronate utilization	-1.49	-2.81	0.02
RBAM_020640	*dapB* - dihydrodipicolinate reductase involved in biosynthesis of lysine and peptidoglycan	-1.52	-2.87	0.01
RBAM_027670	*ytkA* - hypothetical protein	-1.58	-3.00	0.02
RBAM_018470	*dacC* - penicillin-binding carboxypeptidase	-1.65	-3.14	0.03
RBAM_036870	*des* - fatty acid desaturase involved in adaptation of membrane fluidity at low temperatures	-1.68	-3.21	0.03
RBAM_012150	*galK1* - galactokinase involved in galactose utilization	-1.69	-3.24	0.05
B_amylo_FZB42_3830	predicted ncRNA	-1.71	-3.26	0.01
RBAM_017510	hypothetical protein	-1.71	-3.27	0.03

RBAM_003400	*ycbE* - galactarate/glucarate transporter in (proton symport) involved in glucarate uptake	-1.71	-3.28	0.03
RBAM_030260	*liaI* - hypothetical protein	-1.74	-3.34	0.00
RBAM_025130	*folC* - folyl-polyglutamate synthetase involved in biosynthesis of folate	-1.86	-3.64	0.01
RBAM_008360	*malA* - 6-phospho-alpha-glucosidase involved in maltose utilization	-1.87	-3.64	0.03
RBAM_027250	*leuS* - leucyl-tRNA synthetase involved in translation	-1.88	-3.67	0.02
RBAM_021570	*spoIIAB* - anti-sigma F factor involved in control of sporulation	-1.92	-3.79	0.04
RBAM_021700	*ansB* - aspartate ammonia-lyase involved in aspartate degradation	-1.92	-3.79	0.01
RBAM_012080	*penP* - beta-lactamase precursor involved in resistance to beta-lactam antibiotics	-1.97	-3.92	0.00
B_amylo_FZB42_3873	predicted ncRNA	-1.98	-3.96	0.01
RBAM_015190	*ylmC* - conserved hypothetical protein	-2.00	-3.99	0.00
RBAM_006610	*ydjI* - conserved hypothetical protein	-2.15	-4.43	0.00
RBAM_011020	*yitJ* - conserved hypothetical protein	-2.20	-4.58	0.00
RBAM_020080	*degR* - positive effector of DegU-phosphate stability involved in control of DegU activity	-2.29	-4.89	0.02
RBAM_019440	*yodL* - hypothetical protein	-2.31	-4.97	0.01
RBAM_014220	*abh* - transition state regulator	-2.42	-5.34	0.05
RBAM_030250	*liaH* - conserved hypothetical protein involved in protection against daptamycin	-2.47	-5.53	0.00

APPENDIX XXV: Shared up-regulated bacterial genes in the logarithmic phase by N- and P-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	B_amylo_FZB42_3992	predicted ncRNA	0.89	1.85	0.00
P	B_amylo_FZB42_3992	predicted ncRNA	1.04	2.06	0.00
N	RBAM_007430	putative ABC transporter permease	1.05	2.08	0.01
P	RBAM_007430	putative ABC transporter permease	1.20	2.29	0.02
N	RBAM_027830	*ytaB* - conserved hypothetical protein involved in survival of ethanol and salt stresses	1.18	2.27	0.00
P	RBAM_027830	*ytaB* - conserved hypothetical protein involved in survival of ethanol and salt stresses	0.85	1.80	0.01
N	RBAM_034560	hypothetical protein	0.80	1.74	0.01
P	RBAM_034560	hypothetical protein	1.07	2.10	0.00
N	RBAM_017570	hypothetical protein	1.36	2.57	0.03
P	RBAM_017570	hypothetical protein	1.12	2.17	0.00
N	RBAM_021880	*yqjT* - conserved hypothetical protein	1.33	2.51	0.00
P	RBAM_021880	*yqjT* - conserved hypothetical protein	1.42	2.68	0.00
N	RBAM_034440	*arfM* - putative transcriptional regulator involved in regulation of anaerobic genes	0.85	1.80	0.00
P	RBAM_034440	*arfM* - putative transcriptional regulator involved in regulation of anaerobic genes	1.13	2.20	0.00

APPENDIX XXVI: Shared up-regulated bacterial genes in the logarithmic phase by N- and Fe-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	B_amylo_FZB42_3940	predicted ncRNA	1.06	2.08	0.01
Fe	B_amylo_FZB42_3940	predicted ncRNA	0.82	1.77	0.03

APPENDIX XXVII: Shared up-regulated bacterial genes in the logarithmic phase by N- and K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	RBAM_012350	*yjlB* - conserved hypothetical protein	0.95	1.93	0.00
K	RBAM_012350	*yjlB* - conserved hypothetical protein	0.87	1.83	0.00
N	RBAM_019990	*ypiP* - conserved hypothetical protein	1.16	2.23	0.03
K	RBAM_019990	*ypiP* - conserved hypothetical protein	1.35	2.54	0.03
N	B_amylo_FZB42_3984	predicted ncRNA	1.00	2.01	0.01
K	B_amylo_FZB42_3984	predicted ncRNA	0.99	1.99	0.01
N	RBAM_003860	*yczF* - hypothetical protein	0.81	1.75	0.01
K	RBAM_003860	*yczF* - hypothetical protein	0.86	1.81	0.00
N	RBAM_019010	*yozN* - hypothetical protein	0.93	1.91	0.00
K	RBAM_019010	yozN - hypothetical protein	0.82	1.77	0.00

APPENDIX XXVIII: Shared up-regulated bacterial genes in the logarithmic phase by P- and Fe-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
P	RBAM_018880	*yobW* - hypothetical protein involved in sporulation	1.07	2.10	0.03
Fe	RBAM_018880	*yobW* - hypothetical protein involved in sporulation	0.94	1.91	0.01

APPENDIX XXIX: Shared up-regulated bacterial genes in the logarithmic phase by P- and K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
P	RBAM_037880	*ynaF* - conserved hypothetical protein	0.81	1.75	0.02
K	RBAM_037880	*ynaF* - conserved hypothetical protein	0.82	1.76	0.01

APPENDIX XXX: Shared up-regulated bacterial genes in the logarithmic phase by N-, P- and K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	RBAM_008260	hypothetical protein	1.03	2.04	0.02
P	RBAM_008260	hypothetical protein	0.96	1.94	0.01
K	RBAM_008260	hypothetical protein	0.81	1.76	0.01
N	RBAM_034640	hypothetical protein	1.24	2.35	0.00
P	RBAM_034640	hypothetical protein	1.32	2.50	0.04
K	RBAM_034640	hypothetical protein	1.40	2.64	0.02

APPENDIX XXXI: Shared up-regulated bacterial genes in the transitional phase by N- and P-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	RBAM_015820	*rpsP* - 30S ribosomal protein S16 involved in translation	0.87	1.83	0.00
P	RBAM_015820	*rpsP* - 30S ribosomal protein S16 involved in translation	0.87	1.83	0.01
N	RBAM_015380	*pyrF* - orotidine 5'-phosphate decarboxylase involved in pyrimidine biosynthesis	0.95	1.93	0.00
P	RBAM_015380	*pyrF* - orotidine 5'-phosphate decarboxylase involved in pyrimidine biosynthesis	0.87	1.83	0.00
N	RBAM_015370	*pyrD* - dihydroorotate dehydrogenase (catalytic subunit) involved in pyrimidine biosynthesis	1.02	2.02	0.00
P	RBAM_015370	*pyrD* - dihydroorotate dehydrogenase (catalytic subunit)involved in pyrimidine biosynthesis	0.88	1.84	0.01
N	RBAM_002320	*glmS* - l-glutamine-D-fructose-6-phosphate amidotransferase involved in cell wall synthesis	1.14	2.20	0.00
P	RBAM_002320	*glmS* - l-glutamine-D-fructose-6-phosphate amidotransferase involved in cell wall synthesis	1.19	2.28	0.00
N	RBAM_001560	*rplF* - ribosomal protein L6 (BL8) involved in translation	1.13	2.19	0.00
P	RBAM_001560	*rplF* - ribosomal protein L6 (BL8) involved in translation	1.23	2.35	0.00
N	RBAM_001550	*rpsH* - ribosomal protein S8 (BS8) involved in translation	0.91	1.89	0.00
P	RBAM_001550	*rpsH* - ribosomal protein S8 (BS8) involved in translation	0.99	1.98	0.01
N	RBAM_001530	*rplE* - ribosomal protein L5 (BL6) involved in translation	0.96	1.95	0.00
P	RBAM_001530	*rplE* - ribosomal protein L5 (BL6) involved in translation	1.00	2.00	0.00
N	RBAM_001510	*rplN* - ribosomal protein L14 involved in translation	0.97	1.96	0.00
P	RBAM_001510	*rplN* - ribosomal protein L14 involved in translation	0.86	1.82	0.00

APPENDIX XXXII: Shared up-regulated bacterial genes in the transitional phase by N- and Fe-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	RBAM_027300	*bceB* - bacitracin export permease protein involved in bacitracin export	1.01	2.01	0.00
Fe	RBAM_027300	*bceB* - bacitracin export permease protein involved in bacitracin export	0.94	1.92	0.00
N	B_amylo_FZB42_3976	predicted ncRNA	1.37	2.58	0.00
Fe	B_amylo_FZB42_3976	predicted ncRNA	2.09	4.26	0.01

APPENDIX XXXIII: Shared up-regulated bacterial genes in the transitional phase by P- and Fe-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
P	RBAM_032970	*ywtF* - putative transcriptional regulator	1.37	2.58	0.01
Fe	RBAM_032970	*ywtF* - putative transcriptional regulator	1.59	3.01	0.00
P	RBAM_002110	*feuB* - iron-uptake system permease protein involved in iron acquisition	0.99	1.99	0.00
Fe	RBAM_002110	*feuB* - iron-uptake system permease protein involved in iron acquisition	1.01	2.02	0.00

APPENDIX XXXIV: Shared up-regulated bacterial genes in the transitional phase by P- and K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
P	RBAM_000700	*yabQ* - hypothetical protein involved in sporulation	1.33	2.51	0.00
K	RBAM_000700	*yabQ* - hypothetical protein involved in sporulation	0.87	1.83	0.04

APPENDIX XXXV: Shared up-regulated bacterial genes in the transitional phase by Fe- and K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
Fe	RBAM_024270	*yrhI* - conserved hypothetical protein	0.86	1.81	0.05
K	RBAM_024270	*yrhI* - conserved hypothetical protein	1.19	2.28	0.02

APPENDIX XXXVI: Shared up-regulated bacterial genes in the transitional phase by N-, Fe- and K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	RBAM_030200	*gerAC* - nutrient receptor involved in germination response to L-alanine	0.86	1.81	0.00
Fe	RBAM_030200	*gerAC* - nutrient receptor involved in germination response to L-alanine	1.24	2.36	0.00
K	RBAM_030200	*gerAC* - nutrient receptor involved in germination response to L-alanine	0.87	1.83	0.00
N	RBAM_024250	*yybF1* - hypothetical transport protein	1.12	2.18	0.00
Fe	RBAM_024250	*yybF1* - hypothetical transport protein	1.03	2.05	0.00
K	RBAM_024250	*yybF1* - hypothetical transport protein	0.92	1.89	0.01
N	RBAM_006700	*cotA* - spore coat protein (outer) involved in resistance of the spore	1.03	2.04	0.00
Fe	RBAM_006701	*cotA* - spore coat protein (outer) involved in resistance of the spore	1.28	2.43	0.00
K	RBAM_006702	*cotA* - spore coat protein (outer) involved in resistance of the spore	1.07	2.10	0.01
N	RBAM_002040	*gabT1* - 4-aminobutyrate aminotransferase involved in utilization of gamma-amino butyric acid	1.01	2.01	0.00
Fe	RBAM_002041	*gabT1* - 4-aminobutyrate aminotransferase involved in utilization of gamma-amino butyric acid	1.09	2.12	0.00
K	RBAM_002042	*gabT1* - 4-aminobutyrate aminotransferase involved in utilization of gamma-amino butyric acid	0.86	1.82	0.00

APPENDIX XXXVII: Shared up-regulated bacterial genes in the transitional phase by P-, Fe- and K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
P	RBAM_025310	*leuD* - isopropylmalate isomerase small subunit involved in biosynthesis of leucine	1.01	2.01	0.00
Fe	RBAM_025310	*leuD* - isopropylmalate isomerase small subunit involved in biosynthesis of leucine	1.07	2.10	0.00
K	RBAM_025310	*leuD* - isopropylmalate isomerase small subunit involved in biosynthesis of leucine	0.89	1.85	0.00
P	RBAM_009310	*yhcC* - hypothetical protein	1.75	3.37	0.03
Fe	RBAM_009311	*yhcC* - hypothetical protein	2.18	4.54	0.05
K	RBAM_009312	*yhcC* - hypothetical protein	1.97	3.92	0.02

APPENDIX XXXVIII: Shared up-regulated bacterial genes in the transitional phase by N-, P-, Fe- and K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	RBAM_037980	*rpsR* - ribosomal protein S18 involved in translation	1.16	2.23	0.00
P	RBAM_037981	*rpsR* - ribosomal protein S18 involved in translation	0.94	1.92	0.03
Fe	RBAM_037982	*rpsR* - ribosomal protein S18 involved in translation	1.35	2.55	0.04
K	RBAM_037983	*rpsR* - ribosomal protein S18 involved in translation	1.41	2.66	0.00

APPENDIX XXXIX: Shared down-regulated bacterial genes in the logarithmic phase by N- and P -deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	B_amylo_FZB42_3908	predicted ncRNA	-2.03	-4.09	0.02
P	B_amylo_FZB42_3908	predicted ncRNA	-3.22	-9.29	0.00
N	B_amylo_FZB42_3909	predicted ncRNA	-0.93	-1.91	0.02
P	B_amylo_FZB42_3909	predicted ncRNA	-0.86	-1.81	0.02
N	B_amylo_FZB42_3910	predicted ncRNA	-1.84	-3.59	0.00
P	B_amylo_FZB42_3910	predicted ncRNA	-1.37	-2.58	0.00
N	RBAM_000430	*yabB* - conserved hypothetical protein	-1.72	-3.30	0.01
P	RBAM_000430	*yabB* - conserved hypothetical protein	-0.94	-1.92	0.04
N	RBAM_012700	*ykaA* - conserved hypothetical protein	-0.99	-1.98	0.00
P	RBAM_012700	*ykaA* - conserved hypothetical protein	-1.09	-2.13	0.00
N	RBAM_021160	*ypbD* - conserved hypothetical protein	-0.89	-1.85	0.04
P	RBAM_021160	*ypbD* - conserved hypothetical protein	-1.08	-2.12	0.03
N	RBAM_021430	*ypuD* - hypothetical protein	-1.77	-3.41	0.01
P	RBAM_021430	*ypuD* - hypothetical protein	-1.66	-3.16	0.02
N	RBAM_023450	*cshB* - putative ATP-dependent RNA helicase	-1.04	-2.05	0.03
P	RBAM_023450	*cshB* -.putative ATP-dependent RNA helicase	-1.48	-2.79	0.01
N	RBAM_025670	*yshB* - hypothetical protein	-1.21	-2.31	0.00
P	RBAM_025670	*yshB* - hypothetical protein	-0.97	-1.96	0.02

APPENDIX XL: Shared down-regulated bacterial genes in the logarithmic phase by N- and Fe -deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	B_amylo_FZB42_3849	predicted ncRNA	-0.96	-1.95	0.00
Fe	B_amylo_FZB42_3849	predicted ncRNA	-1.01	-2.02	0.00
N	B_amylo_FZB42_4014	predicted ncRNA	-2.85	-7.20	0.00
Fe	B_amylo_FZB42_4014	predicted ncRNA	-2.91	-7.53	0.01
N	RBAM_001100	*mcsB* - modulation of CtsR repression protein involved in regulation of protein degradation	-1.05	-2.07	0.00
Fe	RBAM_001100	*mcsB* - modulation of CtsR repression protein involved in regulation of protein degradation	-1.13	-2.20	0.00
N	RBAM_001110	*clpC* - class III stress response-related ATPase involved in protein degradation	-1.02	-2.03	0.00
Fe	RBAM_001110	*clpC* - class III stress response-related ATPase involved in protein degradation	-0.95	-1.93	0.00
N	RBAM_002150	*ybbD* - putative Beta-hexosaminidase	-0.99	-1.99	0.01
Fe	RBAM_002150	*ybbD* - putative Beta-hexosaminidase	-1.19	-2.28	0.00
N	RBAM_005410	*ydeB* - conserved hypothetical protein	-0.94	-1.92	0.00
Fe	RBAM_005410	*ydeB* - conserved hypothetical protein	-1.32	-2.49	0.00
N	RBAM_005550	hypothetical protein	-1.17	-2.25	0.00
Fe	RBAM_005550	hypothetical protein	-1.13	-2.18	0.00
N	RBAM_005560	conserved hypothetical protein	-1.11	-2.15	0.00
Fe	RBAM_005560	conserved hypothetical protein	-0.99	-1.99	0.00
N	RBAM_007980	*treA* - trehalose-6-phosphate hydrolase involved in trehalose utilization	-0.95	-1.94	0.00
Fe	RBAM_007980	*treA* - trehalose-6-phosphate hydrolase involved in trehalose utilization	-1.39	-2.61	0.02
N	RBAM_011860	*blm* - beta-lactamase II precursor (Penicillinase) (Cephalosporinase)	-1.43	-2.69	0.00
Fe	RBAM_011860	*blm* - beta-lactamase II precursor (Penicillinase) (Cephalosporinase)	-1.15	-2.22	0.00
N	RBAM_012000	hypothetical protein	-1.71	-3.27	0.01
Fe	RBAM_012000	hypothetical protein	-1.14	-2.20	0.00
N	RBAM_013160	*ykoM* - putative transcriptional regulator (MarR family)	-1.01	-2.01	0.00
Fe	RBAM_013160	*ykoM* - putative transcriptional regulator (MarR family)	-0.99	-1.99	0.01
N	RBAM_013890	*abbA* - anti-repressor involved in inhibition of AbrB	-2.22	-4.66	0.00
Fe	RBAM_013890	*abbA* - anti-repressor involved in inhibition of AbrB	-1.46	-2.76	0.00
N	RBAM_015710	*fapR* - transcription factor (Fatty acid and phospholipid biosynthesis regulator)	-1.55	-2.92	0.00
Fe	RBAM_015710	*fapR* - transcription factor (Fatty acid and phospholipid biosynthesis regulator)	-2.00	-4.01	0.03

N	RBAM_016750	*rodZ* - morphogenic protein required for cell shape determination	-0.93	-1.90	0.00
Fe	RBAM_016750	*rodZ* - morphogenic protein required for cell shape determination	-1.12	-2.17	0.05
N	RBAM_016830	*tdh* - L-threonine 3-dehydrogenase involved in threonine utilization	-0.93	-1.91	0.00
Fe	RBAM_016830	*tdh* - L-threonine 3-dehydrogenase involved in threonine utilization	-1.74	-3.35	0.04
N	RBAM_016860	*ymcA* - antagonist of biofilm repression by SinR involved in regulation of biofilm formation	-0.85	-1.80	0.00
Fe	RBAM_016860	*ymcA* - antagonist of biofilm repression by SinR involved in regulation of biofilm formation	-1.03	-2.04	0.00
N	RBAM_018030	*yndH* - hypothetical protein	-2.64	-6.23	0.00
Fe	RBAM_018030	*yndH* - hypothetical protein	-3.52	-11.46	0.02
N	RBAM_020410	*yppF* - hypothetical protein	-0.85	-1.81	0.00
Fe	RBAM_020410	*yppF* - hypothetical protein	-0.87	-1.82	0.00
N	RBAM_020730	*ypiF* - hypothetical protein	-1.07	-2.10	0.00
Fe	RBAM_020730	*ypiF* - hypothetical protein	-1.53	-2.88	0.01
N	RBAM_021290	*resA* - thiol-disulfide oxidoreductase involved in cytochrome c biogenesis	-1.29	-2.45	0.02
Fe	RBAM_021290	*resA* - thiol-disulfide oxidoreductase involved in cytochrome c biogenesis	-1.34	-2.53	0.03
N	RBAM_021850	*yqzH* - hypothetical protein	-1.27	-2.41	0.02
Fe	RBAM_021850	*yqzH* - hypothetical protein	-1.04	-2.06	0.04
N	RBAM_022360	*bcd* - leucine dehydrogenase involved in utilization of branched-chain keto acids	-0.88	-1.84	0.03
Fe	RBAM_022360	*bcd* - leucine dehydrogenase involved in utilization of branched-chain keto acids	-1.13	-2.18	0.01
N	RBAM_022920	*sinI* - sinR antagonist	-1.27	-2.41	0.00
Fe	RBAM_022920	*sinI* - sinR antagonist	-1.92	-3.79	0.01
N	RBAM_026150	*phoP* - two-component response regulator involved in regulation of phosphate metabolism	-1.12	-2.17	0.00
Fe	RBAM_026150	*phoP* - two-component response regulator involved in regulation of phosphate metabolism	-1.72	-3.30	0.00
N	RBAM_026760	putative transcriptional regulator	-1.05	-2.07	0.00
Fe	RBAM_026760	putative transcriptional regulator	-1.23	-2.34	0.02
N	RBAM_027240	*ytwF* - conserved hypothetical protein	-0.93	-1.91	0.03
Fe	RBAM_027240	*ytwF* - conserved hypothetical protein	-0.99	-1.98	0.04
N	RBAM_032460	*yvyD* - conserved hypothetical protein required for survival at low temperatures	-1.42	-2.68	0.00
Fe	RBAM_032460	*yvyD* - conserved hypothetical protein required for survival at low temperatures	-1.75	-3.35	0.03
N	RBAM_035760	*licH* - 6-phospho-beta-glucosidase involved in lichenan utilization	-1.74	-3.35	0.00

Fe	RBAM_035760	*licH* - 6-phospho-beta-glucosidase involved in lichenan utilization	-2.73	-6.62	0.04
N	RBAM_035770	*licA* - phosphotransferase system (PTS) lichenan specific enzyme IIA component involved in lichenan uptake and phosphorylation	-1.86	-3.62	0.00
Fe	RBAM_035770	*licA* - phosphotransferase system (PTS) lichenan specific enzyme IIA component involved in lichenan uptake and phosphorylation	-2.34	-5.06	0.03
N	RBAM_036350	*bglH* - beta-glucosidase involved in salicin utilization	-1.15	-2.22	0.00
Fe	RBAM_036350	*bglH* - beta-glucosidase involved in salicin utilization	-1.18	-2.26	0.04

APPENDIX XLI: Shared down-regulated bacterial genes in the logarithmic phase by N- and K -deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	RBAM_012280	*yjgD* - conserved hypothetical protein involved in survival to ethanol stress	-0.96	-1.95	0.01
K	RBAM_012280	*yjgD* - conserved hypothetical protein involved in survival to ethanol stress	-0.95	-1.93	0.04
N	RBAM_013310	*kinE* -.two-component sensor histidine kinase homolog involved in initiation of sporulation	-1.00	-2.01	0.00
K	RBAM_013310	*kinE* - two-component sensor histidine kinase homolog involved in initiation of sporulation	-1.06	-2.08	0.05
N	RBAM_016060	*fliH* - flagellar assembly protein involved in motility and chemotaxis	-1.29	-2.45	0.00
K	RBAM_016060	*fliH* - flagellar assembly protein involved in motility and chemotaxis	-0.99	-1.99	0.02
N	RBAM_017650	*lexA* - negative transcriptional regulator of the SOS regulon involved in regulation of DNA damage repair	-0.88	-1.84	0.01
K	RBAM_017650	*lexA* - negative transcriptional regulator of the SOS regulon involved in regulation of DNA damage repair	-1.34	-2.53	0.04
N	RBAM019970	*thyB* - thymidylate synthase B involved in biosynthesis of thymidine nucleotides	-1.28	-2.42	0.02
K	RBAM019970	*thyB* - thymidylate synthase B involved in biosynthesis of thymidine nucleotides	-0.98	-1.98	0.02
N	RBAM_023170	*glcK* - glucose kinase involved in phosphorylation of the free glucose moiety of di-and oligosaccharides	-1.03	-2.04	0.02
K	RBAM_023170	*glcK* - glucose kinase involved in phosphorylation of the free glucose moiety of di-and oligosaccharides	-1.28	-2.44	0.04

APPENDIX XLII: Shared down-regulated bacterial genes in the logarithmic phase by P- and Fe -deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
P	RBAM_017340	*xylR* - xylose operon repressor protein involved in regulation of xylan and xylose utilization	-1.35	-2.55	0.04
Fe	RBAM_017340	*xylR* - xylose operon repressor protein involved in regulation of xylan and xylose utilization	-1.24	-2.37	0.03
P	RBAM_022130	*yqjL* - putative hydrolase involved in resistence against paraquat	-0.88	-1.84	0.00
Fe	RBAM_022130	*yqjL* - putative hydrolase involved in resistence against paraquat	-1.13	-2.19	0.01

APPENDIX XLIII: Shared down-regulated bacterial genes in the logarithmic phase by P- and K -deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
P	B_amylo_FZB42_3830	predicted ncRNA	-2.29	-4.89	0.00
K	B_amylo_FZB42_3830	predicted ncRNA	-1.71	-3.26	0.01
P	RBAM_013910	*ccpC* - transcriptional repressor involved in regulation of tricarboxylic acid branch of the TCA cycle	-1.79	-3.45	0.00
K	RBAM_013910	*ccpC* - transcriptional repressor involved in regulation of tricarboxylic acid branch of the TCA cycle	-1.17	-2.25	0.02
P	RBAM_019240	hypothetical protein	-1.06	-2.09	0.02
K	RBAM_019240	hypothetical protein	-1.14	-2.21	0.02
P	RBAM_027250	*leuS* - leucyl-tRNA synthetase involved in translation	-1.24	-2.36	0.05
K	RBAM_027250	*leuS* - leucyl-tRNA synthetase involved in translation	-1.88	-3.67	0.02
P	RBAM_027410	*ytzC* - hypothetical protein	-1.45	-2.74	0.01
K	RBAM_027410	*ytzC* - hypothetical protein	-1.44	-2.71	0.02

APPENDIX XLIV: Shared down-regulated bacterial genes in the logarithmic phase by Fe- and K -deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
Fe	RBAM_003400	*ycbE* - galactarate/glucarate transporter in (proton symport) involved in glucarate uptake	-1.28	-2.42	0.00
K	RBAM_003400	*ycbE* - galactarate/glucarate transporter in (proton symport) involved in glucarate uptake	-1.71	-3.28	0.03
Fe	RBAM_008700	*fhO* - hypothetical protein	-0.94	-1.92	0.00
K	RBAM_008700	*fhO* - hypothetical protein	-0.89	-1.86	0.00
Fe	RBAM_011300	*med* - positive regulator of comK involved in regulation of competence	-1.00	-2.00	0.00
K	RBAM_011300	*med* - positive regulator of comK involved in regulation of competence	-0.95	-1.94	0.00
Fe	RBAM_013620	*zosA* - P-type zinc-transporting ATPase involved in zinc uptake	-0.90	-1.86	0.02
K	RBAM_013620	*zosA* - P-type zinc-transporting ATPase involved in zinc uptake	-0.88	-1.85	0.04
Fe	RBAM_018210	*scoB* - succinyl CoA:3-oxoacid CoA-transferase (subunit B) involved in lipid metabolism	-1.15	-2.22	0.01
K	RBAM_018210	*scoB* - succinyl CoA:3-oxoacid CoA-transferase (subunit B) involved in lipid metabolism	-1.06	-2.09	0.03
Fe	RBAM_018930	*yocC* - hypothetical protein	-1.10	-2.14	0.00
K	RBAM_018930	*yocC* - hypothetical protein	-1.36	-2.56	0.02
Fe	RBAM_022310	*bkdB* - branched-chain alpha-keto acid dehydrogenase E2 subunit (lipoamide acyltransferase) involved in utilization of branched-chain keto acids	-1.09	-2.12	0.00
K	RBAM_022310	*bkdB* - branched-chain alpha-keto acid dehydrogenase E2 subunit (lipoamide acyltransferase) involved in utilization of branched-chain keto acids	-0.95	-1.93	0.04
Fe	RBAM_026370	*argH* - argininosuccinate lyase involved in biosynthesis of arginine	-1.77	-3.42	0.01
K	RBAM_026370	*argH* - argininosuccinate lyase involved in biosynthesis of arginine	-0.96	-1.95	0.05
Fe	RBAM_028990	*yukJ*- conserved hypothetical protein	-0.86	-1.82	0.02
K	RBAM_028990	*yukJ* - conserved hypothetical protein	-0.87	-1.83	0.02
Fe	RBAM_031450	*lutC* - conserved hypothetical protein involved in utilization of lactate	-1.47	-2.78	0.00
K	RBAM_031450	*lutC* - conserved hypothetical protein involved in utilization of lactate	-1.01	-2.01	0.04
Fe	RBAM_031740	*yveG* - hypothetical protein	-0.95	-1.93	0.00
K	RBAM_031740	*yveG* - hypothetical protein	-0.91	-1.87	0.00

APPENDIX XLV: Shared down-regulated bacterial genes in the logarithmic phase by N-, P-, Fe- deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	B_amylo_FZB42_3895	predicted ncRNA	-1.04	-2.05	0.05
P	B_amylo_FZB42_3895	predicted ncRNA	-0.96	-1.94	0.00
Fe	B_amylo_FZB42_3895	predicted ncRNA	-0.89	-1.85	0.00
N	RBAM_009090	*katA* - vegetative catalase involved in detoxification (degradation) of hydrogen peroxide	-2.19	-4.57	0.00
P	RBAM_009090	*katA* - vegetative catalase involved in detoxification (degradation) of hydrogen peroxide	-1.02	-2.03	0.00
Fe	RBAM_009090	*katA* - vegetative catalase involved in detoxification (degradation) of hydrogen peroxide	-1.50	-2.82	0.02
N	RBAM_012010	hypothetical protein	-1.60	-3.03	0.01
P	RBAM_012010	hypothetical protein	-1.80	-3.49	0.01
Fe	RBAM_012010	hypothetical protein	-1.88	-3.68	0.01
N	RBAM_013050	*ispA* - major intracellular serine protease precursor involved in protein degradation	-2.22	-4.65	0.00
P	RBAM_013050	*ispA* - major intracellular serine protease precursor involved in protein degradation	-1.91	-3.76	0.00
Fe	RBAM_013050	*ispA* - major intracellular serine protease precursor involved in protein degradation	-1.18	-2.26	0.01
N	RBAM_018180	*bmyA* - bacillomycin D synthetase A involved in antibiotics production	-1.90	-3.74	0.01
P	RBAM_018180	*bmyA* - bacillomycin D synthetase A involved in antibiotics production	-1.45	-2.73	0.03
Fe	RBAM_018180	*bmyA* - bacillomycin D synthetase A involved in antibiotics production	-1.11	-2.16	0.01
N	RBAM_018540	*ggt* - gamma-glutamyltranspeptidase involved in degradation of poly-glutamate capsules	-1.24	-2.36	0.00
P	RBAM_018540	*ggt* - gamma-glutamyltranspeptidase involved in degradation of poly-glutamate capsules	-0.90	-1.86	0.05
Fe	RBAM_018540	*ggt* - gamma-glutamyltranspeptidase involved in degradation of poly-glutamate capsules	-1.36	-2.58	0.01
N	RBAM_019360	*bglA* - 6-phospho-beta-glucosidase involved in beta-glucoside utilization	-1.48	-2.78	0.02
P	RBAM_019360	*bglA* - 6-phospho-beta-glucosidase involved in beta-glucoside utilization	-1.50	-2.83	0.00
Fe	RBAM_019360	*bglA* - 6-phospho-beta-glucosidase involved in beta-glucoside utilization	-2.09	-4.27	0.01
N	RBAM_036710	*iolH* - inositol utilization protein H involved in myo-inositol catabolism	-2.23	-4.68	0.00
P	RBAM_036710	*iolH* - inositol utilization protein H involved in myo-inositol catabolism	-1.30	-2.47	0.04
Fe	RBAM_036710	*iolH* - inositol utilization protein H involved in myo-inositol catabolism	-2.27	-4.83	0.02

APPENDIX XLVI: Shared down-regulated bacterial genes in the logarithmic phase by N-, Fe-, K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	B_amylo_FZB42_3873	predicted ncRNA	-0.92	-1.89	0.01
Fe	B_amylo_FZB42_3873	predicted ncRNA	-1.48	-2.78	0.00
K	B_amylo_FZB42_3873	predicted ncRNA	-1.98	-3.96	0.01
N	RBAM_004040	*yclM* - aspartokinase III with unknown function	-1.24	-2.36	0.01
Fe	RBAM_004040	*yclM* - aspartokinase III with unknown function	-0.90	-1.87	0.00
K	RBAM_004040	*yclM* - aspartokinase III with unknown function	-1.34	-2.53	0.04
N	RBAM_006610	*ydjI* - conserved hypothetical protein	-2.30	-4.92	0.00
Fe	RBAM_006610	*ydjI* - conserved hypothetical protein	-1.81	-3.50	0.01
K	RBAM_006610	*ydjI* - conserved hypothetical protein	-2.15	-4.43	0.00
N	RBAM_012150	*galK1* - galactokinase involved in galactose utilization	-1.52	-2.87	0.02
Fe	RBAM_012150	*galK1* - galactokinase involved in galactose utilization	-2.45	-5.48	0.01
K	RBAM_012150	*galK1* - galactokinase involved in galactose utilization	-1.69	-3.24	0.05
N	RBAM_014220	*abh* - transition state regulator	-1.36	-2.57	0.00
Fe	RBAM_014220	*abh* - transition state regulator	-1.50	-2.82	0.00
K	RBAM_014220	*abh* - transition state regulator	-2.42	-5.34	0.05
N	RBAM_015190	*ylmC* - conserved hypothetical protein	-1.89	-3.72	0.00
Fe	RBAM_015190	*ylmC* - conserved hypothetical protein	-1.50	-2.83	0.01
K	RBAM_015190	*ylmC* - conserved hypothetical protein	-2.00	-3.99	0.00
N	RBAM_016840	*kbl* - 2-amino-3-ketobutyrate coenzyme A ligase involved in threonine utilization	-0.93	-1.91	0.00
Fe	RBAM_016840	*kbl* - 2-amino-3-ketobutyrate coenzyme A ligase involved in threonine utilization	-1.52	-2.86	0.02
K	RBAM_016840	*kbl* - 2-amino-3-ketobutyrate coenzyme A ligase involved in threonine utilization	-1.05	-2.07	0.05
N	RBAM_018470	*dacC* - penicillin-binding carboxypeptidase	-1.00	-2.00	0.00
Fe	RBAM_018470	*dacC* - penicillin-binding carboxypeptidase	-1.10	-2.14	0.00
K	RBAM_018470	*dacC* - penicillin-binding carboxypeptidase	-1.65	-3.14	0.03
N	RBAM_019060	*dhaS* - aldehyde dehydrogenase	-1.19	-2.28	0.02
Fe	RBAM_019060	*dhaS* - aldehyde dehydrogenase	-1.66	-3.17	0.00
K	RBAM_019060	*dhaS* - aldehyde dehydrogenase	-1.08	-2.12	0.04

N	RBAM_019170	*cwlS* - D,L-endopeptidase, peptidoglycan hydrolase involved in cell wall metabolism	-1.72	-3.29	0.00
Fe	RBAM_019170	*cwlS* - D,L-endopeptidase, peptidoglycan hydrolase involved in cell wall metabolism	-1.33	-2.51	0.00
K	RBAM_019170	*cwlS* - D,L-endopeptidase, peptidoglycan hydrolase involved in cell wall metabolism	-1.24	-2.36	0.00
N	RBAM_019730	*rapA1* - response regulator aspartate phosphatase A involved in control of sporulation initiation	-1.53	-2.88	0.00
Fe	RBAM_019730	*rapA1* - response regulator aspartate phosphatase A involved in control of sporulation initiation	-1.50	-2.82	0.00
K	RBAM_019730	*rapA1* - response regulator aspartate phosphatase A involved in control of sporulation initiation	-0.87	-1.83	0.04
N	RBAM_020080	*degR* - positive effector of DegU-phosphate stability involved in control of DegU activity	-1.48	-2.78	0.02
Fe	RBAM_020080	*degR* - positive effector of DegU-phosphate stability involved in control of DegU activity	-1.75	-3.36	0.00
K	RBAM_020080	*degR* - positive effector of DegU-phosphate stability involved in control of DegU activity	-2.29	-4.89	0.02
N	RBAM_020170	*ypbS* - hypothetical protein	-1.81	-3.51	0.00
Fe	RBAM_020170	*ypbS* - hypothetical protein	-1.43	-2.69	0.04
K	RBAM_020170	*ypbS* - hypothetical protein	-1.07	-2.10	0.02
N	RBAM_021570	*spoIIAB* - anti-sigma F factor involved in control of sporulation	-1.28	-2.43	0.00
Fe	RBAM_021570	*spoIIAB* - anti-sigma F factor involved in control of sporulation	-1.33	-2.51	0.00
K	RBAM_021570	*spoIIAB* - anti-sigma F factor involved in control of sporulation	-1.92	-3.79	0.04
N	RBAM_022350	*buk* - butyrate kinase involved in utilization of branched-chain keto acids	-0.97	-1.97	0.00
Fe	RBAM_022350	*buk* - butyrate kinase involved in utilization of branched-chain keto acids	-1.25	-2.37	0.00
K	RBAM_022350	*buk* - butyrate kinase involved in utilization of branched-chain keto acids	-1.13	-2.18	0.01
N	RBAM_024200	*manP* - phosphotransferase system (PTS) mannose-specificenzyme IIBCA component involved in mannose uptake and phosphorylation, control of ManR activity	-0.94	-1.92	0.00
Fe	RBAM_024200	*manP* - phosphotransferase system (PTS) mannose-specificenzyme IIBCA component involved in mannose uptake and phosphorylation, control of ManR activity	-0.95	-1.94	0.00
K	RBAM_024200	*manP* - phosphotransferase system (PTS) mannose-specificenzyme IIBCA component involved in mannose uptake and phosphorylation, control of ManR activity	-1.03	-2.04	0.03
N	RBAM_027670	*ytkA* - hypothetical protein	-2.25	-4.76	0.00

Fe	RBAM_027670	*ytkA* - hypothetical protein	-2.00	-3.99	0.00
K	RBAM_027670	*ytkA* - hypothetical protein	-1.58	-3.00	0.02
N	RBAM_030250	*liaH* - conserved hypothetical protein involved in protection against daptomycin	-1.28	-2.44	0.00
Fe	RBAM_030250	*liaH* - conserved hypothetical protein involved in protection against daptomycin	-2.11	-4.31	0.00
K	RBAM_030250	*liaH* - conserved hypothetical protein involved in protection against daptomycin	-2.47	-5.53	0.00
N	RBAM_030260	*liaI* - hypothetical protein	-1.09	-2.13	0.00
Fe	RBAM_030260	*liaI* - hypothetical protein	-1.65	-3.13	0.00
K	RBAM_030260	*liaI* - hypothetical protein	-1.74	-3.34	0.00
N	RBAM_034560	hypothetical protein	-2.20	-4.59	0.01
Fe	RBAM_034560	hypothetical protein	-1.34	-2.54	0.02
K	RBAM_034560	hypothetical protein	-0.93	-1.90	0.02
N	RBAM_036760	*iolC* - inositol utilization protein C involved in myo-inositol catabolism	-0.97	-1.96	0.00
Fe	RBAM_036760	*iolC* - inositol utilization protein C involved in myo-inositol catabolism	-1.35	-2.54	0.03
K	RBAM_036760	*iolC* - inositol utilization protein C involved in myo-inositol catabolism	-1.09	-2.13	0.04

APPENDIX XLVII: Shared down-regulated bacterial genes in the logarithmic phase by N-, P-, K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	RBAM_017910	*parC* - DNA topoisomerase IV subunit A involved in chromosome segregation and compaction	-1.37	-2.59	0.00
P	RBAM_017910	*parC* - DNA topoisomerase IV subunit A involved in chromosome segregation and compaction	-1.74	-3.33	0.01
K	RBAM_017910	*parC* - DNA topoisomerase IV subunit A involved in chromosome segregation and compaction	-1.00	-1.99	0.05
N	RBAM_021700	*ansB* - aspartate ammonia-lyase involved in aspartate degradation	-1.44	-2.71	0.00
P	RBAM_021700	*ansB* - aspartate ammonia-lyase involved in aspartate degradation	-0.94	-1.91	0.02
K	RBAM_021700	*ansB* - aspartate ammonia-lyase involved in aspartate degradation	-1.92	-3.79	0.01
N	RBAM_025130	*folC* - folyl-polyglutamate synthetase involved in biosynthesis of folate	-2.16	-4.48	0.00
P	RBAM_025130	*folC* - folyl-polyglutamate synthetase involved in biosynthesis of folate	-1.80	-3.49	0.05
K	RBAM_025130	*folC* - folyl-polyglutamate synthetase involved in biosynthesis of folate	-1.86	-3.64	0.01

APPENDIX XLVIII: Shared down-regulated bacterial genes in the logarithmic phase by P-, Fe-, K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
P	RBAM_008360	*malA* - maltose-6'-phosphate glucosid involved in maltose utilization	-1.59	-3.01	0.00
Fe	RBAM_008360	*malA* - maltose-6'-phosphate glucosid involved in maltose utilization	-3.30	-9.83	0.00
K	RBAM_008360	*malA* - 6-phospho-alpha-glucosidase involved in maltose utilization	-1.87	-3.64	0.03
P	RBAM_012410	*uxaB* - tagaturonate reductase (altronate oxidoreductase) involved in hexuronate utilization	-1.13	-2.19	0.04
Fe	RBAM_012410	*uxaB* - tagaturonate reductase (altronate oxidoreductase) involved in hexuronate utilization	-1.17	-2.25	0.03
K	RBAM_012410	*uxaB* - tagaturonate reductase (altronate oxidoreductase) involved in hexuronate utilization	-1.49	-2.81	0.02
P	RBAM_012470	*xlyB* - N-acetylmuramoyl-L-alanine amidase involved in PBSX prophage-mediated lysis	-1.07	-2.10	0.00
Fe	RBAM_012470	*xlyB* - N-acetylmuramoyl-L-alanine amidase involved in PBSX prophage-mediated lysis	-1.42	-2.68	0.00
K	RBAM_012470	*xlyB* - N-acetylmuramoyl-L-alanine amidase involved in PBSX prophage-mediated lysis	-1.17	-2.25	0.02
P	RBAM012790	*dppC* - dipeptide transport system permease protein involved in uptake of dipeptides	-0.86	-1.82	0.00
Fe	RBAM012790	*dppC* - dipeptide transport system permease protein involved in uptake of dipeptides	-1.31	-2.47	0.02
K	RBAM012790	*dppC* - dipeptide transport system permease protein involved in uptake of dipeptides	-1.27	-2.41	0.01
P	RBAM_013790	hypothetical protein	-0.94	-1.91	0.00
Fe	RBAM_013790	hypothetical protein	-1.44	-2.71	0.00
K	RBAM_013790	hypothetical protein	-1.44	-2.72	0.00
P	RBAM_022330	*bkdAA* - branched-chain alpha-keto acid dehydrogenase involved in utilization of branched-chain keto acids	-0.92	-1.89	0.00
Fe	RBAM_022330	*bkdAA* - branched-chain alpha-keto acid dehydrogenase involved in utilization of branched-chain keto acids	-1.07	-2.11	0.00
K	RBAM_022330	*bkdAA* - branched-chain alpha-keto acid dehydrogenase involved in utilization of branched-chain keto acids	-0.97	-1.95	0.04
P	RBAM_032010	*trxB* - thioredoxin reductase	-1.03	-2.04	0.01
Fe	RBAM_032010	*trxB* - thioredoxin reductase	-1.32	-2.49	0.00
K	RBAM_032010	*trxB* - thioredoxin reductase	-0.96	-1.95	0.00

APPENDIX XLIX: Shared down-regulated bacterial genes in the logarithmic phase by N-, P-, Fe-, K-deficient root exudates

Treatment	Gene ID	Gene and Function	M	Fold-change	p_value
N	RBAM_004120	*ycnE* - conserved hypothetical protein	-1.16	-2.24	0.00
P	RBAM_004120	*ycnE* - conserved hypothetical protein	-1.60	-3.03	0.02
Fe	RBAM_004120	*ycnE* - conserved hypothetical protein	-2.39	-5.23	0.00
K	RBAM_004120	*ycnE* - conserved hypothetical protein	-1.41	-2.66	0.04
N	RBAM_004730	*gsiB* - general stress protein	-1.60	-3.03	0.00
P	RBAM_004730	*gsiB* - general stress protein	-0.92	-1.90	0.00
Fe	RBAM_004730	*gsiB* - general stress protein	-0.92	-1.90	0.00
K	RBAM_004730	*gsiB* - general stress protein	-1.47	-2.78	0.03
N	RBAM_006180	putative transcriptional regulator (gntr family)	-1.08	-2.12	0.00
P	RBAM_006180	putative transcriptional regulator (gntr family)	-1.36	-2.56	0.00
Fe	RBAM_006180	putative transcriptional regulator (gntr family)	-1.81	-3.50	0.00
K	RBAM_006180	putative transcriptional regulator (gntr family)	-1.32	-2.49	0.00
N	RBAM_011020	*yitJ* - conserved hypothetical protein	-2.46	-5.50	0.01
P	RBAM_011020	*yitJ* - conserved hypothetical protein	-1.67	-3.18	0.01
Fe	RBAM_011020	*yitJ* - conserved hypothetical protein	-1.58	-2.99	0.00
K	RBAM_011020	*yitJ* - conserved hypothetical protein	-2.20	-4.58	0.00
N	RBAM_011340	*fabF* - beta-ketoacyl-acyl carrier protein synthase II involved in fatty acid biosynthesis	-1.23	-2.35	0.01
P	RBAM_011340	*fabF* - beta-ketoacyl-acyl carrier protein synthase II involved in fatty acid biosynthesis	-1.58	-3.00	0.00
Fe	RBAM_011340	*fabF* - beta-ketoacyl-acyl carrier protein synthase II involved in fatty acid biosynthesis	-1.89	-3.70	0.05
K	RBAM_011340	*fabF* - beta-ketoacyl-acyl carrier protein synthase II involved in fatty acid biosynthesis	-1.19	-2.29	0.01
N	RBAM_012080	*penP* - beta-lactamase precursor involved in resistance to beta-lactam antibiotics	-1.84	-3.59	0.01
P	RBAM_012080	*penP* - beta-lactamase precursor involved in resistance to beta-lactam antibiotics	-1.64	-3.13	0.01
Fe	RBAM_012080	*penP* - beta-lactamase precursor involved in resistance to beta-lactam antibiotics	-2.66	-6.31	0.00
K	RBAM_012080	*penP* - beta-lactamase precursor involved in resistance to beta-lactam antibiotics	-1.97	-3.92	0.00
N	RBAM_018960	*yocH* - putative cell-wall binding protein	-1.00	-2.00	0.00
P	RBAM_018960	*yocH* - putative cell-wall binding protein	-1.66	-3.16	0.00
Fe	RBAM_018960	*yocH* - putative cell-wall binding protein	-2.20	-4.59	0.02
K	RBAM_018960	*yocH* - putative cell-wall binding protein	-1.45	-2.73	0.00
N	RBAM_019440	*yodL* - hypothetical protein	-1.98	-3.94	0.00

P	RBAM_019440	*yodL* - hypothetical protein	-1.22	-2.34	0.03
Fe	RBAM_019440	*yodL* - hypothetical protein	-2.61	-6.10	0.00
K	RBAM_019440	*yodL* - hypothetical protein	-2.31	-4.97	0.01
N	RBAM_020470	*ponA* - bifunctional glucosyl transferase/ transpeptidase penicillin-binding proteins IA/IB	-1.42	-2.69	0.01
P	RBAM_020470	*ponA* - bifunctional glucosyl transferase/ transpeptidase penicillin-binding proteins IA/IB	-1.18	-2.27	0.01
Fe	RBAM_020470	*ponA* - bifunctional glucosyl transferase/ transpeptidase penicillin-binding proteins IA/IB	-1.02	-2.02	0.04
K	RBAM__020470	*ponA* - bifunctional glucosyl transferase/ transpeptidase penicillin-binding proteins IA/IB	-1.14	-2.21	0.01
N	RBAM_020640	*dapB* - dihydrodipicolinate reductase involved in biosynthesis of lysine and peptidoglycan	-1.70	-3.25	0.00
P	RBAM_020640	*dapB* - dihydrodipicolinate reductase involved in biosynthesis of lysine and peptidoglycan	-1.12	-2.18	0.01
Fe	RBAM_020640	*dapB* - dihydrodipicolinate reductase involved in biosynthesis of lysine and peptidoglycan	-1.33	-2.51	0.04
K	RBAM_020640	*dapB* - dihydrodipicolinate reductase involved in biosynthesis of lysine and peptidoglycan	-1.52	-2.87	0.01
N	RBAM_036590	*yxeA* - hypothetical protein	-1.03	-2.04	0.03
P	RBAM_036590	*yxeA* - hypothetical protein	-2.05	-4.14	0.00
Fe	RBAM_036590	*yxeA* - hypothetical protein	-0.86	-1.82	0.04
K	RBAM_036590	*yxeA* - hypothetical protein	-1.22	-2.32	0.03
N	RBAM_036720	*iolG* - myo-inositol 2-dehydrogenase involved in myo-inositol catabolism	-1.01	-2.01	0.00
P	RBAM_036720	*iolG* - myo-inositol 2-dehydrogenase involved in myo-inositol catabolism	-0.87	-1.83	0.00
Fe	RBAM_036720	*iolG* - myo-inositol 2-dehydrogenase involved in myo-inositol catabolism	-1.23	-2.35	0.01
K	RBAM_036720	*iolG* - myo-inositol 2-dehydrogenase involved in myo-inositol catabolism	-0.87	-1.83	0.02
N	RBAM_036870	*des* - fatty acid desaturase involved in adaptation of membrane fluidity at low temperatures	-2.46	-5.50	0.00
P	RBAM_036870	*des* - fatty acid desaturase involved in adaptation of membrane fluidity at low temperatures	-1.67	-3.18	0.01
Fe	RBAM_036870	*des* - fatty acid desaturase involved in adaptation of membrane fluidity at low temperatures	-1.36	-2.57	0.02
K	RBAM_036870	*des* - fatty acid desaturase involved in adaptation of membrane fluidity at low temperatures	-1.68	-3.21	0.03

APPENDIX L: Most discriminating bacterial transcripts for each nutrient deficiency treatment

N deficiency			**Fold-change**			
Gene	Product and function	Funtion	-N	-P	-Fe	-K
gmuR	transcriptional repressor (GntR family)	Regulation of glucomannan utilization	4.67	1.07	1.07	1.02
ykyB	hypothetical protein	Unknown	2.35	-1.09	1.13	1.19
proJ	glutamate 5-kinase	Biosynthesis of proline	1.91	-1.21	1.09	1.01
RBAM_018700	hypothetical protein	Unknown	2.91	1.40	1.09	1.42
yosT	hypothetical protein	Unknown	1.99	-1.58	-1.11	1.16
spoIVFA	inhibitor of SpoIVFB metalloprotease	Control of SigK activation	2.16	1.26	-1.28	1.20
yxcA	hypothetical protein	Unknown	2.40	-1.05	1.13	1.35
ncRNA_3	predicted non-coding RNA	Unknown	2.58	1.37	1.09	1.45
ywoD	hypothetical protein	Unknown	1.75	1.19	-1.35	-1.20
yfkC	mechanosensitive channel	Resistance to osmotic downshock	1.94	1.34	-1.06	-1.10

P deficiency			**Fold-change**			
Gene	Product and function		-N	-P	-Fe	-K
ymcA	antagonist of biofilm repression by SinR	Regulation of biofilm formation	-3.04	1.60	-1.48	-1.13
yceE	hypothetical protein	Survival to ethanol stress and at low temperatures	-3.66	1.03	-1.46	-1.56
cspB	major cold-shock protein	RNA chaperone	-3.01	1.43	1.25	1.03
ydjI	hypothetical protein	Unknown	-2.21	1.72	1.08	-1.23
rpsH	ribosomal protein S8 (BS8)	Translation	-3.64	1.27	1.51	1.17
rpsR	ribosomal protein S18	Translation	-2.58	1.54	1.26	1.15
yqgA	hypothetical protein	Unknown	-2.31	1.53	-1.03	-1.00
RBAM_011120	hypothetical protein	Unknown	-1.33	1.81	1.09	1.24
rpsG	ribosomal protein S7 (BS7)	Translation	-3.31	1.08	1.11	-1.35
gapA	glyceraldehyde 3-phosphate dehydrogenase	Catabolic enzyme in glycolysis	-1.32	0.29	-0.18	-0.09

Fe deficiency			**Fold-change**			
Gene	Function		-N	-P	-Fe	-K
rpsH	ribosomal protein S8 (BS8)	Translation	-3.64	1.27	1.51	1.17
rplX	ribosomal protein L24 (BL23)	Translation	-3.01	-1.01	1.33	1.09
ncRNA_6	predicted non-coding RNA	Unknown	-2.69	1.27	1.52	1.27
rpsG	ribosomal protein S7 (BS7)	Translation	-3.31	1.08	1.11	-1.35
rplP	ribosomal protein L16	Translation	-2.57	1.16	1.41	1.06
cspB	major cold-shock protein	RNA chaperone	-3.01	1.43	1.25	1.03
rplE	ribosomal protein L5 (BL6)	Translation	-2.45	-1.19	1.38	1.08
infA	translation initiation factor IF-I	Translation	-2.29	1.12	1.58	1.60
RBAM_004030	hypothetical protein	Unknown	-1.08	1.16	3.55	-1.29
rplV	ribosomal protein L10 (BL5)	Translation	-2.67	-1.00	1.21	-1.09

K deficiency			**Fold-change**			
Gene	Function		-N	-P	-Fe	-K
rpsH	ribosomal protein S8 (BS8)	Translation	-3.64	1.27	1.51	1.17
infA	translation initiation factor IF-I	Translation	-2.29	1.12	1.58	1.60
ncRNA_6	predicted non-coding RNA	Unknown	-2.69	1.27	1.52	1.27
rplX	ribosomal protein L24 (BL23)	Translation	-3.01	-1.01	1.33	1.09
ncRNA_2	predicted non-coding RNA	Unknown	-2.42	1.16	1.33	1.30
rpsQ	ribosomal protein S17 (BS16)	Translation	-2.67	1.17	1.30	1.21
cspB	major cold-shock protein	RNA chaperone	-3.01	1.43	1.25	1.03
ilvH	acetolactate synthase	Biosynthesis of branched-chain amino acids	-2.40	1.02	1.01	1.20
rplE	ribosomal protein L5 (BL6)	Translation	-2.45	-1.19	1.38	1.08
rplJ	ribosomal protein L14	Translation	-2.11	1.56	1.63	1.39

APPENDIX LI: Functional groups of differentially expressed bacterial genes by different nutrient-deficient maize root exudates

Functional groups	N deficiency				P deficiency				Fe deficiency				K deficiency			
	OD 1.0		OD 3.0		OD 1.0		OD 3.0		OD 1.0		OD 3.0		OD 1.0		OD 3.0	
	up	down	up	down	up	down	up	Down	up	down	up	down	up	down	up	down
Cell wall	0	1	1	3	1	0	1	2	0	0	0	4	0	0	0	3
Transport/binding proteins and lipoproteins	4	5	4	7	2	0	7	3	2	0	7	10	0	0	2	4
Sensors (signal transduction)	0	1	0	2	0	0	1	1	0	0	0	1	0	0	0	3
Membrane bioenergetics (electron transport chain and ATP synthase)	0	4	2	1	0	0	0	1	0	0	1	4	1	0	0	1
Motility and chemotaxis	1	1	0	5	1	0	10	0	0	0	0	0	0	0	0	1
Protein secretion	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Cell division	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Sporulation	4	1	1	6	3	0	0	1	1	0	1	5	0	0	1	2
Germination	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0
Transformation/competence	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	1
Metabolism of carbohydrates and related molecules	2	5	0	14	0	0	2	7	0	0	1	16	0	0	0	6
Metabolism of amino acids and related molecules	1	3	2	10	0	0	3	5	0	0	2	7	0	0	2	6
Metabolism of nucleotides and nucleic acids	0	1	2	1	0	0	3	1	0	0	1	1	0	0	0	1
Metabolism of lipids	0	1	1	6	0	0	0	4	0	0	0	9	0	0	0	6
Metabolism of coenzymes and prosthetic groups	1	0	0	3	1	0	1	2	0	0	0	3	0	0	0	2
Metabolism of phosphate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metabolism of sulfur	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DNA replication	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0
DNA restriction/modification	2	0	0	1	0	0	0	2	0	0	0	0	0	0	1	0

and repair																
DNA recombination	0	0	0	1	0	0	0	2	0	0	1	0	0	0	0	1
DNA packaging and segregation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RNA synthesis	6	6	0	18	0	0	4	4	0	0	4	10	1	0	1	5
RNA modification	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Protein synthesis	0	32	12	1	2	0	9	2	0	0	2	0	0	0	1	1
Protein modification	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
Protein folding	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Adaptation to atypical conditions	1	2	0	5	0	0	0	1	0	0	0	4	0	0	0	2
Detoxification	0	3	0	3	1	0	0	2	0	0	1	5	0	0	0	1
Antibiotic production	0	1	0	4	0	0	0	1	0	0	0	1	0	0	0	0
Phage-related functions	1	1	0	0	0	0	1	1	0	0	0	1	0	0	0	1
Transposon and IS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0
From *B. subtilis*	21	22	1	33	5	1	10	14	1	1	9	29	8	0	2	21
From other organisms	5	1	0	7	2	0	1	1	0	0	1	3	2	0	0	2
No similarity	6	2	0	7	5	0	0	4	1	0	1	5	3	0	0	2
ncRNA	8	10	1	11	2	0	0	7	1	1	2	4	4	0	1	2
Sum of genes	64	108	28	155	27	1	55	72	7	2	35	125	19	0	12	74

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