You Are What You Can Find to Eat:  
Bacterial Metabolism in the Rhizosphere

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Abstract
Metabolism is the underpinning force that sustains life. Within the rhizosphere it is a cyclic process, with substrates flowing between different compartments of the complete soil-plant-microbe system. The physiochemical and structural environment of the rhizosphere is shaped by a combination of plant genotype and soil type, both of which strongly impact the microbial community structure. External influences such as seasonality, the degree of water saturation and anthropomorphic inputs also play a role. Together these factors influence the flux of metabolites through the rhizosphere community, which in turn impacts on plant growth, development and disease. In this review, the focus is on metabolism within the bacterial population of the rhizosphere, since this group covers every type of plant-microbe interaction: from obligately symbiotic to destructively pathogenic, and includes those have little or no direct impact on plant hosts. The focus of the review is on metabolic functions that occur in the rhizosphere either during bacteria-plant interactions or bacteria-bacteria interactions and mainly covers heterotrophic metabolism of organic substrates. As such, many of the autotrophic (and phototrophic) reactions of inorganic compounds are not included.

Metabolic pathways of bacteria
Broadly speaking, heterotrophic metabolism in bacteria is based on carbon- or nitrogen-containing organic compounds, i.e. carbohydrates (complexed or simple) and amino acids, whereas autotrophic metabolism is based on inorganic carbon or nitrogen. Bacteria can use multiple pathways to oxidise glucose: in addition to glycolysis, the hexose monophosphate shunt and the Entner-Doudoroff pathway can also be utilised, usually dependent on environmental parameters and lifestyle type. Some bacterial species lack key enzymes for all three pathways, such as Azotobacter and most Pseudomonas species that utilize the Entner-Doudoroff pathway for glucose catabolism, in contrast to the Enterobacteriaceae, as they lack the phosphofructokinase required for conversion to fructose (Jurtshuk P Jr., 1996) (T G Lessie and P V Phibbs, 1984). Metabolic flexibility is often associated with saprophytic pseudomonads due to the wide range of metabolites that they are likely to encounter in soil, exemplified by the bioremediation strain of Pseudomonas putida, which has been shown to have the capacity to use 911 metabolites (Nogales et al., 2008). Nitrate reduction in
heterotrophic bacteria is a feature of anaerobic metabolism that allows $\text{NO}_2^-$ ion to serve as a terminal electron acceptor (Jurtshuk P Jr., 1996), and the physiologic type of nitrate reductase forms the basis for metabolic separation of some species. While autotrophic bacteria still use CO$_2$ as a carbon source for growth, nitrogen is derived from $\text{NH}_3$, $\text{NO}_3^-$ (nitrification and denitrification, respectively) or $\text{N}_2$ (nitrogen-fixing). A second group can oxidize sulphur compounds such as $\text{H}_2\text{S}$, $\text{S}_2^-$, and $\text{S}_2\text{O}_3^-$, although they are not strictly autotrophic like some nitrifiers. Representatives of both groups are found in soil and can associate with the rhizosphere. However, a critical aspect of these reactions is that they are very sensitive to the presence oxygen and some such as denitrification and nitrogen-fixation, only occur under anaerobic conditions (Jurtshuk P Jr., 1996).

**Functional plant-bacteria interactions**

Rhizosphere-associated bacteria can be broadly split into functional groups dependent on how they interact with plant hosts, ranging from beneficial to overtly pathogenic. For those that have an intimately symbiotic relationship with the host, e.g. the rhizobia, the basis for the interaction is mutual exchange of substrates. This has been well described for fixation of atmospheric nitrogen and exchange of ammonia for carbohydrates. At the other end of the spectrum are the pathogens, although even for this group, the basis for development of disease is in the acquisition of nutrients from the plant host. Finally, in the middle are a diverse group classed as generalists or opportunists that perhaps receives less attention than the symbionts or pathogens. Cutting across these categorisations, different groups of rhizosphere bacteria are either shown to be nutritionally flexible or have evolved to exploit very specific ecological niches and associated substrates.

**Beneficial interactors**

Plant-microbe interactions that are classed as 'beneficial' normally relate to positive impacts on plant growth and development. This group of bacteria are generally termed plant growth promoting bacteria (PGP) and since the majority inhabit the rhizosphere or rhizoplane, they are termed PGP-rhizo-bacteria (PGPR). Metabolic flux frequently forms the basis to the interactions and whole system transcriptomic approaches have shown that over 50 % of the differentially expressed genes of respective bacteria and plant partners can be involved in metabolic processes, e.g. for a PGPR

*Burkholderia* spp. and sugar cane host (Paungfoo-Lonhienne et al., 2016). One of the best-known beneficial interaction is in biological nitrogen fixation, where the basis of the symbiotic interaction is rooted in metabolism and exchange of nutrients. In essence, it involves encapsulation of rhizobia in bacteroids (specialised compartments in plant root cells), where they carry out the conversion of atmospheric nitrogen to ammonia, in exchange for plant-derived carbohydrates. Since it has been expertly explained in more detail elsewhere, the reader is referred to excellent reviews on nitrogen fixation such as (Dixon and Kahn, 2004; Hayat et al., 2010; Frans J. de Bruijn, 2015).

Others rhizospheric bacteria (herein termed rhizobacteria), such as PGPR do not have such an intimate dependency on their hosts, but can still play a beneficial role albeit with more complex functional interactions. A major taxonomic group in the rhizosphere are the Firmicutes, which includes the model Gram positive species, *Bacillus subtilis*. Members of the *Bacillus* genus are capable of functions that benefit the plant host directly, such as mineral nutrient solubilisation, e.g. through the production of enzymes that solubilise organic phosphorus, mainly stored as insoluble myo-inositol hexaphosphate or phytate, into a plant usable form. They can also secret phytohormone mimics to induce plant growth and have indirect effects such as biocontrol and antibiosis that act on other members of the rhizosphere community in an antagonistic manner (Francis et al., 2010). Furthermore, PGPR can alter the plant defence response, which in turn impacts other members of the microbial community, again in a negative, competitive manner. Within this group, the spectrum of interactions is likely to be broad and specific to a particular bacteria-plant system, with different degrees of benefit to the host plant.

The pseudomonads comprise another major taxonomic group within the rhizosphere. Individual genotypes, even within a single species, are capable of a wide range of functional interactions with plant hosts, exemplified by *Pseudomonas fluorescens*. This is a diverse species that consisting of a complex of sub-species (Scales et al., 2014). On one hand, there are isolates that are known to promote plant growth (Silby et al., 2009; Redondo-Nieto et al., 2013) and some are available commercially as agricultural inputs. The basis to their functional activity, like some of the *Bacillus* species, is wide-ranging, dependent on a variety of traits including phytohormone production, biofilm
formation, siderophore activity and direct antimicrobial competition via production of antimicrobial compounds. Isolate-specific differences are evident, e.g. in their ability to tap into phytohormone signalling, where some isolates encode genes for IAA synthesis, while others produce ACC deaminases that promote root elongation (Shen et al., 2013). At the other end of the spectrum, some Ps. fluorescens isolates are able to cause soft-rot disease via the production of secreted enzymes to degrade pectin and other cell wall components (Silby et al., 2011). Much attention has been given to this organism as a model species for different metabolic attributes, from nitrogen fixation (Haahtela et al., 1983) to denitrification (Redondo-Nieto et al., 2013), and its ability to generate similar levels of ATP under different substrate limitations and stresses (Silby et al., 2009; Appanna et al., 2016).

**Opportunists**

One group of bacteria appear to be related to the PGPRs because of their ability to fix atmospheric nitrogen, although the metabolic flux appears to be tipped in favour of the bacteria. They are distinct from the 'ineffective' rhizobia that either cannot or do not fix nitrogen in a mutualistic fashion and still infect legumes (Denison and Kiers, 2004), but instead exist as free-living diazotrophs. While there are those that clearly play a beneficial role, e.g. *Herbaspirillum* spp. for which fixation of nitrogen in association with non-leguminous crop hosts has been demonstrated (James, 2000), there are others that for which the interaction is less likely to be of benefit to the plant hosts, e.g. for *Azotobacter* and *Klebsiella* spp.. *Klebsiella pneumonia* is probably best known for its ability to cause disease in humans as an opportunistic nosocomial pathogen, but some isolates are equally at home on plants (Brown and Seidler, 1973; Haahtela et al., 1986; Falomir et al., 2013) and other species, such as *K. oxytoca* are well known members of the soil and plant community (Bagley, 1985). Some isolates of *K. pneumonia* (and *K. oxytoca*) that have been isolated from plants encode genes for nitrogen fixation and are classed as diazotrophic, e.g. *K. pneumoniae* isolate Kp342 (Dong et al., 2003a; Fouts et al., 2008). Indeed, its interaction with plant hosts induces a host response (Iniguez et al., 2005) but the extent to which nutrient exchange takes place is less well established beyond an interaction with a particular variety of wheat (Red Baron) (Iniguez et al., 2004). It is possible that instead, these genes provide an extension of the bacterial metabolic capacity in environments where ammonia is lacking and insufficient oxygen induces an anaerobic physiological response, and thus play little or no beneficial role in the plant-bacteria interaction. Indeed, the minimal gene set for nitrogen fixation is present in at least 149 diazotrophic species (Dos Santos et al., 2012), including some species that have a well-characterised phytopathogenic interaction with the plant hosts, for example *Pectobacterium atrosepticum*, a species that causes devastating disease on potato (Bell et al., 2004). Together this supports the concept of an extended metabolic capacity that is induced under challenging physiochemical conditions.

*K. pneumoniae* and other rhizosphere bacteria that are nutritionally flexible form a functional group that are defined as generalists or could be termed 'opportunistic'. Broadly speaking, they neither benefit nor harm the plant hosts, but instead are attracted to the rhizosphere as a nutrient-rich ecological niche. Bacteria within this group are made up diverse taxonomic groups and are capable of a broad range of metabolic functions, able to exploit not only plant-derived rhizodeposits, but substrates released by other members of the community (Badri et al., 2009; Bakker et al., 2013). Other members of this group include some species that although they are likely to be present at very low densities, garner a good deal of attention. These include bacteria that cause disease to human and animal hosts, e.g. pathogenic *Escherichia coli* and non-typhoidal *Salmonella enterica* (Holden et al., 2009; Holden et al., 2015).

Plants were not considered to be hosts for bacteria that are commonly associated with animals until relatively recently. It was originally thought that foodborne illness as a result of consumption of contaminated fruit and vegetables had only arisen through transient transmission of foodborne pathogens on the food products. This would be akin to viruses and parasites that can only complete their replication cycle within a susceptible animal host, but have still been shown to be transmitted into the food chain via edible crops (Chancellor et al., 2006; Amoros et al., 2010; Macarisin et al., 2010; Made et al., 2013; Swinkels et al., 2014; Einoder-Moreno et al., 2016). However, numerous studies have shown that human pathogenic bacteria can interact with plants and use them as hosts, which by definition requires cell mass growth and division (Holden et al., 2015). Given the richness of species associated with the rhizosphere, it is perhaps not surprising that it was recognised as a reservoir for human pathogenic bacteria (Berg et al., 2005), although there are differences in colonisation ability between
the species, e.g. for a lab-adapted isolate of *E. coli* that compares relatively poorly to *S. enterica* serovar Cubana or an endophytic isolate of *K. pneumoniae* (Dong et al., 2003b). Colonisation in the opposite direction, from plants to humans has also been reported, e.g. for some isolates of well-characterised rhizobacteria such as *Ps. fluorescens* that behave as opportunistic human pathogens (Scales et al., 2014), and other human pathogens such as *Listeria monocytogenes* and *Pseudomonas aeruginosa* that are considered to be ‘environmental’ or soil-derived (Stover et al., 2000; Freitag et al., 2009). Therefore, these bacteria also fit within the group of rhizosphere metabolic opportunists.

*E. coli* and *S. enterica* are well-characterised model bacteria (Neidhardt and Curtiss, 1999) for which their metabolic capabilities have been thoroughly researched and are probably the best known of all bacteria. Given their metabolic flexibility, these bacteria can also be classed as ‘opportunist’ colonisers of plants. However, much of what we have learnt has been derived at temperatures that are relevant to mammalian hosts, which can be 20 °C higher or more than edible crops grown in temperate zones, thereby raising questions about relevance. Nonetheless, these are mesophilic bacteria capable of growth over a wide temperature range encompassing most plant growth requirements. Modelling based on multiple genomes of *E. coli* isolates, including established pathotypes has resulted in the generation of metabolic models for a pangenome and a core genome (Baumler et al., 2011; Monk et al., 2013). The *E. coli* EHEC pathotype that belongs to foodborne group and are frequently associated with contamination of edible crop species (Holden et al., 2009), encode unique genes for metabolic reactions that are relevant to plant-derived substrates, including salicylate hydroxylase, gentisate 1,2-dioxygenase, sucrose transport and fucose synthetase (Baumler et al., 2011). The function of these gene products points to a role for catabolism of plant derived metabolites and although *E. coli* are normally associated with vegetarian or omnivorous mammals and hence have access to plant-derived material in the animal gut, there is an obvious need to understand their response to plant hosts directly.

**Phytopathogens**

Research on plant pathogens generally focuses on the mechanisms of disease and how it can be prevented or reduced. As with pathogens of animals, it accepted that the underlying driver of pathogenesis is metabolism as a means to exploit the host as a source of metabolic substrates. This can be manifested by phytopathogens in the production of specialised enzymes to break cell walls, or in manipulation of the host defence to facilitate colonisation. The vast majority of characterised phytopathogens cause symptomatic damage to aerial plant tissue, e.g. fruit bodies or leaves and as such any reported interaction within the rhizosphere is either absent or if it occurs, is overlooked. One group that is recognised to cause damage to below-ground tissues are the soft-rot erwiniae, which cause a major economic threat to potato stolon production. The soft-rot erwiniae produce a plethora of enzymes, targeting not just pectin, but also cellulose in the plant cell wall (Harris et al., 1998). In fact, this metabolic function has been exploited in biotechnology as part of co-cultures used to reduce recalcitrant substrates to bio-ethanol (Grohmann et al., 1998). Development of symptomatic disease from cell-wall degradation has been shown to be a direct response to population density, e.g. for *Pectobacterium atrosepticum*, and production of cell-wall degrading enzymes only occurs in a quorum-dependent manner when the population becomes sufficiently large that there is a need to increase acquisition of nutrients through the active release of cytoplasmic contents (Toth and Birch, 2005). In a similar manner, quorum sensing-dependent colonisation and pathogenicity has been shown to occur for *Ps. aeruginosa*, resulting in necrotic lesions on the root tips of sweet basil and *Arabidopsis thaliana* (Walker et al., 2004).

Plants are not without their own defences, and responses to bacterial molecular patterns has been well established as a first-line defensive strategy, followed by more specific responses that counteract the function of bacterial effectors (Jones and Dangl, 2006). As part of the response, plants also produce secondary metabolites as active anti-microbial compounds, such as the inhibition of quorum sensing, which could prevent phytopathogens reaching numbers that cause cellular destruction. One of these is a phenolic antimicrobial compound, curcumin, which at sub-inhibitory concentrations has been shown to reduce *Ps. aeruginosa* (isolate PA01) pathogenicity on *A. thaliana* by interfering with quorum sensing (Rudrappa and Bais, 2008). However, it is notable that a pathway for curcumin catabolism via a curcumin/dihydrocurcumin reductase has been identified in *E. coli* (Hassaninasab et al., 2011), and indeed this enzyme was found to be induced in *E. coli* O157:H7 in response to spinach root exudates (Crozier et al.,
Bacterial Metabolism in the Rhizosphere (Holden, 2016). The genes for degradation of gentisate, a product of salicylic acid, were similarly induced. This raises the intriguing possibility that opportunists such as *E. coli* are able to exploit aspects of plant immunity for metabolism, although this has yet to be shown.

Metabolism of plant-associated bacteria is an active area of research and has reached the stage where metabolic networks can be constructed. These range from research for biotechnology to a fundamental understanding of the pathways involved. Examples include the identification of bactericides from *Pectobacterium carotovorum* for agricultural use (Wang et al., 2015); identification of differences between pathogenic and non-pathogenic pseudomonads (Mithani et al., 2011); the process of denitrification using *Ps. fluorescens* as a model (Arat et al., 2015); and for bioethanol production from plant-based feedstocks for *B. subtilis* (Qi et al., 2014). Metabolic pathway analysis can be reconstructed from gene expression data to examine substrate utilisation for the foodborne pathogen *E. coli* O157:H7 with different edible crop tissue types, such as spinach root exudates (Figure 1).

**Role of the plant host**

Technical advances in sequencing technology have facilitated detailed community and microbiome analysis, which is revolutionising microbiology. With the appropriate approaches and analysis, a more detailed picture of plant-microbe interactions is emerging, at not just a taxonomic but also functional level. What has become clear is that the rhizosphere-associated microbiota effectively increase the functional diversity of the rhizosphere by orders of magnitude (Bakker et al., 2013). One of

![Figure 1. KEGG pathway analysis of *E. coli* O157:H7 transcriptomic response to spinach root exudates. Gene expression microarray data derived from incubation of *E. coli* O157:H7 (isolate Sakai) in spinach root exudates for 1 hour (Crozier et al., 2016) was analysed with the KEGG pathways tools (Okuda et al., 2008). Genes that correspond to KEGG orthologs were mapped to the metabolic pathways KEGG map for *E. coli* K-12 (reference isolate MG1655). The fold-change gene expression data (i.e. exudates compared to the no-plant control) were log-transformed and scaled to a red (high) / blue (low) colour scheme. All other reactions/compounds were greyed-out (unpublished, courtesy of Leighton Pritchard, James Hutton Institute).](image)
these key aspects is driven by bacterial metabolism (Hacquard et al., 2015). One of the most profound findings is the strength of influence that rhizodeposits exert on microbial community composition and hence function (Wagner et al., 2016). Substrate-driven community recruitment has been reported for model species, A. thaliana, as well as non-model species such as wild oat (Avena fatua) (Bulgarelli et al., 2013). Microbiome analysis of the rhizosphere from different accessions of barley (wild and domesticated), showed a significant host genotype-dependent impact. However, the difference between barely genotypes was not particularly large, presumably because of their recent ancestry, in comparison to larger Family-level differences comparing barley with A. thaliana (Bulgarelli et al., 2015). Analysis of the functional groups in the barley rhizosphere community revealed enrichment in genes involved in plant-microbe interactions, e.g. in virulence and adherence, but also in siderophores and phosphotransferase systems (Bulgarelli et al., 2015). Logical questions that arise from such community-level research are in availability and localisation of plant-derived substrates.

Substrate availability

Poly saccharides
Various techniques have been applied to taking a bottom-up (genes to population) approach to identify the genetic basis to colonisation of the rhizosphere. One of the earlier reports took a positive selection approach, using in vivo expression technology, to show the contribution of metabolism-associated genes for Ps. fluorescens during colonisation of rhizosphere of sugar beet seedlings (Rainey, 1999). In a similar manner, whole genome transcriptomic analysis has been carried out to determine the response of foodborne pathogens to plants or plant extracts, focusing almost entirely on E. coli O157:H7 (Bergholz et al., 2009; Kyle et al., 2010; Fink et al., 2012; Hou et al., 2012; Hou et al., 2013; Landstorfer et al., 2014; Crozier et al., 2016; Linden et al., 2016). Although this global gene expression approach enables reconstruction of metabolic pathways, it is surprisingly difficult to find consistencies between the responses. This is likely due to differences in the experimental set-up that alter the physiochemical environment, one of the most important aspects of which is temperature. However, together the data support the view that these bacteria can function as opportunistic plant colonisers and that there is specificity in their response, dependent on the plant species and tissue type.

The plant cell wall represents a major evolutionary difference between plants and animals and as a consequence, genomic differences that relate to utilisation of cell-wall metabolites can serve as a basis for differentiation of plant- and animal-association of bacteria. The differences between the animal and plant kingdoms that relate to cell wall glycans as potential metabolites are in their mechanism of secretion; the linkages between glycans; and the glycans themselves. In plants, unique modification of N-glycans in the Golgi occur at the trimming stage (Etzler ME, 2009). After the addition of N-acetylglucosamine to the distal mannose of the core by N-acetylglucosamine transferase I (GnT-I), two specific plant modifications occur: the addition of xylose in β1-2 linkage to the core β-mannose, which is unique to plants; and the addition of fucose in α1-3-linkage to the asparagine-linked N-acetylglucosamine residue, which has also been found in invertebrates (Etzler ME, 2009). L-arabinose, like xylose, is a pentose that is unique to plants and green algae, and depending on the plant species, can account for 5 - 10 % of plant cell wall glycans (Kotake et al., 2016). It is mainly located in pectin as side chains on hennogalacturonan I (RG-I) and hennogalacturonan II (RG-II), and in arabinogalactans, O-linked hydroxylproline-rich glycoproteins (Mohnen, 2008). In addition to the presence of xylose on complex or hybrid N-linked glycoproteins, it is also present as xyloglucan, the major component of hemicellulose in higher plants, and as a minor component of pectin in xylogalacturonan (Etzler ME, 2009). Biosynthesis of L-arabinose occurs from epimerization of UDP-D-xylose (Burget et al., 2003). The action of phytopathogen cell-wall degrading enzymes provides access to this resource, e.g. the xylanolytic system, which is ubiquitous in lignocellulose-degrading microbes and has been well-characterised for the phytopathogenic xanthomonads (Santos et al., 2014).

In bacteria, metabolism of xylose is intrinsically linked to arabinose, since the low affinity transporter (AraE) can accommodate either pentose, and the master regulator (AraC) controls expression of both sets of metabolic genes, as shown for E. coli (Desai and Rao, 2010). Catabolite repression results in hierarchical utilisation of firstly arabinose followed by xylose. In E. coli O157:H7 the araBAD and xylBA loci were upregulated in response to spinach root exudates after just one hour (Crozier et al., 2016),
but downregulated after two days colonisation of lettuce roots (Linden et al., 2016), indicating a transient and dynamic response. Functional ability to catabolise arabinose has also been demonstrated during colonisation of the pea root rhizosphere by *Rhizobium leguminosarum* biovar *viciae*, using a signature-tagged mutagenesis approach that identified essential rhizosphere-colonisation genes (Garcia-Fraile et al., 2015). *Ps. fluorescens* colonisation of rhizosphere of sugar beet seedlings resulted in induction of genes for metabolism of xylose as well as complex N-compounds and sensory systems (Rainey, 1999), although subsequent characterisation of the genes revealed specificity in their functional roles on different species, e.g. for histidine sensing and utilisation (Zhang et al., 2006).

**Amino acids and cyclic compounds**

Roots secrete an array of biologically active compounds, including ions, water, oxygen, enzymes, mucilage and primary and secondary metabolites (Bais et al., 2006). This provides an enviable niche inhabited a rich diversity and density of microorganisms (Bakker et al., 2013). The rhizosphere of numerous plant species has been shown to support the growth of foodborne pathogenic bacteria, (Jablonske et al., 2005; Ibeke et al., 2009; Quilliam et al., 2012; Wright et al., 2013) (Wright *et al* MBT paper in press)(Semenov et al., 2010; Kisluk and Yaron, 2012; Mendes et al., 2013). Root exudates play an important role in attracting bacteria to the rhizosphere and are often metabolites in their own right (Huang et al., 2014). Amino acids released from alfalfa seedling exudates (tryptophan, methionine, lysine, and phenylalanine) were found to be depleted in the presence of *S. enterica* indicative of *de novo* amino acid metabolism (Kwan et al., 2015). Amino acid transport and metabolism has been shown to be one of the largest classes of shared genes in catabolism of plant-derived substrates, in a genomic comparison of PGPR *Ps. fluorescens* isolates. For three isolates in the *Ps. fluorescens* species complex (i.e. not *sensu stricto*) (GP72, Pf-5 and M18), the number of shared genes exceeds 500 (Shen et al., 2013). Genes for the catabolism of aromatic amino acids phenylalanine and tyrosine, as well as oxygenases for other aromatic compounds are well represented in the *Ps. fluorescens* group, and to a lesser extent also in the related rhizobacteria, *Ps. putida* (Shen et al., 2013).

**Micronutrients**

PGPR bacteria can contribute to plant growth through the solubilisation of phosphorus into plant-available forms. For *Ps. fluorescens* isolates, this has been demonstrated from enzyme activity of non-specific phosphatases, inositol phosphate phosphatases and C-P lyases (Shen et al., 2013). Similar functional solubilisation has been shown for Gram-positive PGPR, including isolates from *Bacillus*, *Brevibacterium*, *Sarcina*, *Paenibacillus*, *Corynebacterium* and *Micrococcus* species (Francis et al., 2010).

Iron limitation is known to be a factor affecting colonisation of plant-associated bacteria, e.g. *Ps. aeruginosa* (Sulochana et al., 2014), *Erwinia amylovora* (Smits and Duffy, 2011) and *Ps. syringae* pv. *tabaci* (Taguchi et al., 2010). Iron was found to be depleted in alfalfa exudates in the presence of *S. enterica* and correlated with increased enterobactin expression (Hao et al., 2012). A similar limitation of Fe**3+** was apparent for *E. coli* O157:H7 inoculated in root spinach exudates, resulting in induction of systems for ferric iron and haem transport, via enterobactin and Chu transport system respectively (Crozier et al., 2016). These studies have relied on extracts or plants grown under hydroponics systems, and there are inherent technical difficulties for such molecular mechanistic data from the roots and rhizosphere of soil/compost-grown plants. However, competition for iron has been postulated to be one of the possible mechanisms for the beneficial effects demonstrated by PGPR on phytopathogenic microbes (Wensing et al., 2010; Yu et al., 2011).

Excess micronutrients, such as heavy metals, can occur from anthropomorphic activities resulting in pollution. Plants that are capable of growing in polluted sites are termed metal hyperaccumulators, and are dependent on the metabolic functions of rhizobacteria for their resistance (Visioli et al., 2015). There are various examples of heavy metal accumulation with some evidence for links to the plant defence response by incorporation of the compounds to levels that are toxic to phytopathogens (Fones and Preston, 2013). There are also strong drivers within industrial biotechnology to identify rhizobacteria that can aid in bioremediation. For example, mobilisation of copper by a PGPR species, *Ps. putida* has been demonstrated in *Elsholtzia splendens*, a hyperaccumulator used in phytoremediation, by promoting copper redistribution in the plant root and translocation from root to shoot (Xu et al., 2015).
Substrate sensing

Metabolism and growth potential are inherently tied in to responsive changes in gene expression. Despite the nutritional abundances in the rhizosphere, it is a limiting environment in some respects, inducing physiological stress responses and resulting in restricted growth rates. Niche separation or partitioning is an accepted concept in soil microbiology (Lennon et al., 2012), and growth characteristics of soil bacteria have shown distinct phyla and order level differences in their utilisation of labile and recalcitrant carbon-based substrates (e.g. glycine / sucrose 'vs' cellulose / lignin / tannin-protein) (Goldfarb et al., 2011). As such, successful colonisation and establishment requires responsive sensing of the surrounding environment to obtain physical access to the appropriate substrates, followed by effective and efficient re-routing of the metabolic circuitry to best exploit the resources.

Chemotaxis

Bacteria respond to the presence of a substrate by moving down a gradient toward it. For the majority of species, this motility is flagella-driven via induction of chemotaxis genes (Sourjik and Wingreen, 2012), and a role for flagella in plant-associated bacteria has been well established in promoting root colonisation, e.g. for Ps. fluorescens (De Weger et al., 1987). In symbiotic bacteria, there are a number of chemoreceptors that aid targeting of the bacteria host interaction, for example with Sinorhizobium meliloti in sensing its host Medicago sativa (Webb et al., 2016). Once the interaction has been established and bacteria growth commences, this function is down-regulated, as shown for Ps. fluorescens growing in the presence of sugarbeet (B. vulgaris L.) root exudates (Mark et al., 2005). The presence of flagella was shown to contribute to survival of S. enterica serovar Dublin in manure-amended soil (Olsen et al., 2012) and a chemotactic response occurred for S. enterica serovar Typhimurium on lettuce leaves (Kroupitski et al., 2009). Both serovars, Typhimurium and Dublin were shown to exhibit chemotaxis towards, and induction of metabolism genes in response to the presence of root exudates from lettuce plants (Klerks et al., 2007).

The concept of direct sensing or responding to physiochemical changes has been well documented with description of riboswitches. These are natural aptamers imbedded in the gene leader sequences (5'UTR) that selectively bind small molecules such as second messengers, and have been reported for numerous metabolic genes, e.g. vitamins and amino acids (Nudler and Mironov, 2004). In a recent study in Ps. putida, in vivo characterisation of the riboswitch that binds the active form of thiamine (vitamin B1, TPP) to represses the expression of thiamine-related genes, showed that the riboswitch acted at the translational level by interfering with RBS-ribosome recognition (D’Arrigo et al., 2016). Riboswitch function has been exploited in synthetic biology for a range of uses, including the potential removal of agri-chemicals or other pollutants. The chemotaxis locus cheX in E. coli was reprogrammed to respond to the herbicide atrazine, and incorporation of genes for metabolism of atrazine generated a strain that is capable of sensing, chemotaxis and degradation of the compound (Sinha et al., 2010).

Two-component systems

Two-component systems are the primary mechanism for sensing substrates. They act at the transcriptional level, via sensing and signal transduction, often involving a cascade of regulation from the global transcriptional response-regulator. For example, the barA-uvrY system (and its orthologues) is widespread in bacteria and is involved in the regulation of multiple responses including metabolism (Sahu et al., 2003). In the γ-Proteobacteria it controls expression of the carbon storage regulator (csr) / repressor of stationary phase metabolites (rsm) system, a widespread regulatory metabolic network, by binding regulatory elements in an antagonistic manner (Zere et al., 2015). A role for rsm has been shown for Ps. fluorescens (Valverde et al., 2004), but it is generally reported for this genus in the context of biofilm formation, for example for a plant-growth promoting isolate of Ps. putida (Huertas-Rosales et al., 2016). Another well described two-component regulatory system is ntrB-ntrC, which is activated under nitrogen limiting conditions for nitrogen uptake and metabolism. In Ps. fluorescens, its link to motility was elegantly demonstrated in an experimental evolution approach using a non-motile mutant that lacks both flagella and viscosin motility. Following repeated culturing on motility medium the strain became partly motile via mutation in the ntrB gene, which resulted in over-activation of nitrogen regulation, uptake and metabolism genes. This was followed by a compensatory mutation in ntrC, which reduced the expression of nitrogen uptake and metabolism genes further up-regulated flagellar and chemotaxis gene expression to WT levels (Taylor et al., 2015).
Growth rates and translation

Substrate sensing and induction of the appropriate metabolic pathways is fundamental to successful establishment on the plant hosts and growth is inherently tied into transcription and translation. Alterations in the physiochemical environment impact directly on ribosome structure and function and therefore have a direct impact on effective translation. Functioning bacterial ribosomes are the 70S particles that comprise two subunits, 30S and 50S. In *E. coli*, the small subunit, 30S, is made of 16S rRNA (1,542 nt) and 21 ribosomal proteins (r-proteins), while the large subunit, 50S, is composed of two rRNAs, 23S (2,904 nt) and 5S (120 nt) rRNA, and 33 proteins (Kaczanowska and Rydén-Aulin, 2007). RNA chaperones, helicases and ribosome-dependent GTPases play a role in assisting with RNA folding, by resolving and destabilising incorrect RNA structures. As such, some RNA helicases are involved in ribosome biogenesis, e.g. CsdA, a cold-shock inducible ATP-independent RNA helicase, is a member of the DEAD-box family and is involved in 50S biogenesis (Kaczanowska and Rydén-Aulin, 2007). This protein was found to be induced in *E. coli* O157:H7 (accession # ECs4043) on exposure to spinach leaf lysates when the bacteria were able to grow, but was repressed in a spinach root exudate extract that did not support growth (Crozier et al., 2016). It had been established that under these conditions, the bacterial cultures did not experience any temperature shifts, including a cold shock, but the shift from minimal synthetic medium to plant extracts did result in induction of some of the cold-stress genes. This was indicative of translational stalling by the ribosomes are the metabolic pathways were re-routed to adjust to the different metabolites and explains a role for CsdA. Furthermore, RNA chaperones that are linked to translation inhibition, CspA and CspG, were induced, coincident with induction of spoT, a marker of the stringent response (Crozier et al., 2016). A similar adjustment was observed for *E. coli* O157:H7 on contact with growing lettuce plants (Linden et al., 2016).

Growth rate correlates with the amount of functional ribosomes, not just the total amount of ribosomes. In stationary phase, excess ribosomes are stored as 100S particles, which are dimers of 70S. Conversion of the 70S ribosomes to the 100S is regarded as a control mechanism, storing unused ribosomes in an inactive form, and also protecting ribosomes from degradation by proteases and nucleases induced in stationary phase (Wada, 1998). The ribosome modulation factor (RMF) plays a key role ribosome function and translation inhibition by RMF occurs partly as a result of loss of amino acyl tRNA binding on conversion to 100S ribosomal particles. Dissociation of 100S and release of RMF to 70S ribosomes regains high translation activity. *rmf* mRNA accumulates in the transition from log to stationary phase and continues in stationary phase. However, *rmf* can also be detected during exponential phase in slow-growing cells so that level of expression is inversely related to growth rate (Wada, 1998). This correlation was seen for *E. coli* O157:H7 on exposure to plant extracts that either did or did not support growth, where *rmf* levels were significantly repressed or induced, respectively (Crozier et al., 2016).

Measurement of growth rates of rhizosphere bacteria is challenging and data vary depending on the approach taken, but it fair to say that doubling times are slow in comparison to growth under laboratory conditions. For example, generation times in the order of 106 hours have been reported for *Pseudomonas* species on sugar beet seedlings in sterilised soil (Christensen et al., 1989). Although this data contrasts with data obtained from modelling with ~ 4.6 cells per day for *Pseudomonas* species or ~ 0.6 for *Bacillus* species and one - four cells per day for most species, (Watt et al., 2006), the generation times are substantially greater than seen in animals and are known to be notoriously difficult to measure accurately. A similarly slow generation time was recorded for *E. coli* O157:H7 colonisation of ~ 1.3 day *Nicotiana benthamiana* (leaf apoplast), albeit at a different tissue site from the rhizosphere (Kaths paper), which contrasts with a generation time for the same bacteria in bovine mucus of ~ 3.4 hrs (Bai et al., 2011).

Conclusion and future developments

Detailed molecular analysis of individual systems has elucidated many of the metabolic processes that occur for rhizobacteria. Although the rhizosphere remains a technically challenging habitat to study, this reductionist approach has yielded many pieces of the puzzle to show the dynamic nature of metabolism within the rhizosphere and the flow of metabolites through the system. The research has now reached a stage where these individual pieces can be put together to produce a more coherent picture that shows how different members of the community capable of different metabolic functions combine to generate a complete and functioning rhizosphere, appropriate to any given habitat.
Technological advances in microbiology, such as deep sequencing and in situ spectroscopy have opened our eyes to the extraordinary diversity of microbiota associated with the rhizosphere. Although there is a good understanding of gross-level functions, principally in metabolic processes, metagenomics approaches are on the cusp of providing rhizosphere community-level functions in far greater detail. Key areas for future research relate to crop health and productivity, bioremediation of contaminated land, and bio-mining of rhizosphere-derived secondary metabolites and compounds for the pharmaceutical and biotechnology industries.

A promising technological advance is in the application of single cell approaches within a mixed community. Combining Raman spectroscopy with stable isotope labelling has been used to speciate bacteria and determine metabolic functions, for example uncovering a role for an unculturable species (Acidovorax sp.) in the degradation of polycyclic aromatic hydrocarbons in groundwater (Huang et al., 2009). Technical limitations that arise from the lack of some carbon-labelled substrates, such as citrate, have been overcome by the addition of labelled water and a reverse-label approach, where the reversion from labelled to unlabelled cells is detected. This has been successfully used to show that in a mixed culture, E. coli, which does not encode citrate catabolism genes, can profit from the ability of Acinetobacter baylyi to metabolise citrate, and grow (Wang et al., 2016). Although this elegant demonstration was carried out under laboratory conditions, it has the potential to be developed for in situ examination in complex environments such as the rhizosphere.

Work on below-ground interactions has always been hampered by technical challenges working with the soil matrix, e.g. the presence of inhibitors that impact any PCR-based examination of rhizosphere bacteria (Holmes et al., 2014), as well as in situ work on the roots and rhizosphere. Various techniques have been applied for in situ visualisation, with varying degrees of resolution. However, the development of artificial substrates that permit normal root development hold much promise. Transparent soil particles that have the same refractive index of water and have surface ionic exchange properties, allows seamless penetration of light for microscopic examination and have been used to examine in situ rhizosphere colonisation for E. coli on lettuce seedlings (Downie et al., 2012). An obvious extension of this is for use with mixed rhizosphere communities, labelled with different fluorescent reporters.

Technological developments have brought together mixed disciplines with the aim of better understanding rhizosphere functions and interactions. Future questions, whether on exploitation for crop productivity or in control of pathogens for crop health, will rely on multi-disciplinary teams to continue to uncover hitherto unknown functions for this fascinating habitat.

References


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