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Chemotaxis signaling systems in model beneficial plant–bacteria associations

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Abstract Beneficial plant–microbe associations play critical roles in plant health. Bacterial chemotaxis provides a competitive advantage to motile flagellated bacteria in colonization of plant root surfaces, which is a prerequisite for the establishment of beneficial associations. Chemotaxis signaling enables motile soil bacteria to sense and respond to gradients of chemical compounds released by plant roots. This process allows bacteria to actively swim towards plant roots and is thus critical for competitive root surface colonization. The complete genome sequences of several plant-associated bacterial species indicate the presence of multiple chemotaxis systems and a large number of chemoreceptors. Further, most soil bacteria are motile and capable of chemotaxis, and chemotaxis-encoding genes are enriched in the bacteria found in the rhizosphere compared to the bulk soil. This review compares the architecture and diversity of chemotaxis signaling systems in model beneficial plant-associated bacteria and discusses their relevance to the rhizosphere lifestyle. While it is unclear how controlling chemotaxis via multiple parallel chemotaxis systems provides a competitive advantage to certain bacterial species, the presence of a larger number of chemoreceptors is likely to contribute to the ability of motile bacteria to survive in the soil and to compete for root surface colonization.

Keywords Flagella · Motility · Nitrogen fixation · Rhizosphere · Signal transduction · Symbiosis

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Introduction

Plant growth and productivity depend on the soil type and architecture, and on the activity of diverse microbes associated with plant roots. A range of associations between microbes and plant roots, from pathogenic or symbiotic to commensals, can be established. For soil bacteria, which live in spatially and temporally heterogeneous environments, the ability to locate niches that support optimum growth in the rhizosphere is often critical to their survival. Abundant experimental evidence shows that chemotaxis, the ability of motile bacteria to direct their movement in gradients of chemorepellents and chemoattractants, enhances the ability of soil bacteria to colonize the roots of diverse plant hosts (Ames and Bergman 1981; Bais et al. 2006; Bauer and Caetano-Anollés 1990; Berendsen et al. 2012; Caetano-Anollés et al. 1988b; Dharmatilake and Bauer 1992; Gulash et al. 1984; Reinhold et al. 1985). Furthermore, the genomes of most soil bacteria analyzed to date contain chemotaxis and motility genes, an observation that emphasizes the competitive advantage of chemotaxis in this environment.

A nutrient gradient is formed in the soil by plant-released root exudates and rhizodeposits, which chemotactically attract diverse motile bacteria (Badri et al. 2009; Bais et al. 2004; Bais et al. 2006). Root colonization may be initiated at the root hair zones, the root tips and the points of emergence of secondary roots, suggesting that

these sites release copious amount of exudates [e.g., (Gulash et al. 1984; McDougall and Rovira 1970; Vande Broek et al. 1998)]. Root exudates composition may vary with plant species, stage of development, or environmental conditions, leading to the recruitment and growth stimulation of different members of the rhizosphere microbial communities (Badri et al. 2009; Dennis et al. 2010; Lakshmanan et al. 2014). Recent evidence also indicates that plants may actively recruit specific microbes to the rhizosphere, including those supporting plant growth under conditions of stress, via modulation of root exudates composition (Berendsen et al. 2012; Lakshmanan et al. 2014; Walker et al. 2003).

Many motile soil bacteria recruited to the rhizosphere via chemotaxis toward root exudates are beneficial to plant productivity (Berendsen et al. 2012; Walker et al. 2003). Chemotaxis has been studied in detail and characterized at the molecular level in several beneficial soil bacteria. This review focuses on sensing and signaling during chemotaxis in the following widespread rhizosphere beneficial bacteria that are motile by flagella, namely *Azospirillum brasilense*, *Rhizobium leguminosarum* and *Sinorhizobium meliloti*. Their chemotaxis systems represent a range of structural characteristics that are widespread in soil and rhizosphere bacteria. Despite the importance of beneficial *Pseudomonas* species in the rhizosphere, their chemotaxis systems will not be covered here. Instead we refer to a recent review article by Sampedro et al. (2015). The present review illustrates the diversity of chemotaxis systems, including sensing, signal transduction and regulation, and their role in the establishment of beneficial associations between soil bacteria and plant roots.

Chemotaxis paradigm and diversity in beneficial plant associated bacteria

Chemotaxis in the model organism *Escherichia coli*

The chemotaxis signal transduction system (Che) was first described in *Escherichia coli*. A number of behavioral, genetic, biochemical and biophysical studies conducted over several decades on the *E. coli* Che system led to a detailed molecular understanding of how chemotaxis functions to navigate this bacterium in gradients of various chemicals (Hazelbauer 2012; Parkinson et al. 2015). Analysis of chemotaxis in several other bacterial species, followed by comparative genomics in recent years, established the conservation of basic chemotaxis principles identified in *E. coli* (Wadhams and Armitage 2004). In *E. coli*, chemoreceptors (called methyl-accepting chemotaxis proteins or chemotaxis transducers) are located in the polar regions of motile cells where they form large membrane-bound arrays that relay sensory information to interacting cytoplasmic proteins. The cytoplasmic chemotaxis signal transduction system comprises a histidine kinase named CheA, a scaffolding protein, CheW, that bridges CheA and the signaling domain of chemoreceptors, and the response regulator, CheY, which is phosphorylated upon phosphate transfer from phospho-CheA. The major signaling output of the chemotaxis pathway regulates the phosphorylation state of the CheY response regulator. CheY$\sim$P ultimately controls the direction of flagellar motor rotation via interaction with the flagellar switch complex, and thus the probability of switching the direction of rotation of the flagellar motors and the occurrence of tumbles that reorient the cell in a new swimming direction. In *E. coli*, environmental cues are sensed by five dedicated chemoreceptors (Parkinson et al. 2015). All chemoreceptors contain a highly conserved domain (HCD) flanked by two methylation helices comprising a cytoplasmic signaling module. Upon attractant or repellent binding to the periplasmic domain of transmembrane chemoreceptors, a conformational change occurs and is transmitted through the membrane to the cytoplasmic signaling domain. This induced conformational change modulates the kinase activity of CheA. In addition, the combined activity of a constitutively active methyltransferase, named CheR, and a methylesterase, which is activated by transfer of phosphate from CheA$\sim$P, reset the sensitivity of chemoreceptors following ligand-induced conformational changes. Methylation and demethylation of chemoreceptors by CheR and CheB$\sim$P is kinetically slower than phospho group transfer between CheA$\sim$P and CheY, introducing a short time delay between chemotaxis signaling excitation and adaptation and functions as the bacterial chemotaxis "memory" (Wadhams and Armitage 2004).

Additional chemotaxis components in beneficial plant associated bacteria

Most sequenced bacterial genomes, especially of species found in soils and sediments, possess an average of two or more chemotaxis pathways (Buchan et al. 2010) (Table 1). Chemotaxis signaling is thus prevalent in bacteria occupying these environments and likely to provide a competitive advantage. A recent phylogenomics analysis (Wuichet and Zhulin 2010) identified 18 classes of chemotaxis systems: 16 classes comprise Che systems that control flagellar-based motility (F1 through F16 classes), one class includes Che systems controlling type IV pili motility (Tfp class) and one includes Che systems that control cellular functions other than motility (alternative cellular function or ACF class), such as cyst differentiation (Berleman and Bauer 2005) or development (Kirby and Zusman 2003).

Table 1 Genus, species and strain diversity in chemotaxis pathways and number of chemoreceptors in the complete genome sequences of closely related beneficial plant-associated bacteria

Species	Strain	Number of predicted *mcp* genes	Number of chemotaxis systems	Genome size (kb)
Azorhizobium caulinodans	ORS 571	43	1	5.4
Agrobacterium radiobacter	K84	19	1	7.3
Agrobacterium sp.	H13-3	22	2	5.6
Agrobacterium tumefaciens	C58	20	1	5.7
Agrobacterium vitis	S4	43	2	6.3
Azospirillum amazonense	Y2	40	3	6.7
Azospirillum brasilense	Sp245	51	4	7.5
Azospirillum brasilense	Az39	49	4	7.4
Azospirillum lipoferum	4B	63	5	6.8
Azospirillum thiophilum	DSM21654	55	4	7.6
Azospirillum sp.	B510	89	6	7.6
Bradyrhizobium japonicum	USDA 110	36	3	9.1
Bradyrhizobium japonicum	USDA 6	30	3	9.2
Bradyrhizobium sp.	BTAi1	60	5	8.5
Bradyrhizobium sp.	ORS278	50	4	7.5
Rhizobium etli	CIAT 652	27	2	6.4
Rhizobium etli	CFN42	26	2	6.5
Rhizobium leguminosarum bv. *viciae*	3841	25	2	7.8
Rhizobium leguminosarum bv. *trifolii*	WSM1325	29	2	7.4
Rhizobium leguminosarum bv. *trifolii*	WU95	28	2	7
Rhizobium leguminosarum bv. *viciae*	USDA 2370	18	1	5.2
Rhizobium leucaenae	USDA9039	22	2	6.7
Rhizobium lupini	HPC(L)	16	1	5.3
Rhizobium phaseoli	Ch24-10	29	2	7.11
Rhizobium tropici	CIAT 899	14	1	6.7
Rhizobium sp.	IRBG74	21	1	5.5
Sinorhizobium fredii	NGR234	18	2	6.9
Sinorhizobium fredii	HH103	18	2	7.8
Sinorhizobium medicae	WSM419	9	2	6.8
Sinorhizobium meliloti	1021	9	2	6.7
Sinorhizobium meliloti	GR4	8	2	7.1
Sinorhizobium meliloti	Rm41	9	2	7.1
Sinorhizobium meliloti	2011	9	2	6.7

Since orthologous *che* operons appear to perform different functions in various organisms, functional assignment of chemotaxis systems based on sequence alone is not straightforward: for example, the F5 chemotaxis pathway named Che1 regulates all flagellar-dependent taxis responses in *Rhodospirillum centenum*, while the orthologous F5 chemotaxis system in *A. brasilense*, also called Che1, has a minor role in controlling taxis responses (Bible et al. 2008, 2012; Hauwaerts et al. 2002). Interestingly, the F7 class of chemotaxis systems is prevalent in rhizosphere bacteria (Buchan et al. 2010; Wisniewski-Dyé et al. 2011), suggesting that specific characteristics of the F7 Che

system provide enhanced chemotaxis in this environment. The genomes of *A. brasilense* (Wisniewski-Dyé et al. 2011), *R. leguminosarum* (Young et al. 2006) and *S. meliloti* (Galibert et al. 2001) encode two, two and four Che systems, respectively. All three species utilize a single, F7 class chemotaxis system as the major system controlling chemotaxis responses and competitive root colonization (see below) (Fig. 1).

In addition to multiple chemotaxis systems, there is a significant variation in the number and type of chemotaxis proteins and receptors encoded in the genomes of plant-associated bacteria (Table 1). One significant variation is
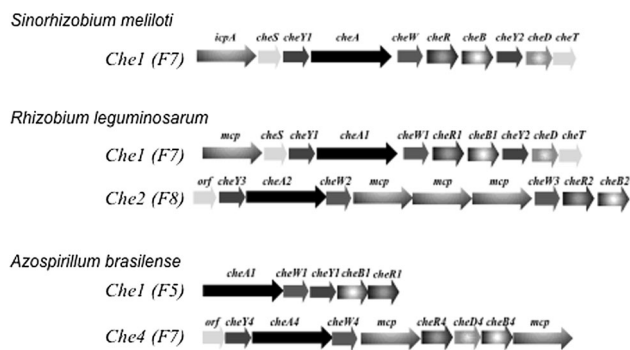
Fig. 1 Architecture of the *che* operons controlling chemotaxis responses. The gene content of *che* systems from *Sinorhizobium meliloti* (*top*), *Rhizobium leguminosarum* (*middle*) and *Azospirillum brasilense* (*bottom*) are shown as arrows indicating the direction of transcription. The operon flagellar class is indicated in *brackets* next to each operon's name. See text for details on this classification

the occurrence of more than one homolog of CheY. For example, *S. meliloti* possesses two, *R. leguminosarum* has three, while *A. brasilense* has seven CheY homologs. Not all CheY homologs directly affect the flagellar motors. In *S. meliloti*, CheY2 controls flagellar motor rotation while CheY1, which also interacts with CheA, acts as a phosphate sink to promote signal termination (Sourjik and Schmitt 1996, 1998). In addition, there are differences in flagellar motor function: *R. leguminosarum* and *S. meliloti* possess a unidirectional flagellar motor while *A. brasilense* possesses a bidirectional motor, similar to that of *E. coli*. Motor directionality correlates with different effects of CheY∼P, the output of the signaling pathway, on flagellar rotation. The output of the chemotaxis pathway triggers a change in the rotational speed of the flagellar motors in *S. meliloti* (Attmannspacher et al. 2005; Platzer et al. 1997) and *R. leguminosarum* (Miller et al. 2007). Therefore, tumbles are produced by asynchronous rotational speed of individual flagella in these species (Scharf 2002). In *A. brasilense*, chemotaxis signaling causes a change in both the swimming speed and the direction of flagellar rotation (Bible et al. 2012). In addition to the core chemotaxis components, genes encoding accessory proteins that are not found in the *E. coli* Che system are also present in the genome of many plant associated bacteria. Such proteins include homologs of the CheC phosphatase, which promotes signal termination by enhancing CheY∼P dephosphorylation and the CheD deamidase, which modifies conserved residues in the signaling domain of chemoreceptors and modulates their activity, as experimentally shown in *B. subtilis* (Rosario et al. 1995; Kristich and Ordal 2002). CheD is found in the F7 class Che system and thus it is present in the genome of *S. meliloti*, *R. leguminosarum* and *A. brasilense* (Fig. 1), but only the two latter species possess CheC, which is encoded outside of the *che* operons. The diversity in chemotaxis systems of beneficial

plant-associated bacteria thus includes a greater number of Che pathways, chemoreceptors and ancillary proteins for signal termination and adaptation. The increased complexity of chemotaxis signaling compared to the *E. coli* Che system is widespread in other soil and plant-associated bacteria, suggesting that additional chemotaxis components provide a specific competitive advantage in these environments.

Chemotaxis systems in *S. meliloti*

There are two chemotaxis systems. The chromosomal *che1* operon (F7 class) contains ten genes and in-frame deletions in any of these genes result in abolished or diminished chemotaxis (Dogra et al. 2012; Meier et al. 2007; Sourjik and Schmitt 1996; Scharf, unpublished results). In addition to the canonical chemotaxis genes, the *che1* operon contains a gene coding for the receptor-modifying deamidase CheD, the chemoreceptor gene, *icpA*, and two open reading frames coding for novel proteins, CheS and CheT. CheS and CheT have no counterparts in enteric bacteria, but display similarities to unassigned genes in other members of alpha-proteobacteria Ulrich and Zhulin 2009). CheS facilitates an efficient drainage of the phosphate sink by forming a tight complex with the kinase CheA, which allows an accelerated dephosphorylation of CheY1 (Dogra et al. 2012). A *cheT* deletion strain has the same phenotype as a *cheY2* or *cheA* deletion strain, but its exact function is currently under investigation (Scharf, unpublished results).

The *che2* operon (ACF class) is located on the pSymA plasmid and contains five core chemotaxis genes, namely *cheR*, *cheW*, *mcpS*, *cheA-REC* (encoding a hybrid protein with a REC domain fused to the C-terminus of CheA), and *cheB* (Barnett et al. 2001; Galibert et al. 2001; Meier et al. 2007). In-frame deletion of *mcpS* has no effect on chemotaxis (Meier et al. 2007). In addition, analysis of translational fusions of McpS with green fluorescent protein and transcriptional fusions of the upstream region of *che2* with a *lacZ* reporter gene gave no indication of gene expression under liquid culture growth (Meier and Scharf 2009). Therefore, chemotaxis in *S. meliloti* appears to be mediated by one system, Che1, whereas a role of Che2 in chemotaxis can be excluded.

Chemotaxis systems in *R. leguminosarum*

Rhizobium leguminosarum also possesses two chemotaxis systems, Che1 (F7 class) and Che2 (F8 class) (Fig. 1). In contrast to *S. meliloti*, both *che1* and *che2* are expressed in liquid cultures of *R. leguminosarum* (Miller et al. 2007). The *R. leguminosarum* Che1 is orthologous to the *S. meliloti* Che1 and essential for chemotaxis because mutations in *che1* yield a null phenotype (Miller et al. 2007).

The role of Che2 in chemotaxis is more subtle and, likely, indirect: a mutation abolishing Che2 function has no significant effect on chemotaxis, but over-expressing $cheB_2$ from this pathway affects chemotaxis, probably via chemoreceptor modification. This suggests that Che2 has a role in fine-tuning the chemotaxis response mediated by Che1 signaling. However, the direct signaling output of Che2 remains unknown.

Chemotaxis systems in *A. brasilense*

The genome of *A. brasilense* encodes four Che pathways named Che1, Che2, Che3 and Che4. Phylogenetic analysis assigned Che1, Che2, and Che4 to flagellar motility Che pathways of the F5, F9, and F7 classes, respectively, while Che3 is an ACF-type pathway (Wisniewski-Dyé et al. 2011). Che1 comprises a full set of chemotaxis proteins, including CheA-REC (hybrid CheA harboring a C-terminal REC domain), CheY, CheW, CheB and CheR (Hauwaerts et al. 2002). Experimental evidence indicates that Che1 controls a transient increase in swimming speed in response to attractants (Bible et al. 2008, 2012) but does not change swimming direction directly (Bible et al. 2008), suggesting that another chemotaxis system provides this function. Consistently, the Che1 system has a minor role, if any, in plant root colonization (Siuti et al. 2011), though chemotaxis is essential for colonization of the rhizosphere by *A. brasilense* (Greer-Phillips et al. 2004). While the identity of the major chemotaxis pathway has not been experimentally demonstrated, Che4 is the most likely candidate for the following reasons: *che2* is not expressed under laboratory conditions (Xie and Alexandre, unpublished results) and *che3* is predicted to belong to the ACF class of chemotaxis systems (Wisniewski-Dyé et al. 2011). Therefore, at least two chemotaxis systems control chemotaxis responses in *A. brasilense*, which parallels the suggested regulation of chemotaxis in *R. leguminosarum* (Fig. 1). The underlying advantage provided by two chemotaxis systems is not obvious given that chemotaxis is efficient in species using only one system, such as *E. coli* or *S. meliloti*. Both *A. brasilense* and *R. leguminosarum*, possess a greater number of chemoreceptors than *E. coli* or *S. meliloti*, which could suggest a threshold in the number of chemoreceptors above which additional Che systems enhance chemotaxis responses.

Regulation of chemotaxis and flagellar gene expression in plant associated bacteria

Chemotaxis and motility provide a competitive advantage in colonization of the root surface. Thus, expression of flagellar and chemotaxis genes is strictly coordinated. In addition to the structural diversity of chemotaxis systems, plant-associated bacteria differ from *E. coli* in their expression patterns of flagellar and chemotaxis genes. These differences may be directly related to their metabolic versatility and rhizosphere lifestyle.

In *S. meliloti*, all *che* genes, with the exception of genes encoding for chemoreceptors, flagellar (*fla*, *flg*, *flh*, and *fli*), motility (*mot*), and regulatory genes (*visN*, *visR*, *rem*, *flbT*) are clustered in one contiguous chromosomal region, the flagellar regulon (Galibert et al. 2001; Sourjik et al. 1998). The expression of genes in the flagellar regulon is organized as a four-class hierarchy: class IA comprises the master regulatory genes, *visN* and *visR*; class IB, the transcription factor encoding *rem*; class II, controlled by Rem, includes flagellar assembly and motility genes; and class III contains flagellin and chemotaxis genes requiring class II for expression (Fig. 2a). The LuxR-type global transcription activator VisNR is constitutively expressed during liquid culture growth (Sourjik et al. 2000). In contrast, expression of the OmpR-like transcription factor Rem is confined to exponential growth and it thereby acts as a temporal determinant of swimming motility (Rotter et al. 2006). Motility in *S. meliloti* is also controlled by factors outside of the flagellar regulon. Upstream of VisNR, flagellar motility and chemotaxis is repressed by the Sin/ExpR quorum-sensing-based transcriptional regulation program (Hoang et al. 2008; McIntosh et al. 2008, 2009; Zatakia et al. 2014) and through the ExoR/ExoS/ChvI pathway (Yao et al. 2004). Cell density is thus a key controlling factor of motility and chemotaxis in this species. In addition, the Ros-like zinc finger protein MucR, a regulator of exopolysaccharide (EPS) production, inhibits expression of *rem* (Bahlawane et al. 2008), while the symbiosis regulator CbrA positively controls transcription of *visN* and *visR* (Gibson et al. 2007). Lastly, the small protein EmmA and the two-component system EmmB-EmmC are found to regulate motility, EPS production, and nodule formation. Interestingly, *emm* appears to not only affect motility through VisNR, but may have multiple targets in the motility pathway (Morris and González 2009). Thus, coupling of these regulatory systems provides a dynamic and precise control of cellular processes important for host interaction (Charoenpanich et al. 2013; Janczarek 2011). In particular, the integrated control of motility and chemotaxis by quorum sensing, master regulators of EPS and nodulation ensures that cells remain motile until they initiate symbiosis or establish a dense biofilm on the root surfaces of non-legumes.

The regulation of expression of chemotaxis and motility genes in *R. leguminosarum* exhibits a few differences from *S. meliloti*. While the basic VisN/R—Rem pathway is conserved and required for expression of the major flagellin genes and the primary (*che1*) chemotaxis operon, the secondary (*che2*) chemotaxis operons, some *mcp* genes,

Fig. 2 Regulatory cascades controlling expression of chemotaxis and motility genes in *S. meliloti* (**a**) and *R. leguminosarum* (**b**). The stepwise assembly of flagella is reflected by a regulatory cascade of four classes. Operons are indicated as *horizontal arrows*, the corresponding gene products as *ellipsoids*. Positive and negative transcription controls are shown as *black arrows* and *blunt ended lines*, respectively, and translation to gene products as *open arrows*. Positive transcription controls are shown as *vertical black arrows* and translation to gene products as *open arrows*
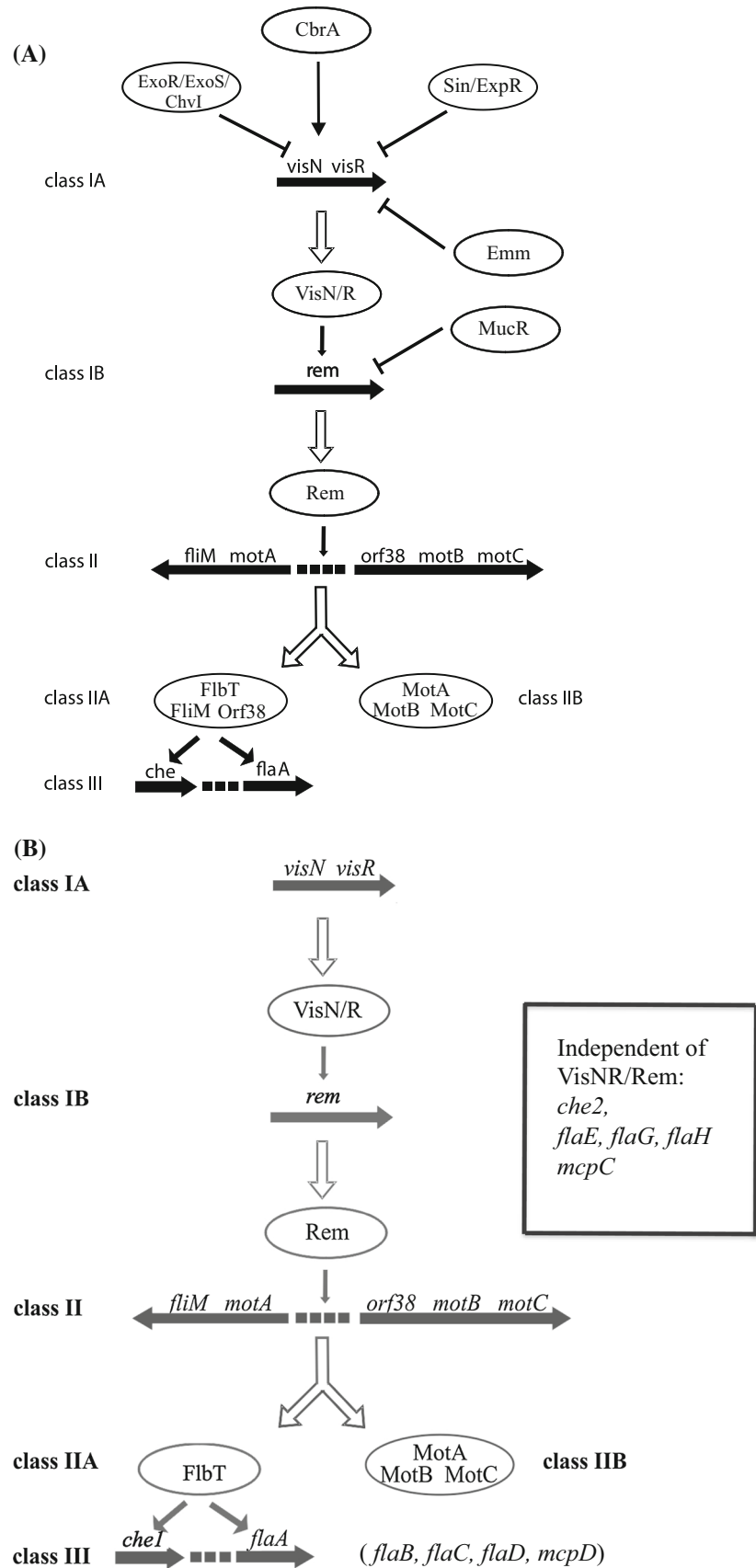
and the minor flagellin genes are not dependent on this regulatory cascade (Tambalo et al. 2010) (Fig. 2b). In contrast to *S. meliloti*, the expression of the Rem-dependent genes remains high in late exponential and stationary phase, and evidence for down-regulation through quorum sensing has not been found. All chemotaxis and motility genes examined, including the regulatory genes *visN*, *visR* and *rem*, are down-regulated in the nodule (Tambalo et al. 2010; Yost et al. 2004), although the nodulation stage at which regulation occurs remains unknown. This down-regulation was also observed in microarray studies of both *S. meliloti* (Becker et al. 2004; Capela et al. 2006) and *R. leguminosarum* (Karunakaran et al. 2009). Interestingly, the mechanism of down-regulation is independent of known symbiotic regulators.

Little information is available regarding the regulation of flagellar and chemotaxis gene expression in *A. brasilense*. This bacterium is motile by means of two types of flagella: a single polar flagellum mediates motility in liquid environments (swimming) and peritrichous lateral flagella are produced in response to growth on surfaces to promote motility under viscous conditions (Moens et al. 1995, 1996). *A. brasilense* cells are motile under all growth conditions tested, and loss of motility only occurs upon exposure to persistent and severe nutrient starvation and metabolic stress (Sadasivan and Neyra 1985) underscoring the role of motility in this species. This pattern of expression parallels that of the *che1* and *che4* chemotaxis systems, which are also constitutively expressed in *A. brasilense* (Xie and Alexandre, unpublished results). The *A. brasilense* genome lacks evidence of a complete quorum sensing system, making a regulation of motility and chemotaxis by cell density unlikely (Wisniewski-Dyé et al. 2011). While chemotaxis has been linked to swimming motility, there is no experimental evidence indicating that chemotaxis controls lateral-flagella dependent swarming. Since *A. brasilense* cells are motile under most conditions, motility and chemotaxis may play an even greater role in the ability of these bacteria to survive in the soil and the rhizosphere. *A. brasilense* is a versatile plant-associated bacterium that can establish in the rhizosphere of diverse plants (Steenhoudt and Vanderleyden 2000). Motility and chemotaxis under changing conditions could further enhance the competitive colonization abilities of *A. brasilense* in diverse rhizospheres.

A variety of cues are sensed by beneficial plant associated bacteria

In plant-associated bacteria, the cues that stimulate a chemotaxis response are expected to include compounds found in root exudates or on the root surfaces since chemotaxis toward crude root exudates has been demonstrated in several plant associated bacterial species (Caetano-Anollés et al. 1992; Dharmatilake and Bauer 1992; Caetano-Anollés et al. 1988a, b; Reinhold et al. 1985; Barbour et al. 1991; Caetano-Anollés et al. 1992; Gulash et al. 1984; Heinrich and Hess 1985; Mandimba et al. 1986). The composition of root exudates is highly complex and varies with environmental conditions and plant development stages, complicating their detailed characterization. However, numerous components, some being common and others rather unique to certain plants species have been identified (Bais et al. 2004, 2006; Caetano-Anollés et al. 1988a, b; Mandal et al. 2010; Uren 2000). Generally, the root mucilage that contains polysaccharides is abundantly produced by root caps and is also present in the exudates secreted by root tips. Meristems and elongation zones preferentially contain rapidly oxidized organic compounds such as sugars, organic acids and amino acids (Dennis et al. 2010; Walker et al. 2003). Sensing of organic acids is widespread in beneficial soil bacteria, including *S. meliloti*, *R. leguminosarum*, *A. brasilense*, and several species of *Pseudomonas*, for which specific receptors for a range of structurally different organic acids have been identified (Sampedro et al. 2015). *S. meliloti* (Bringhurst and Gage 2002), *R. leguminosarum* (Poole et al. 1994), and *A. brasilense* (Mukherjee and Ghosh 1987) use organic acids as catabolite repressors. Organic acids thus represent key metabolic regulators for adaptation to the rhizosphere and may explain the widespread occurrence of organic acid chemotaxis in plant-associated bacteria (Alexandre et al. 2000; Meier et al. 2007; Miller et al. 2007; Robinson and Bauer 1993). In addition to organic acids, these beneficial bacteria sense sugars and sugar alcohols (Alexandre et al. 2000; Bowra and Dilworth 1981; Burg et al. 1982; Meier et al. 2007; Miller et al. 2007), which are also present in root exudates of different plants. Sensing of these molecules typically occurs indirectly via the phosphotransferase system and/or periplasmic binding proteins, which then bind to corresponding chemoreceptors (Hazelbauer et al. 2008; Neumann et al. 2012; Wadhams and Armitage 2004). For example, the periplasmic binding protein ChvE specifically binds galactose and contributes to both metabolism and chemotaxis in *A. brasilense* (Van Bastelaere et al. 1999), but the interacting chemoreceptor is not known. Amino acids are relatively strong attractants for *S. meliloti* (Götz et al. 1982; Meier et al. 2007; Van Bastelaere et al. 1999) and *R. leguminosarum* (Miller et al. 2007) but are weak attractants for *A. brasilense* (Alexandre et al. 2000). *S. meliloti* and *R. leguminosarum* fix nitrogen only under symbiotic conditions and must rely on other nitrogen sources, including amino acids, under free-living conditions. In contrast, *A. brasilense* fixes nitrogen under free-living conditions, an ability that could explain the weak

chemostimulatory effect of amino acids in this species. Chemotaxis toward flavonoids, which are host root phenolic compounds that stimulate expression of nodulation genes in bacteria, was proposed for *S. meliloti* (Caetano-Anollés et al. 1988a; Dharmatilake and Bauer 1992) and *R. leguminosarum* (Armitage et al. 1988). Chemotaxis towards flavonoids was reported to occur in the nanomolar concentration range, which would imply chemoreceptor-mediated sensing. However, these results could not be reproduced in later studies (Webb and Scharf, unpublished results; Miller, Hynes and Alexandre, unpublished results). Therefore, the role, if any, of flavonoids released in root exudates to specifically attract motile rhizobia to the legume rhizosphere remains to be demonstrated.

Chemoreceptors and ligand specificity for plant association

Signal transduction during bacterial chemotaxis is initiated by the detection of extracellular cues via dedicated receptors. Chemoreceptors are structurally and functionally modular: they possess an N-terminal sensory domain, typically exposed to the extracellular environment and a highly conserved C-terminal domain required for signal transduction (Hazelbauer and Lai 2010). The cues sensed by chemoreceptors thus determine the environment toward which a bacterium may move. Bacteria possessing a greater number of receptors would be predicted to navigate a greater variety of chemical gradients. The number of chemoreceptors encoded in the genomes of plant-associated bacteria varies, but how these differences are reflected in distinct behaviors in the rhizosphere or other environments has not been demonstrated experimentally (Table 1).

Sinorhizobium meliloti possesses nine chemoreceptors; eight of them are involved in sensing environmental stimuli to direct flagellar motor rotation. Similar to what is observed for other sequenced genomes, the majority of the receptor genes has a monocistronic organization and are scattered throughout the genome. Recently, McpU has been identified as a proline sensor mediating chemotaxis towards its alfalfa host (Webb et al. 2014). Further investigations also suggest that McpU functions as general amino acid receptor (Webb and Scharf, unpublished results). In contrast, the function of the remaining seven chemoreceptors is not known and subject of current investigations.

Although ligands for various chemoreceptors in *R. leguminosarum* have not been identified, it is clear through nodule competition assays that chemoreceptors have a biological role in establishing a successful association with the plant. Mutations in *mcpB* and *mcpC* genes cause significant decreases in the ability of the mutants to form nodules on peas when challenged with a wild-type competitor strain. This finding suggests that both receptors detect some specific compound(s) exuded by plant roots and regulate movement towards roots, and explicitly, appropriate infection sites (Yost et al. 1998). Intriguingly, it has been found that *mcpC* mutants do not have a competitive disadvantage when inoculated on some other legume hosts of *R. leguminosarum* (Yost and Hynes, unpublished results), indicating that a different attractant spectrum is present in the rhizosphere of various plant species. Furthermore, the study suggests that the symbiotic competition phenotype of mutants in other *mcp* genes (*mcpG*, *mcpD*) (Yost et al. 1998, 2003) should be reexamined using different host plant species and cultivars, to detect plant specific effects.

Similar to *S. meliloti* and *R. leguminosarum*, the ligand specificity of most chemoreceptors encoded within the genome of *A. brasilense* is not known. However, the critical role of Tlp1 in promoting plant–root colonization indicates that chemotaxis modulates plant–root surface colonization by *A. brasilense*. Experimental evidence shows that the expression pattern of chemoreceptors parallels their function in chemotaxis. In particular, receptor expression is being upregulated under conditions where the cue(s) it senses is prevalent (Xie et al. 2010; Russell and Alexandre, unpublished results). This could represent a strategy to enhance sensitivity to a particular cue and could function as a reinforcing signal for sustained chemotaxis. However, since transcriptomic studies performed in *R. leguminosarum* failed to identify any *mcp* genes significantly up-regulated in the rhizosphere (Karunakaran et al. 2009), this is probably not a universal strategy.

Conclusion: challenges and outlook

Bacterial chemotaxis promotes the recruitment of motile soil bacteria to the roots of plants and it is thus critical for the establishment of many associations of bacteria with the roots of plants. Sensing of specific chemoeffectors exuded by roots are likely chemostimulatory and identifying such cues could represent a strategy to specifically recruit beneficial bacteria to enhance plant growth. However, the ligand specificity of most chemoreceptors is unknown and identifying the active fraction(s) of compounds within root exudates that specifically attract bacteria remains challenging. Overcoming these limitations would provide tools for the rationale design of plants or manipulation of growth conditions for enhancing the recruitment of beneficial bacteria to the rhizosphere. This is one of the many approaches needed to achieve sustainable agriculture and ensure future food securities.

Author's contribution BES, MFH and GMA wrote and revised the manuscript and designed the figures and tables.

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