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REVIEW ARTICLE

Rhizobacterial mediation of plant hormone status

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Abstract

Plant growth-promoting rhizobacteria are commonly found in the rhizosphere (adjacent to the root surface) and may promote plant growth via several diverse mechanisms, including the production or degradation of the major groups of plant hormones that regulate plant growth and development. Although rhizobacterial production of plant hormones seems relatively widespread (as judged from physico-chemical measurements of hormones in bacterial culture media), evidence continues to accumulate, particularly from seedlings grown under gnotobiotic conditions, that rhizobacteria can modify plant hormone status. Since many rhizobacteria can impact on more than one hormone group, bacterial mutants in hormone production/degradation and plant mutants in hormone sensitivity have been useful to establish the importance of particular signalling pathways. Although plant roots exude many potential substrates for rhizobacterial growth, including plant hormones or their precursors, limited progress has been made in determining whether root hormone efflux can select for particular rhizobacterial traits. Rhizobacterial mediation of plant hormone status not only has local effects on root elongation and architecture, thus mediating water and nutrient capture, but can also affect plant root-to-shoot hormonal signalling that regulates leaf growth and gas exchange. Renewed emphasis on providing sufficient food for a growing world population, while minimising environmental impacts of agriculture because of overuse of fertilisers and irrigation water, will stimulate the commercialisation of rhizobacterial inoculants (including those that alter plant hormone status) to sustain crop growth and yield. Combining rhizobacterial traits (or species) that impact on plant hormone status thereby modifying root architecture (to capture existing soil resources) with traits that make additional resources available (e.g. nitrogen fixation, phosphate solubilisation) may enhance the sustainability of agriculture.

Introduction

Improving the resource use efficiency of the world's major crops is clearly key to deliver a safe, secure food supply to a rising global population. A recent report has advocated the 'sustainable intensification of agriculture' while minimising harmful impacts on cropping ecosystems (Royal Society, 2009), and it is incumbent on plant scientists to deliver this goal. One major area of crop improvement that has hitherto been comparatively neglected is the role of the plant root system in maximising resource (water, nutrients) capture (Lynch, 2007). However, it is important to recognise that the rhizosphere (the area of the soil adjacent to the root surface) is biologically diverse, and that rhizosphere organisms can play a major role in plant resource capture (see other papers in this volume). Most attention has focussed on certain bacterial genera that can fix atmospheric nitrogen within a specialised host organ (the legume nodule), and certain fungal genera (mycorrhizae) whose hyphal networks ramify throughout the soil and within the plant and seem particularly important in plant acquisition of relatively immobile nutrients such as phosphorus (P). Rhizosphere bacteria can also play important roles in plant resource capture.

Plant growth-promoting rhizobacteria (PGPR) are commonly found in the rhizosphere and can also be isolated from internal plant tissues, the so-called 'endophytic bacteria'. Often a species may be isolated both internally and externally, thus *Azospirillum* spp. are generally assumed to be rhizosphere bacteria, but are also commonly found as endophytes, *albeit* sometimes at lower densities (Rothballer *et al*., 2003). Similarly, *Gluconacetobacter (Acetobacter) diazotrophicus* is often assumed to be an 'obligate endophyte', but can occur in large numbers on the surface of sugarcane roots (James & Olivares, 1998). Regardless of the localisation of PGPR, they may promote plant growth via several diverse mechanisms (although it is conceivable that bacterial niche may influence plant response). Firstly, biocontrol occurs when PGPR decrease root pathogen infection by producing antibiotics or competitively excluding other rhizosphere organisms by consuming nutrients, or by inducing systemic resistance to combat foliar disease (Lugtenberg & Kamilova, 2009). Secondly, biofertilisation occurs when PGPR improve plant nutrient status by associative nitrogen fixation, P solubilisation, producing siderophores thus increasing Fe availability, directly stimulating plant ion uptake and/or transport systems, increasing root proton efflux, altering the permeability and arrangement of root cortical cells via pectinolytic activity and transforming nutrients in the rhizosphere thus increasing their bio-availability (Dobbelaere *et al*., 2003; Vessey, 2003; Mantelin & Touraine, 2004). Thirdly, hormonal effects occur when PGPR either produce or metabolise chemical signalling compounds that directly impact on plant growth and functioning (Costacurta & Vanderleyden, 1995; Frankenberger & Arshad, 1995). Although hormonal mechanisms do not directly make more water or nutrients available to the plant, they can alter root elongation and architecture, thus increasing the volume of soil explored by the plant and thus indirectly increase the capture of plant resources already in the soil. This has undoubtedly contributed to the recent proliferation of articles on hormonal impacts of rhizobacteria on plants (Fig. 1), as agronomists and plant scientists within both private and public institutes aim to improve crop resource use efficiency. While it is important to recognise that the mechanisms outlined above are not mutually exclusive (e.g. many hormone-producing rhizobacteria also fix nitrogen and solubilise P) (Belimov *et al*., 2001; Dey *et al*., 2004; Ahmad *et al*., 2008), this review focusses on rhizobacterial impacts on plant hormone status and the physiological consequences.

Plant hormones [abscisic acid (ABA), auxins, cytokinins (CKs), ethylene, gibberellins (GAs), jasmonic acid (JA), salicylic acid (SA)] regulate multiple physiological processes including root initiation, elongation, architecture and root hair formation. They typically operate in complex networks involving cross-talk and feedback (Woodward & Bartel, 2005; Swarup *et al*., 2007; Fukaki & Tasaka, 2009), thus it can be difficult to establish the role of a particular hormone in plant response. Similarly, many PGPR have the potential to affect multiple plant hormone groups (Table 1), although it is not always clear whether the capacity of bacteria to produce hormones *in vitro* actually alters plant hormone concentration *in vivo*. Although PGPR can directly affect rhizosphere hormone concentrations (by uptake of hormones or their precursors as carbon and nitrogen sources, and efflux of hormones synthesised by the bacteria), there is increasing evidence that PGPR affect root hormone concentrations (Table 1), and can also alter root-to-shoot long-distance signalling (Dodd, 2005; Belimov *et al*., 2009) to mediate shoot hormone status.

In this review, for each major hormone group, their biosynthesis and impacts on root growth are considered only superficially since comprehensive reviews on plant hormone biosynthesis (Kende, 1993; Taylor *et al*., 2000; Woodward & Bartel, 2005; Kudo *et al*., 2010) and molecular mechanisms of regulating root growth (Casson & Lindsey, 2003; Fukaki & Tasaka, 2009) already exist. Instead, this review aims to link rhizobacterial mediation of plant hormone status with changes in root growth and architecture, and ultimately plant performance.

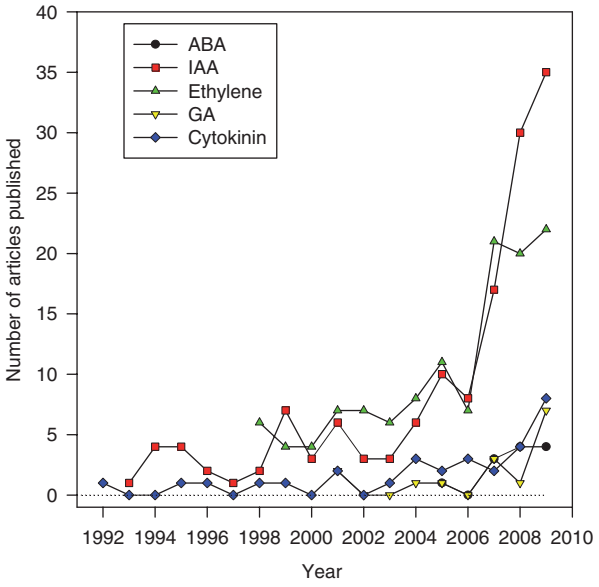

Figure 1 Number of articles published per year in Web of Science for the terms 'rhizobacteria' and 'hormone' where hormone refers to abscisic acid (ABA), cytokinin(s), ethylene, gibberellin and indole-3-acetic acid (IAA). Salicylic acid (SA) and jasmonic acid (JA) are omitted for the sake of clarity.

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Table 1 Examples of rhizobacterial effects on phytohormone concentrations of culture media and plants

Rhizobacterial Species	Culture Filtrate	*In Planta In Vitro*	*In Planta Ex Vitro*	References
Achromobacter xylosoxidans Cm3	↑ IAA[b]			Belimov *et al*. (2001)
Achromobacter xylosoxidans Ps27	↑ ABA, GA_3, IAA[d]			Sgroy *et al*. (2009)
Achromobacter xylosoxidans SF2	↑ JA, ABA[e]			Forchetti *et al*. (2007)
Acinetobacter calcoaceticus SE370	↑ $GA_{1, 3, 4, 9, 12}$, $GA_{15, 20, 24, 53}$[d]			Kang *et al*. (2009)
Agrobacterium radiobacter 10	↑ IAA[b]			Belimov & Dietz (2000)
Arthrobacter mysorens 7	↑ IAA[b]			Belimov & Dietz (2000)
Azospirillum brasilense Sp13t, SR2	↑ IAA, GA, Z[a]			Tien *et al*. (1979)
Azospirillum brasilense 200, 245	↑ IAA[e]			Kravchenko *et al*. (1993)
Azospirillum brasilense FT 326	↑ IAA[d]	↑ IAA (r/s)[d] ↑ C_2H_4 (s)[d]		Ribaudo *et al*. (2006)
Azospirillum brasilense Az39	↑ GA_3, IAA[d] ↑Z[e]			Perrig *et al*. (2007) Cassán *et al*. (2009)
Azospirillum brasilense Cd	↑ GA_3, IAA[d] ↑Z[e]			Perrig *et al*. (2007)
Azospirillum brasilense Sp245	↑ ABA[d]	↑ ABA[d]		Cohen *et al*. (2008)
Azospirillum lipoferum USA59b			↑ ABA[d]	Cohen *et al*. (2009)
Azospirillum lipoferum 137	↑ IAA[b]			Belimov & Dietz (2000)
Bacillus cereus MJ-1	↑ $GA_{1, 3, 4, 7, 9}$, $GA_{12, 19, 20, 24}$, $GA_{34, 36, 44, 53}$[d]			Joo *et al*. (2004)
Bacillus licheniformis Ps14	↑ ABA, GA_3, IAA[d]			Sgroy *et al*. (2009)
Bacillus licheniformis CECT 5106	↑ IAA, ↑ GA_1, GA_3, GA_4, GA_{20}[d]			Gutierrez-Manero *et al*. (2001)
Bacillus macroides Ps19	↑ $GA_{1, 3, 4, 5, 7}$, $GA_{8, 9, 12, 19, 20}$, $GA_{24, 34, 36, 44, 53}$[d]			Joo *et al*. (2004)
Bacillus pumilis CECT 5105	↑ IAA, ↑ GA_1, GA_3, GA_4, GA_{20}[d]			Gutierrez-Manero *et al*. (2001)
Bacillus pumilus Ps19	↑ ABA, GA_3, IAA, Z[d]			Sgroy *et al*. (2009)
Bacillus pumilus Cj-69	↑ $GA_{1, 3, 4, 7, 9}$, $GA_{12, 19, 20, 24}$, $GA_{34, 36, 44, 53}$[d]			Joo *et al*. (2004)
Bacillus sp.	↑ IAA, GA_3[c]			Islam *et al*. (2009)
Bacillus subtilis IB22			↑ tZ, tZR[c] ↓ ABA[c]	Arkhipova *et al*. (2007)
Bacillus subtilis Ps8	↑ ABA, GA_3, IAA, Z[d]			Sgroy *et al*. (2009)
Brevibacterium halotolerans Ps9	↑ ABA, GA_3, Z[d]			Sgroy *et al*. (2009)
Brevundimonas sp. RFNB15, RFNB32	↑ IAA[c]			Islam *et al*. (2009)
Burkholderia cepacia Ral 3	↑ DHZR, iPA, tZR[c]			De Salamone *et al*. (2001)
Burkholderia sp. KCTC 11096BP	↑ $GA_{1,3,4}$[d]			Joo *et al*. (2009)
Burkholderia sp. RFNB11, RFNB12, RFNB16	↑ IAA[c]			Islam *et al*. (2009)
Corynebacterium sp.	↓ ABA[d]			Hasegawa *et al*. (1984)
Flavobacterium sp. L30	↑ IAA[b]			Belimov & Dietz (2000)
Herbaspirillum sp. RFNB20, RFNB26, RFNB30	↑ IAA[c]			Islam *et al*. (2009)
Lysinibacillus fusiformis Ps7	↑ ABA, GA_3, IAA[d]			Sgroy *et al*. (2009)
Methylobacterium extorquens CBMB120, CMBM130	↑ IAA, iPA, tZR[c]			Madhaiyan *et al*. (2006)
Methylobacterium fujisawaense CBMB20, CMBM110	↑ IAA, iPA, tZR[c]	↑ IAA, iPA, tZR[c] ↓ ACC/C_2H_4[d]		Madhaiyan *et al*. (2006)
Paenibacillus polymyxa B2	↑ iP[d]			Timmusk *et al*. (1999)
Paenibacillus polymyxa Lp6, Pw2	↑ IAA[b]		NE DHZR[c] ↑ IAA[c]	Bent *et al*. (2001)
Paenibacillus sp. RFNB4	↑ IAA[c]			Islam *et al*. (2009)
Pantoea agglomerans Z143	↑ iP, IPA[d] Other CKs[d]			Omer *et al*. (2004*b*)
Pseudomonas brassicacearum Am3	↑ IAA[b]			Belimov *et al*. (2001)
Pseudomonas chlororaphis 63-28	↑ DHZR, iPA, tZR[c]			De Salamone *et al*. (2001)

Table 1 Continued

Rhizobacterial Species	Culture Filtrate	*In Planta* *In Pitro*	*In Planta* *Ex Vitro*	References
Pseudomonas fluorescens M20	↑ IAA[b]		↑ DHZR[c] NE IAA[c]	Bent *et al*. (2001)
Pseudomonas fluorescens G20-18, G8-32, GR12-2	↑ DHZR, iPA, tZR[c]			De Salamone *et al*. (2001)
Pseudomonas oryzihabitans Ep4	↑ IAA[b]			Belimov *et al*. (2001)
Pseudomonas putida Rs198			↓ ABA[a], ↑ IAA[d]	Yao *et al*. (2010)
Pseudomonas putida GR12-2	↑ DHZR, iPA, tZR[c]			De Salamone *et al*. (2001)
Pseudomonas putida GR12-2		↓ ACC[e]		Penrose *et al*. (2001)
Pseudomonas putida Ps30	↑ ABA, IAA, Z[d]			Sgroy *et al*. (2009)
Rhodococcus sp. 4N-4	↑ IAA[b]			Belimov *et al*. (2005)
Serratia sp. RFNB17, RFNB18, RFNB19	↑ IAA[c]			Islam *et al*. (2009)
Sphingomonas sp. RFNB22, RFNB28	↑ IAA[c]			Islam *et al*. (2009)
Variovorax paradoxus (8 strains)	↑ IAA[b]			Belimov *et al*. (2005)
Xanthomonas sp. RFNB24	↑ IAA[c]			Islam *et al*. (2009)

Effects are increases (↑), decreases (↓) or no statistically significant effect (NE) where (r/s) indicates roots and shoots, respectively.
Detection of phytohormones was via
[a]bioassay techniques,
[b]colourimetric techniques,
[c]enzyme-linked immunosorbent assay (ELISA),
[d]gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS), or
[e]high performance liquid chromatography-ultraviolet detection (HPLC-UV).
Phytohormones are as follows: ABA, abscisic acid; ACC, 1-aminocyclopropane carboxylic acid; CK, cytokinin; C_2H_4, ethylene; DHZR, dihydrozeatin riboside; GA_X, gibberellic $acid_X$; IAA, indole-3-acetic acid; iP, isopentenyladenine; iPA, isopentenyladenine-9-riboside; JA, jasmonic acid; tZR, *trans*-zeatin riboside; tZ, *trans*-zeatin; Z, zeatin.

Particular attention is given to the ability of PGPR to affect plant growth via metabolising phytohormones in the rhizosphere (Table 2; rhizobacteria degrading the ethylene precursor ACC are summarised in Belimov, 2009 – supplementary Table S1 is available), since this aspect of plant–bacteria interactions has received comparatively little attention in the literature.

Abscisic acid

Water stress dramatically stimulates plant ABA biosynthesis (e.g. Dodd, 2007) and partially closes the stomata (an adaptive response to conserve water), with ABA concentrations in a given plant compartment depending on local synthesis (determined by cellular turgor), metabolism and import (from either xylem or phloem). Abscisic acid biosynthesis begins with the oxidative cleavage of the carotenoids 9′-*cis*-violaxanthin or 9′-*cis*-neoxanthin to xanthoxin by the plastid enzymes 9-*cis*-epoxycarotenoid dioxygenases (NCEDs). Xanthoxin is converted to abscisic aldehyde by xanthoxin oxidase, then abscisic aldehyde oxidase catalyses conversion of abscisic aldehyde to ABA (reviewed in Taylor *et al*., 2000). During water stress, activities of the above-mentioned enzymes and their mRNA transcript abundance increases in both leaves and roots. In the roots, xanthophylls are in low abundance and zeaxanthin epoxidation to violaxanthin via zeaxanthin epoxidase (ZEP) might be a further regulatory step of water stress-induced ABA biosynthesis (Taylor *et al*., 2000).

The role of ABA in mediating root elongation depends on substrate (and thus plant) water potential, Ψ: ABA accumulation inhibits elongation of well-watered roots ($\Psi = -0.03$ MPa) and in hydroponics (Fig. 2), but maintains elongation of roots growing in substrates at low water potential ($\Psi = -1.6$ MPa), at least partially by suppressing excessive ethylene synthesis which can inhibit growth (Sharp & LeNoble, 2002). Although ABA-deficient mutants generally have lower root growth rates and less root biomass (Munns & Cramer, 1996), total root length of the ABA-deficient *aba2-1* and *aba3-1* mutants of *Arabidopsis thaliana* was fourfold higher than wild-type (WT) plants *in vitro*, and the inhibition of lateral root formation by osmotic stress ($\Psi\pi = -0.6$ MPa) was less in these mutants (Deak & Malamy, 2005). Exogenous ABA application mimicked the inhibition of lateral root development caused by osmotic stress (Guo *et al*., 2009), although the exogenous ABA concentrations required to elicit this inhibition (1 μM in *A. thaliana*, 10 μM in *Arachis hypogea*) were several orders of magnitude higher than typically found in the rhizosphere (1–10 nM, Hartung *et al*., 1996). Detailed physiological and morphological

Table 2 Examples of *in vitro* phytohormone degradation by bacteria

Bacterial Species	Hormone	References
Alcaligenes sp.	IAA	Claus & Kutzner (1983)
Alcaligenes sp.	IAA	Libbert & Risch (1969)
Achromobacter sp.	IAA	Libbert & Risch (1969)
Arthrobacter sp. SN17, DF14, SF27	SA	Plotnikova *et al*. (2001)
Arthrobacter sp.	IAA	Mino (1970)
Azospirillum brasilense Cd	GA_{20}	Cassán *et al*. (2001)
Azospirillum lipoferum USA	GA_{20}	Cassán *et al*. (2001)
Bacillus sp. SN501	SA	Plotnikova *et al*. (2001)
Bacillus sp.	IAA	Libbert & Risch (1969)
Bradyrhizobium japonicum TA3, TA5, TA11	IAA	Egebo *et al*. (1991)
Bradyrhizobium japonicum 110	IAA	Jensen *et al*. (1995)
Burkholderia sp. 383	IAA	Leveau & Gerards (2008)
Corynebacterium sp. K3	Ethylene	Coleman *et al*. (2002)
Corynebacterium sp.	ABA	Hasegawa *et al*. (1984)
Flavobacterium sp.	IAA	Libbert & Risch (1969)
Mycobacterium sp. E3	Ethylene	Elsgaard (1998)
Mycobacterium sp. E20, 32, S, T1, T2	Ethylene	De Bont (1976)
Mycobacterium sp. K1	Ethylene	Coleman *et al*. (2002)
Mycobacterium sp. JS60, JS61, JS616, JS617	Ethylene	Coleman *et al*. (2002)
Marinomonas sp. MWYL1	IAA	Leveau & Gerards (2008)
Nocardioides sp. JS614	Ethylene	Coleman *et al*. (2002)
Pseudomonas butanovora	SA	Kesserü *et al*. (2005)
Pseudomonas fluorescens HK44	SA	Silva *et al*. (2007)
Pseudomonas putida 1290	IAA	Leveau & Lindow (2005)
Pseudomonas putida GB-1	IAA	Leveau & Gerards (2008)
Pseudomonas putida g15f, g20f, g24f, NS7, NS11, NS12, NS15, NS17, NS18, NS20, NS22, NS24	SA	Sasonova *et al*. (2008)
Pseudomonas savastanoi pIAA1	IAA	Roberto *et al*. (1990)
Pseudomonas sp. SN11, SN21, SN101, G51	SA	Plotnikova *et al*. (2001)
Pseudomonas sp. DL1b	Ethylene	Coleman *et al*. (2002)
Pseudomonas spp.	IAA	Libbert & Risch (1969)
Pseudomonas spp. (27 different strains)	SA	Grishchenkov *et al*. (2003)
Rhodococcus sp. RHA1	IAA	Leveau & Gerards (2008)
Rhodococcus sp. SN31, DB11, G10	SA	Plotnikova *et al*. (2001)
Serratia marcescens	SA	Jaiswal & Thakur (2007)
Serratia proteamaculans B1	BA, 8-OHBA	Taylor *et al*. (2006)
Sphingomonas wittichii RW1	IAA	Leveau & Gerards (2008)
Unidentified bacterium RD4	Ethylene	Elsgaard & Andersen (1998)

Phytohormones are as follows: ABA, abscisic acid; BA, N^6-benzyladenine; IAA, indole-3-acetic acid; 8-OHBA, 8-hydroxy-N^6-benzyladenine; SA, salicylic acid.

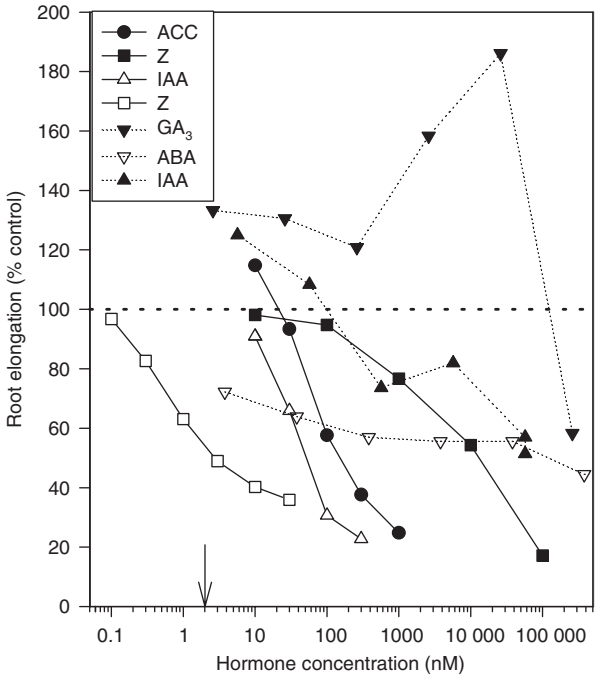

Figure 2 Primary root elongation of non-nodulated faba bean (*Vicia faba*), pea (*Pisum sativum*) and wheat (*Triticum aestivum*) in hydroponic solutions containing abscisic acid (ABA) (▽), 1-aminocyclopropane-carboxylic acid (ACC) (●), GA_3 (▼), indole-3-acetic acid (IAA) (△, ▲) and zeatin (□, ■). Data were redrawn from El-Antably and Larsen (1974) (*V. faba* – ▽, ▼, ▲), Bertell and Eliasson (1992) (*P. sativum* – ■), Eliasson *et al*. (1989) (*P. sativum* – ●, △) and Stenlid (1982) (*T. aestivum* – □). Arrow on the *x*-axis indicates representative rhizosphere ABA concentrations (from Hartung *et al*., 1996).

studies are required to establish whether the inhibition of lateral root development in drying soil is a direct response to increased endogenous ABA accumulation, or an indirect effect of slower primary root elongation.

Several rhizobacteria produce ABA in culture media or mediate plant ABA status (Table 1). Although the biochemical mechanisms by which ABA is produced *in planta* have been well characterised (Taylor *et al*., 2000), as has fungal ABA production (Siewers *et al*., 2006), the biochemical mechanism of bacterial ABA production does not appear to have been investigated. Nevertheless, the existence of bacterial genome sequences for carotenoid cleavage oxygenase homologues (Marasco & Schmidt-Dannert, 2008) provides a possible mechanism for bacterial ABA production.

Various *Azospirillum brasilense* strains such as Az 39 and Cd (Perrig *et al*., 2007) and Sp245 (Cohen *et al*., 2008) produced ABA *in vitro* when grown on defined media. Abscisic acid production of strain Sp245 increased eightfold [per colony-forming unit (CFU)] when the osmotic potential of the medium ($\Psi\pi$) was lowered

from −0.2 to −0.7 MPa by the addition of 100 mM NaCl (Cohen *et al*., 2008). Furthermore, three endophytic sunflower (*Helianthus annuus*) bacteria (including one identified as *Achromobacter xylosoxidans*) increased the ABA concentration of the culture medium when $\Psi\pi$ was lowered from 0 to −2.03 MPa by adding polyethylene glycol (Forchetti *et al*., 2007). While medium $\Psi\pi$ obviously determines bacterial ABA production, there may be residual effects of the growth environment, since *Azospirillum* isolates from water-stressed conditions produced more ABA *in vitro* than strains isolated from well-watered plants (Ilyas & Bano, 2010) although the underlying mechanisms remain unclear.

Addition of a concentrated *A. brasilense* suspension (10 μL of a bacterial suspension containing 10^6 CFU mL^{-1}) to *A. thaliana* seedlings grown *in vitro* approximately doubled tissue ABA concentrations (Cohen *et al*., 2008), indicating that ABA-producing PGPR can potentially augment plant ABA concentrations. Similarly, aseptic inoculation of maize (*Zea mays*) seedlings with *Azospirillum lipoferum* USA59b doubled tissue ABA concentrations when well-irrigated pot-grown plants were harvested 45 days after inoculation (Cohen *et al*., 2009). The persistence of this increased ABA concentration was attributed to endophytic colonisation of the maize plants by *Azospirillum*. To what extent the differences in bacterial colonisation between root and shoot (two orders of magnitude lower bacterial colonisation of plant aerial parts) resulted in differences in tissue ABA concentration is unknown, although shoot growth promotion (11%) was less than root growth promotion (48%) of inoculated plants under well-watered conditions (Cohen *et al*., 2009). Higher leaf water potential and relative water content (RWC) of wheat (*Triticum aestivum*) plants inoculated with *A. brasilense* Sp245 than uninoculated controls, when exposed to drought (Creus *et al*., 2004), may result from bacterial ABA production partially closing the stomata thus attenuating water deficit. However, it will be important to determine whether effects of *Azospirillum* inoculation on plant water relations are attributable to bacterial ABA production, or whether inoculation alters the sensitivity of physiological processes (e.g. stomatal closure) to soil drying.

Improved growth of *Azospirillum* inoculated plants has also been attributed to bacterial gibberellic acid (GA) production (Perrig *et al*., 2007). The relative importance of ABA and GA production was assessed by foliar spraying of maize seedlings with fluridone (ABA biosynthesis inhibitor) or prohexadione (GA biosynthesis inhibitor) or their combination (Cohen *et al*., 2009). *A. lipoferum* restored (or increased) both root and shoot growth of well-irrigated fluridone-treated plants, and root (but not shoot) growth of prohexadione-treated plants, but could not restore growth of plants sprayed with both inhibitors. At least part of this growth recovery of inoculated plants may be attributed to increased leaf RWC, but hydraulic mechanisms cannot be solely responsible for growth recovery as plants sprayed with both inhibitors had a normal RWC (Cohen *et al*., 2009). Paired measurements of leaf water relations, photosynthesis and phytohormones in the same leaf (Dodd, 2007) will be essential to dissect the role of bacterial ABA production in the improved growth of *Azospirillum* inoculated plants.

The relative ease with which ABA can be measured using immunological techniques and its importance in controlling plant water loss and growth (Dodd *et al*., 2009*a*) has allowed its routine measurement in other experiments with PGPR, even when there may appear no *a priori* reason (such as hormonal analyses of liquid culture media) to suggest that a rhizobacterial species may alter plant ABA relations. Soil inoculation with the 1-aminocyclopropane-carboxylic acid (ACC) deaminase-containing rhizobacterium *Variovorax paradoxus* 5C-2 increased pea (*Pisum sativum*) growth and yield in both well watered and drying soil, but also increased xylem ABA concentrations in drying soil (Belimov *et al*., 2009). However, xylem ABA concentration of both inoculated and uninoculated maize plants increased similarly as leaf water potential decreased, and hormone flow modelling of well-watered maize plants showed that inoculation decreased phloem flow of ABA to the root (Dodd *et al*., 2009*b*). It was suggested that the enhanced xylem ABA concentration in the pea experiments was not a direct rhizobacterial impact *per se*, but resulted from increased shoot growth (and hence transpiration) of inoculated plants likely causing additional soil drying (Belimov *et al*., 2009). When rhizobacterial inoculation accelerates plant development, including a developmental control is valuable to allow comparisons of plant hormone relations in similarly sized plants. Since one of the functions of ABA is to restrict ethylene synthesis (Sharp & LeNoble, 2002; Dodd *et al*., 2009*a*), the decreased ethylene evolution of plants inoculated with ACC deaminase (ACCd)-containing rhizobacteria (Mayak *et al*., 2004) might cause feedback regulation of ABA levels. This proposition should be more thoroughly evaluated, especially in view of interest in using ACCd-containing rhizobacteria to ameliorate plant responses to soil drying (Dey *et al*., 2004; Mayak *et al*., 2004; Belimov *et al*., 2009).

Another unsuspected effect of PGPR inoculation on plant ABA relations occurred when the CK-producing PGPR *Bacillus subtilis* IB-22 was applied to sand-grown lettuce (*Lactuca sativa*) seedlings: this doubled both shoot CK and ABA concentrations of well-watered plants, but prevented any increase in shoot ABA accumulation induced by soil drying (Arkhipova *et al*., 2007). However, bacterial

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inoculation had no effect on stomatal conductance or leaf RWC or root hormone concentrations. The greater impact of rhizobacterial inoculation on shoot than root hormone concentrations suggests considerable root-to-shoot signalling of phytohormones in inoculated plants, but more detailed measurements of xylem hormone concentration or hormone flow modelling studies are required to substantiate that systemic ABA signalling is altered by *Bacillus* inoculation. Alternatively, exposure of *A. thaliana in vitro* to the volatiles generated by *B. subtilis* GB03 downregulated shoot (but not root) ABA concentrations, and the ABA biosynthetic transcripts *AtZEP*, *AtNCED3* and *AtNCED4* in the shoots without plant–bacterial contact (Zhang *et al*., 2008). While this decreased ABA concentration increased photosynthetic efficiency (apparently independently of any stomatal effects), the extent to which these mechanisms operate *ex vitro* requires further work.

Immersing cotton (*Gossypium hirsutum*) seeds in suspensions of *Pseudomonas putida* Rs-198 (10^9 CFU mL^{-1} for 6 h) prior to planting increased seedling biomass accumulation by 10 and 19% in saline and non-saline soil, respectively, and prevented any salinity-induced ABA accumulation in cotton seedlings (Yao *et al*., 2010). However, the physiological impact of this change in ABA accumulation remains to be resolved, especially since growth promotion was also associated with a 30–50% increase in leaf indole-3-acetic acid (IAA) concentration, irrespective of salinity. Furthermore, other strains of *P. putida* contain ACCd (Glick *et al*., 1994) and/or produce exopolysaccharides that can improve root–soil contact (Sandhya *et al*., 2009), potentially attenuating root water stress. It is therefore difficult to unequivocally establish whether these rhizobacterial changes in plant ABA relations are involved in mediating plant responses to drought or salt stress.

Although plant-associated bacteria can apparently synthesise ABA (Table 1), less is known about microbial metabolism of this phytohormone (Frankenberger & Arshad, 1995). To the best of our knowledge, a solitary report indicated bacterial ABA degradation in a *Corynebacterium* sp. isolated from ABA-amended soil, which converted ABA to dehydrovomifoliol [(±)-1′-hydroxy-4′-keto-α-ionone] *in vitro* (Hasegawa *et al*., 1984). This provides a possible biochemical mechanism for a 30–40% degradation of radioactive ABA after its introduction to soil (Hartung *et al*., 1996). Recently, we have also isolated several ABA-degrading bacterial strains from the rice (*Oryza sativa*) rhizosphere via incubation of root samples on a selective nutrient media (Belimov & Dodd, unpublished data). Further work will investigate the possible role of these ABA-utilising rhizosphere bacteria in plant–bacteria interactions.

In conclusion, an increasing number of rhizobacterial strains appear capable of synthesising ABA in defined culture media, especially when exposed to osmotic stress. To what extent this occurs in nature (where changes in the water relations of bacterial niches will occur more slowly than in batch culture), and whether this alters ABA concentrations *in planta* requires further work. The dependence of plant ABA concentrations on both soil water content and leaf water potential (Dodd, 2007) requires 2 (± irrigation) by 2 (± rhizobacteria, and preferably their ABA-deficient mutants) experiments to resolve not only whether rhizobacteria affect plant ABA relations, but also whether they affect the sensitivity of plant response to ABA. These experiments are necessary to resolve whether ABA production/degradation by rhizobacteria, or indirect rhizobacterial alteration of plant ABA synthesis and metabolism, will have positive or negative impact on plants experiencing environmental stress.

Auxins

The most important natural auxins seem to be IAA, indole-3-butyric acid and phenoxyacetic acid, with most IAA stored as conjugated (probably inactive) forms such as ester conjugates (predominantly in monocotyledonous plants) or amide conjugates (predominantly in dicotyledonous plants) (Woodward & Bartel, 2005). Indole-3-acetic acid can be synthesised via tryptophan-dependent and tryptophan-independent pathways, although the significance of the latter pathway continues to be debated (Cohen *et al*., 2003). Bacterial IAA production was recently comprehensively reviewed (Spaepen *et al*., 2007) with at least five independent pathways identified, although some pathways were reported only in individual bacterial species. Typically, PGPR synthesise IAA via the indole-3-pyruvate pathway utilising the enzyme indole-3-pyruvate decarboxylase encoded by *ipdC*, with *ipdC* expression and IAA production occurring in the stationary phase, induced by exogenous tryptophan (Ryu & Patten, 2008). A microbial biosensor demonstrated higher rhizosphere tryptophan concentrations 12–16 cm from the root tip of *Avena barbata* in the zone of lateral root proliferation (Jaeger *et al*., 1999). Spatial separation of high populations of IAA-producing bacteria from the primary root elongation zone implies that some bacterially supplied auxin is transported towards the root tip to moderate growth.

Effects of auxin on root development are highly dependent on the particular auxin applied and the responsiveness of the genotype to auxin. Addition of 200 or 5000 nM IAA to the nutrient solution of hydroponically grown common bean (*Phaseolus vulgaris*) increased root dry weight and the number of basal roots by 1.9- and

1.4-fold, respectively, in one genotype, but had no effect on another (Remans *et al.*, 2008). In other legumes, inhibition of primary root elongation by IAA was dose-dependent (Fig. 2) with stimulation of faba bean (*Vicia faba*) root elongation at IAA concentrations <100 nM, but growth inhibition at concentrations >500 nM (El-Antably & Larsen, 1974) while pea (*P. sativum*) primary root elongation was inhibited by IAA concentrations between 10 and 500 nM (Eliasson *et al.*, 1989).

Of the major groups of plant hormones, there has been more interest in rhizobacterial production of auxins than any other (Frankenberger & Arshad, 1995; Fig. 1). While auxin production is clearly a widespread bacterial property (with up to 80% of species within some rhizobacterial genera producing auxin) (Ahmad *et al.*, 2008), this interest has undoubtedly been stimulated by the adoption of a standardised colourimetric assay for indole production (Ehman, 1977) in the presence of the auxin precursor, tryptophan, in the medium. Although this assay also detects intermediates of the IAA biosynthetic pathway (Glickmann & Dessaux, 1995), a survey of 15 bacterial species revealed that colourimetric and gas chromatography–mass spectrometry assays of bacterial auxin production were correlated and that both variables were correlated with endogenous IAA concentration of wheat seedlings (Ali *et al.*, 2009). Consequently, characterising rhizobacteria for their plant growth-promoting properties usually includes assays of indole production (Cattelan *et al.*, 1992; Belimov *et al.*, 2001; Dey *et al.*, 2004) and a wide range of rhizobacterial species have been identified as auxin producers (Table 1).

The role of auxin in plant–bacteria interactions has been addressed using bacterial genotypes with higher or lower IAA production. Although the auxin producer *P. putida* GR12-2 stimulated canola (*Brassica juncea*) root elongation, genotypes with higher levels of bacterial IAA production (up to four times higher than WT) had no additional effect on root elongation or lost their growth-promoting effects (Xie *et al.*, 1996). Similarly, an IAA-deficient mutant of *P. putida* GR12-2 constructed by insertional mutagenesis lost its growth-promoting effect on canola primary root elongation even though it had similar root colonisation as the WT strain (Patten & Glick, 2002), suggesting an optimal level of bacterial IAA production to stimulate root elongation. Similar approaches have investigated the role of IAA in the wheat root growth response to *A. brasilense* Sp245. An IAA-deficient mutant with 10% of WT auxin production abolished a dose-dependent inhibitory effect on wheat root elongation (Dobbelaere *et al.*, 1999) while enhancing IAA production (1.5-fold in culture media) by upregulating *ipdC* expression using constitutive or plant-inducible promoters further inhibited wheat root length at lower inoculum concentrations than the WT strain (Spaepen *et al.*, 2008). Furthermore, strains of the auxin-producing PGPR *Bacillus amyloliquefaciens* FZB42 with knockout mutations in genes probably involved in IAA metabolism (putative IAA transacetylase, putative nitrilase) were less efficient in promoting plant growth (Idriss *et al.*, 2007). Thus, while the absolute effect of auxin on plant response depended on the plant–microbe combination, moderating auxin production within a given rhizobacterial genotype had dose-dependent effects on plant growth.

An alternative evaluation of plant responses to bacterial-produced auxin used auxin-resistant plants. *Pseudomonas thivervalensis* MLG45 inhibited root length of WT *A. thaliana* seedlings by 70%, but did not inhibit root growth of the auxin-resistant mutant *aux1-100* (Persello-Cartieaux *et al.*, 2001). In contrast, a range of other hormonal mutants (ABA-insensitive *abi1*, *abi2* and *abi3*; CK-insensitive mutants *cdd1*, *cei1* and *cic1*; ethylene-insensitive *ein2-1* and *etr1-3*; gibberellin-insensitive *gai*; jasmonic-acid-insensitive *jar1-1*) showed a WT response to inoculation with *P. thivervalensis*, indicating the specificity of auxin-mediated root growth inhibition by this bacterium. Similar approaches have been used to investigate the interaction of other rhizobacteria with *A. thaliana*, due to the range of hormone-insensitive mutants in this plant species.

Although bacterial auxin production was associated with decreased wheat root length, the same bacteria increased lateral root number (Ali *et al.*, 2009). Despite the inhibitory effect of auxin on root growth, auxin-producing bacteria often stimulate shoot growth. For example, upregulating bacterial auxin production in *A. brasilense* Sp245 increased leaf length and shoot dry weight at least 10% more than the WT strain (Spaepen *et al.*, 2008). Across a range of PGPR, leaf IAA concentration of 14-day-old-wheat seedlings grown *in vitro* was correlated with shoot fresh weight in pot trials (Fig. 3, Ali *et al.*, 2009). Thus, bacterial auxin production remains a desirable trait in screening for PGPR activity, although inhibition of root length by auxin may be an undesirable side-effect.

However, the impact of bacterial auxin production on plant root growth likely depends not only on endogenous root auxin levels, but also on the existence of other bacterial characteristics (such as ACCd, see discussion under Ethylene section) that may mitigate auxin's impact. Auxin increases ethylene synthesis by upregulating activity of the rate-limiting enzyme [ACC synthase (ACS)] in the ethylene biosynthetic pathway (Kende, 1993). Recently, this hypothesis was substantiated with the IAA-producing PGPR *A. brasilense* FT 236, which almost doubled tomato shoot ethylene production, ACS activity and *LeACS2* gene expression (Ribaudo *et al.*, 2006)

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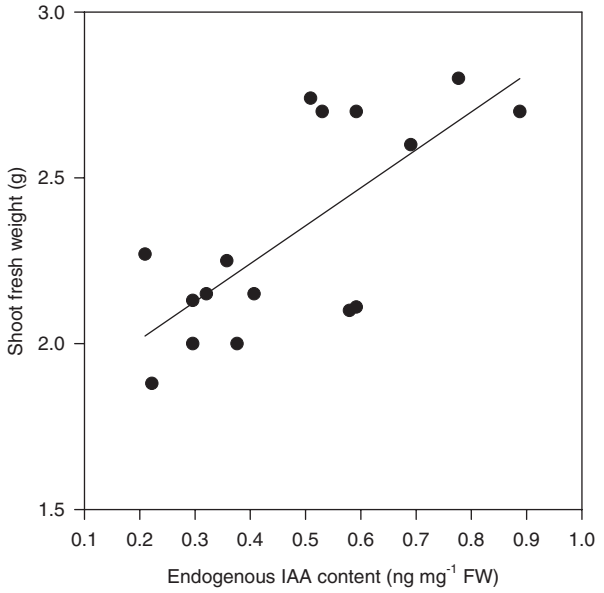

Figure 3 Correlation between endogenous indole-3-acetic acid (IAA) content of wheat and shoot fresh weight under axenic conditions. Each point represents a different bacterial species and a linear regression was fitted in SigmaPlot for Windows 2.01 (plotted from data in Ali *et al*., 2009).

and increased root hair development without inhibiting root elongation. Exogenous ethylene application mimicked the effects of *A. brasilense* FT 236 on root hair development, whereas blocking ethylene action with 1-methylcyclopropane decreased the positive effects of PGPR inoculation in root development. Bacterial mutants of this strain (in auxin production) are required to confirm auxin-mediated upregulation of plant ethylene status.

Biodegradation of IAA in soils was repeatedly studied (reviewed by Frankenberger & Arshad, 1995), suggesting that bacterial IAA metabolism is a widespread trait (Table 2). Gene clusters regulating IAA catabolism were identified in various bacterial families like *Burkholderia*, *Marinomonas*, *Pseudomonas*, *Rhodococcus* and *Sphingomonas* (Leveau & Gerards, 2008). As highlighted previously (Leveau & Lindow, 2005), the ecological significance of bacterial IAA catabolism has received little attention although the regulation of plant–bacteria symbiosis formation and function by IAA (Pii *et al*., 2007; Spaepen *et al*., 2007) clearly indicates that bacterial auxin metabolism may play important roles in mediating interactions with plants. It has been suggested (Leveau & Lindow, 2005) that (a) IAA-producing and IAA-degrading bacteria can interact in the rhizosphere to establish specific IAA concentrations outside and inside the plant; (b) the plant may regulate the composition of the rhizobacterial community by secreting IAA into the rhizosphere; (c) IAA-degrading bacteria may protect the plant from pathogen infection by 'mopping up' root surface IAA, a known signal molecule in phytopathogenesis. Indeed, co-inoculation with the IAA-degrading strain *P. putida* 1290R reduced inhibition of radish (*Raphanus sativus*) root growth caused by the IAA-overproducing *Rahnella aquaticus* and *Pseudomonas syringae* (Leveau & Lindow, 2005). The relative activity of IAA-producing and IAA-degrading bacteria may prevent extreme rhizosphere IAA concentrations thus mediating plant growth and preventing pathogen attack. Moreover, the same bacterial strain may possess both IAA biosynthesis and degradation traits (Egebo *et al*., 1991; Leveau & Lindow, 2005) allowing bacterial modulation and tuning to specific IAA concentrations in plant roots depending on partner genotypes and environmental conditions. Future experiments with mutant bacteria with altered IAA degradation are needed to establish the physiological significance of these hypotheses.

In conclusion, while rhizobacterial auxin production is clearly a widespread trait, its impacts have been difficult to assess as IAA-producing bacteria often produce other plant hormones. Creating bacterial mutants with altered IAA production, and using auxin-resistant plant mutants, have confirmed the importance of auxin in selected plant–bacteria interactions. While IAA-producing bacteria clearly alter root elongation and root architecture, it is not clear whether impacts of these bacteria on shoot growth are because of direct long-distance IAA signalling, or indirect effects of altered root system performance on water and nutrient capture. Further investigation of the role of bacterial IAA degradation as a potential mechanism of both positive and negative plant–bacteria interactions seems warranted.

Cytokinins

Most naturally occurring CKs are N^6-substituted adenine molecules with a branched 5-carbon side chain, such as *cis*-zeatin (cZ), *trans*-zeatin (tZ) and isopentenyladenine (iP). Although the importance of various pathways of CK biosynthesis varies according to plant tissue and environmental conditions, the formation of N^6-(Δ^2-isopentenyl)adenosine-5′-monophosphate (iP ribotide) from dimethylallyl diphosphate (DMAPP) and adenosine-5′-monophosphate catalysed by isopentenyltransferase (IPT), with subsequent hydroxylation to tZ ribotide, are important steps (Kudo *et al*., 2010). Biosynthesis of cZ from tRNA and DMAPP is catalysed by tRNA-IPTs (Kudo *et al*., 2010). Cytokinins are produced in plant meristematic regions including the roots, with different *IPT* genes showing different spatial regulation (Takei *et al*., 2004), and are transported in both the xylem and the phloem.

Experiments with exogenous applications of both natural and synthetic CKs (often in much higher concentrations than expected in the rhizosphere) to growing media (Fig. 2), and the creation of transgenic plants with altered CK biosynthesis or metabolism, have led to the general conclusion that CKs inhibit root growth. At least part of this growth inhibition may be via CK stimulation of ethylene production (Cary *et al*., 1995), since 1 μM benzylaminopurine (BAP) in vermiculite significantly increased root ethylene evolution and 15 μM BAP decreased pea primary root elongation and lateral root number by about 20% (suggesting the latter is dependent on the former) and lateral root elongation by 50% (Lorteau *et al*., 2001). Constitutive transgenic overexpression of *IPT* decreased root mass resulting in transient wilting (Hewelt *et al*., 1994). Furthermore, constitutive overexpression of CK oxidase increased root biomass by 60% even though shoot biomass was decreased by 30% (Werner *et al*., 2001).

Prior to the isolation of plant *IPT* genes for *de novo* CK synthesis, it was argued that plant CK status was maintained by endophytic bacteria known as pink-pigmented facultative methylotrophs or PPFMs (Holland, 1997). The bacterial endophyte *M. extorquens* produced adenine and adenine derivatives (Pirttilä *et al*., 2004) but not other commonly produced hormones (IAA, GA, Z were all below detection limit). In contrast, Korean isolates of both *Methylobacterium extorquens* and *M. fujisawaense* produced IAA in culture (independently of the presence of tryptophan) and both tZ and iPA, which increased total cytokinin concentrations of canola seedlings threefold (Madhaiyan *et al*., 2006). Bacterial IAA production by such endophytes (Omer *et al*., 2004*a*; Madhaiyan *et al*., 2006) indicates their impacts on plant growth are not only by altering CK homeostasis.

Several PGPR increased CK concentrations of culture media (Table 1). Using immunoaffinity chromatography of culture media before and after inoculation, *Paenabacillus polymyxa* B2 was shown to produce iP after the late stationary phase of growth, and to metabolise iPA present in the culture medium up to the logarithmic growth phase (Timmusk *et al*., 1999). Although other strains of *P. polymyxa* (L6, Pw2) had no impact on root dihydrozeatin riboside (DHZR) concentration of lodgepole pine seedlings, *Pseudomonas fluorescens* M20 increased root DHZR concentration by 2.7-fold. However, all three bacterial strains increased pine root growth, perhaps suggesting that bacterial IAA production (found in all three rhizobacteria *in vitro*) may have had a dominant effect on root growth (Bent *et al*., 2001).

However, in some cases, CK-producing PGPR negatively affected root growth. Although no significant changes in root CK concentrations were detected following inoculation with the CK-producing PGPR *B. subtilis* IB-22, lettuce root length was decreased by 20% (Arkhipova *et al*., 2007), potentially threatening the long-term productivity of plants in soil inoculated with this organism. However, in these short-term (2 weeks) experiments with plants grown under nutritionally optimal conditions but with constrained root systems, the negative impact of CKs on root elongation did not prevent a stimulatory effect of CKs on shoot growth, probably mediated by increased cell division and cell wall extensibility (Rayle *et al*., 1982). Root growth inhibition by CKs may be partially attributed to increased ethylene production (Lorteau *et al*., 2001), thus bacterial mechanisms of decreasing root ethylene production (discussed below) may determine whether a CK-producing PGPR has positive or negative impact on root growth. Since co-occurrence of bacterial CK production and ACCd activity has yet to be identified (Table 1), it would be desirable to screen CK-producing rhizobacteria for ACCd activity.

To our knowledge, only one report relates to rhizobacterial CK metabolism: *Serratia proteamaculans* B1 metabolised the synthetic CK N^6-benzyladenine as a carbon source via the enzyme xanthine dehydrogenase (Taylor *et al*., 2006). Surprisingly, this organism was the only one among 60 000 clones of soil/rhizosphere bacteria screened for utilisation of N^6-benzyladenine *in vitro*. This unlikely result may be because of the 'enrichment' procedure applied: before bacterial isolation, soil suspensions were amended with milled plant materials (but not with pure cytokinins!) and shaken for 2 weeks. This procedure might enrich non-target microorganisms and eliminate CK-degrading bacteria. Further attempts should be made to isolate CK-degrading rhizobacteria, in view of the generally negative impacts of CKs on root growth. From a historical standpoint of plant–microbe interactions, it might be speculated that such rhizobacteria may stimulate CK exudation into the rhizosphere, thus minimising the impact of CK-producing rhizobacteria.

Ethylene

The gaseous hydrocarbon ethylene, most popularly associated with fruit ripening, is involved in multiple physiological roles *in planta* and like most other plant hormones can promote or inhibit growth depending on the cell type and plant species (Pierik *et al*., 2006). Typically, plant ethylene production is upregulated in response to environmental stresses such as waterlogging, excess heavy metals and soil compaction (Morgan & Drew, 1997). *In planta*, the first step of ethylene biosynthesis is the conversion of *S*-adenosylmethionine to the immediate ethylene precursor ACC, catalysed by the enzyme ACS. 1-aminocyclopropane-carboxylic acid can be conjugated to malonyl-ACC (Peiser & Yang, 1998), de-aminated

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(McDonnell *et al*., 2009) or oxidised (catalysed by the enzyme ACC oxidase) to give ethylene, carbon dioxide and cyanide (Yang & Hoffman, 1984). The relative importance of these pathways varies according to the plant tissue and environmental conditions.

Although ethylene is usually regarded as an inhibitor of primary root elongation (Swarup *et al*., 2007) and lateral root formation (Negi *et al*., 2010), it has positive impacts on root hair formation and aerenchyma formation (Pierik *et al*., 2006). Ethylene-insensitive mutants have been used to investigate plant–bacteria interactions: stimulation of root hair length by ACCd-containing rhizobacteria was comparable in both WT and the ethylene-insensitive *ein2-1 A. thaliana* mutant, suggesting that stimulation of root hair length was independent of ethylene (Contesto *et al*., 2008). Similar root growth of *ein2-1*, *etr1-3* and WT *A. thaliana* seedlings in response to the IAA-producing *P. thivervalensis* discounted a role for ethylene in root growth inhibition (Persello-Cartieaux *et al*., 2001). Likewise, similar promotion of shoot biomass and lateral root number, and inhibition of primary root length, of *ein2-1*, *etr1-3* and WT *A. thaliana* seedlings in response to *Bacillus megaterium* discounted a role for ethylene in these responses (López-Bucio *et al*., 2007).

Although rhizobacteria can produce ethylene when supplied with methionine in culture media, recent attention has focussed on rhizobacterial mediation of plant ethylene status via the enzyme ACCd that degrades the ethylene precursor ACC (Klee *et al*., 1991; Glick *et al*., 1998). Defined protocols for the isolation of ACCd-containing organisms (Glick *et al*., 1995; Penrose & Glick, 2003), coupled with their generally positive impacts on plant growth (Glick *et al*., 2007) has undoubtedly stimulated the isolation of these bacteria (Fig. 1). ACC deaminase has been identified in 34 bacterial genera (Belimov, 2009) including such well-known PGPR as *A. brasilense* Sp245 (Blaha *et al*., 2006). Since a dynamic equilibrium of ACC concentration exists between root, rhizosphere and bacterium, bacterial uptake of rhizospheric ACC (for use as a carbon and nitrogen source) decreases root ACC concentration and root ethylene evolution and can increase root growth *in vitro* (Glick *et al*., 1998; Penrose *et al*., 2001). Inoculation with the ACCd-containing organism *M. fujisawaense* not only decreased root ACC concentration, but also upregulated canola root ACS activity and decreased root ACC oxidase activity (Madhaiyan *et al*., 2006). A short-term (3 weeks) trial indicated that inoculation of pepper and tomato gnotobiotic seedlings with the ACCd-containing bacteria *Achromobacter piechaudii* ARV8 decreased stress-induced whole plant ethylene evolution and improved recovery of plants when watering was resumed (Mayak *et al*., 2004). It now appears that effects of ACCd-containing bacteria are not restricted to the root system, as the ACCd-containing bacteria *V. paradoxus* 5C-2 attenuated a drought-induced increase in xylem ACC concentration in pea, thus increasing plant yield and water use efficiency by promoting vegetative growth (Belimov *et al*., 2009).

The importance of bacterial ACCd in plant growth promotion has been demonstrated by experiments showing ACCd-containing bacteria stimulated plant growth while mutants in the same genetic background but lacking ACCd did not, for canola primary root elongation under gnotobiotic conditions (Glick *et al*., 1994, 1997; Li *et al*., 2000; Madhaiyan *et al*., 2006) and canola (Glick *et al*., 1997) and pea (Belimov *et al*., 2009) seedling root and shoot growth in pot trials. An ACCd-deficient mutant (T8-1) of *Pseudomonas brassicacearum* Am3 had a dose-dependent negative impact on tomato (*Lycopersicon esculentum*) primary root growth *in vitro*, but the WT strain did not (Belimov *et al*., 2007). However, WT- and ACCd-deficient mutants of four different bacteria (*Phyllobacterium brassicacearum* STM196, *P. putida* UW4, *Rhizobium leguminosarum* bv. *viciae* 128C53K, *Mesorhizobium loti* MAFF303099) had similar effects on primary root length, total lateral root length and lateral root number of *A. thaliana* seedlings grown *in vitro* (Contesto *et al*., 2008). Although all these bacteria stimulated root hair length by two- to three-fold, the ACCd-deficient mutants further stimulated root hair length by 15–40% (Contesto *et al*., 2008) indicating a negative impact of bacterial ACCd on root hair elongation. Similar results were obtained in a gnotobiotic system of tomato and *P. brassicacearum* Am3 and its mutant T8-1 (Belimov & Dodd, unpublished data). The functional significance of these contrasting effects of bacterial ACCd on root elongation (stimulation or no effect) and root hair elongation (inhibition) on total nutrient uptake requires further evaluation, especially since increased root hair length can improve P uptake (Gahoonia & Nielsen, 2004). Furthermore, ACCd-containing bacteria failed to promote root elongation of P-deficient rape seedlings having a low ethylene evolution rate, and positive bacterial effects on plant growth decreased in P-deficient soil (Belimov *et al*., 2002).

Although effects of ACCd-containing organisms on plant growth have been well studied, impacts of ethylene metabolising microorganisms on plant growth have received little attention. While ethylene is actively metabolised in soils (Abeles *et al*., 1971; Smith *et al*., 1973; Cornforth, 1975; Arshad & Frankenberger, 1991), there are few reports on the isolation and characterisation of ethylene-degrading bacteria (Table 2). Of five *Mycobacterium* strains isolated from soil (De Bont, 1976), *Mycobacterium* sp. E20 converted ethylene to ethylene oxide, which was metabolised via acetyl-CoA to epoxide

(De Bont & Harder, 1978). Soil inoculation with the unidentified ethylene-degrading bacterium RD-4 rapidly decreased exogenous ethylene concentrations surrounding potted *Begonia elatior* plants to below the detection limit, and thus lowered bud drop of the ethylene-treated plants (Elsgaard & Andersen, 1998). Since this strain was also successfully used in a biofilter for ethylene removal from soil, the role of such rhizobacteria in mediating plant tolerance to environmental stresses (that stimulate ethylene production) seems worthy of further study.

Gibberellins

Gibberellins (GAs) are diterpenes constituted of four isoprene units derived from *ent*-kaurene formed by cyclisation of geranylgeranyl pyrophosphate (Bomke & Tudzynski, 2009). Many plants contain a mixture of different GAs, and at least 130 gibberellins have been isolated from natural sources. Cleavage of the ring system results in loss of activity. The biochemical mechanisms for bacterial gibberellin production are largely similar to those in plants, although fewer biochemical steps have been unequivocally demonstrated (Bottini *et al.*, 2004).

Mutant plants that are GA-deficient generally show decreased lateral root number and length, a phenotype that is apparently independent of photo-assimilate supply from the shoot and increases in severity as root GA levels decrease (Yaxley *et al.*, 2001). These mutational analyses support more classical studies of GA addition to intact roots, which show promotion of primary root elongation across a wide concentration range (Fig. 2, El-Antably & Larsen, 1974). It is therefore not surprising that bacterial GA production has been viewed as a growth-promoting trait, and a number of PGPR produce GAs in culture media (Table 1). Rhizobacterial production and metabolism of GAs was recently comprehensively reviewed (Bottini *et al.*, 2004), thus this section aims only to highlight key results, and the *in planta* effects of GA-producing rhizobacteria. Rhizobacterial GA production was first suggested by incubating lettuce hypocotyls in the culture medium of *A. brasilense* Sp13t SR2 (grown under stationary conditions for 7 days in nitrogen-free medium): this bioassay suggested concentrations of 0.05 μg mL^{-1} of GA_3 equivalents, while concentrations of authentic GA_3 an order of magnitude lower increased lateral root number in pearl millet (Tien *et al.*, 1979). Gas chromatography–mass spectrometry unequivocally identified GA_1 and GA_3 in culture media of *A. lipoferum* (Bottini *et al.*, 1989) and *A. brasilense* (Janzen *et al.*, 1992), with the levels detected depending on the culture medium. Culture media of the auxin-producing rhizobacteria *Bacillus pumilis* and *Bacillus licheniformis* also contained high levels of GA_1, GA_3, GA_4 and GA_{20}, and topical application of concentrated culture media to the shoot apex of alder (*Alnus glutinosa*) partially reversed the inhibition of stem elongation and leaf expansion induced by the GA biosynthesis inhibitor paclobutrazol (Gutierrez-Manero *et al.*, 2001). Similarly, *A. lipoferum* USA5b and *A. brasilense* Cd promoted sheath elongation of GA-deficient dwarf rice, when the inoculated seedlings had been supplied with glucosyl ester GA precursors (Cassán *et al.*, 2001). Despite this long history of research into rhizobacterial GA production, and more recent discoveries of new bacterial species producing physiologically active GAs (Table 1), unequivocal demonstration that rhizobacteria can increase plant GA levels is, somewhat surprisingly, lacking. In addition, although rhizobacteria such as *Azospirillum* are capable of metabolising GAs *in vitro* and in association with plants (reviewed by Bottini *et al.*, 2004) there is no evidence that they use these substances as a nutrient source, suggesting that the biochemical mechanisms underlying biodegradation of GAs in soil requires further attention.

Jasmonic acid

Despite intense interest in PGPR triggering plant induced systemic resistance (ISR) to insect attack and plant disease via JA-dependent pathways (reviewed in Van der Ent *et al.*, 2009), ISR is not always associated with enhanced plant JA concentrations (Pieterse *et al.*, 2000). This suggests that effects of ISR-producing PGPR are mediated by altered JA sensitivity as supported by numerous studies with JA-insensitive mutants (reviewed in Van der Ent *et al.*, 2009). Despite this, certain PGPR can produce JA (and its precursor 12-oxo-phytodienoic acid) in culture media at very low water potential (Forchetti *et al.*, 2007), although it is not certain how widespread this trait is. It will be interesting to determine whether JA-producing bacteria can increase plant JA concentrations and/or affect ISR responses, and whether rhizobacteria can metabolise JA.

Salicylic acid

Analogous to the role of JA in plant–bacteria interactions, induction of ISR by PGPR via SA-dependent pathways seems to be independent of rhizobacterial SA production (Press *et al.*, 1997; Ran *et al.*, 2005). Although initial work revealed that nanogram amounts of SA produced by *Pseudomonas aeruginosa* 7NSK2 triggered ISR in common bean (De Meyer *et al.*, 1999), a mutant strain of the same organism unable to produce SA also triggered ISR in *A. thaliana*, while three pseudomonad species were still able to trigger ISR in SA non-accumulating transgenic plants (Ran *et al.*, 2005). Furthermore, mutants of *Serratia marcescens* 90-166 that did not produce detectable

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amounts of SA still retained ISR, while a mutant that did not induce ISR still produced SA, confirming that bacterial SA biosynthesis was not the primary mediator of ISR (Press *et al*., 1997). Many naphthalene-degrading bacteria of the genus *Pseudomonas* catabolise SA to catechol and then cleave it via the meta- or ortho-pathway, or oxidise it through the gentisic acid pathway to Krebs cycle intermediates (Yen & Serdar, 1988). However, the role of these bacteria in mediating plant SA status during plant–microbe interactions has not been studied.

Conclusions

As discussed above, elucidating the role of individual phytohormones in plant responses to PGPR has used plant mutants that are hormone insensitive, bacterial mutants with altered capacity for phytohormone synthesis and/or fractionation of bacterial culture filtrates to determine whether physiological effects can be explained by particular fractions (corresponding to known plant hormones). Despite these efforts, the abundant evidence that PGPR produce (Table 1) or metabolise (Table 2) phytohormones *in vitro* has not always been translated into measurements of hormone concentrations (or sensitivity) *in planta* (Table 1), in part since multi-analyte plant hormone analysis (a necessity since many PGPR produce more than one plant hormone, and synergistic or antagonistic hormone interactions exist in plants) is time-consuming. Future research should aim to link root colonisation by PGPR (bacterial quantification) in the field, with rhizosphere and plant hormone concentrations, to determine whether the potential for rhizobacterial mediation of plant hormone status (Table 1) and growth is actually expressed following soil inoculation with PGPR. Although rhizobacterial hormone production has received more attention, the role of rhizobacterial hormone degradation may be particularly important, and may partially account for unsuccessful attempts in applying hormone-producing PGPR to stimulate plant growth.

Further research efforts are required in this area, to allow the sustainable intensification of agriculture despite decreasing availability of water (because of the climate change and competition from other users) and nutrients (because of unacceptable environmental pollution resulting from nutrient leaching). Consequently, many authors have advocated the use of PGPR, including those that mediate plant hormone status reviewed here, to sustain crop yields despite decreased nutrient (Adesemoye *et al*., 2009) and water (Dodd, 2009) inputs. In spite of considerable (arguably misplaced) optimism that PGPR could contribute additional nitrogen resources to the plants, as distinct from 'mining' existing soil resources (reviewed in Andrews *et al*., 2003), it seems that PGPR that impact on plant hormone status will most likely be used in conjunction with 'proven technologies' such as mycorrhizae (to augment plant P uptake) and nodulating bacteria (to fix nitrogen). Despite some successes from such co-inoculation (Gamalero *et al*., 2008; Adesemoye *et al*., 2009), developing compatible microbial mixtures will remain an academic and commercial challenge, because of its usual empirical methodology and the prospect of microbial antagonism. Nevertheless, experiments demonstrating that application of ACCd-containing rhizobacteria to field soils enhanced legume nodulation by indigenous rhizobia (Belimov *et al*., 2009) suggest that rhizobacteria that mediate plant hormone status may prove a viable technology as independent inocula.

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References

Abeles F.B., Craker L.E., Forrence L.E., Leather G.R. (1971) Fate of air pollutants: removal of ethylene, sulfur dioxide, and nitrogen dioxide by soil. *Science*, **173**, 914–916.

Adesemoye A.O., Torbert H.A., Kloepper J.W. (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microbial Ecology*, **58**, 921–929.

Ahmad F., Ahmad I., Khan M.S. (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting characteristics. *Microbiological Research*, **163**, 173–181.

Ali B., Sabri A.N., Ljung K., Hasnain S. (2009) Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of *Triticum aestivum* L. *Letters in Applied Microbiology*, **48**, 542–547.

Andrews M., James E.K., Cummings S.P., Zavalin A.A., Vinogradova L.V., McKenzie B.A. (2003) Use of nitrogen fixing bacteria inoculants as a substitute for nitrogen fertiliser for dryland graminaceous crops: progress made, mechanisms of action and future potential. *Symbiosis*, **35**, 209–229.

Arkhipova T.N., Prinsen E., Veselov S.U., Martynenko E.V., Melentiev A.I., Kudoyarova G.R. (2007) Cytokinin producing bacteria enhance plant growth in drying soil. *Plant and Soil*, **292**, 305–315.

Arshad N., Frankenberger W.T. (1991) Microbial production of plant hormones. *Plant and Soil*, **133**, 1–8.

Belimov A.A. (2009) Interactions between associative bacteria and plants depending on biotic and abiotic factors. DSc Thesis. ARRIAM, Saint-Petersburg, p. 356 (in Russian).

Belimov A.A., Dietz K.-J. (2000) Effect of associative bacteria on element composition of barley seedlings grown in solution culture at toxic cadmium concentrations. *Microbiological Research*, **155**, 113–121.

Belimov A.A., Safronova V.I., Sergeyeva T.A., Egorova T.N., Matveyeva V.A., Tsyganov V.E., Borisov A.Y., Tikhonovich I.A., Kluge C., Preisfeld A., Dietz K.-J., Stepanok V.V. (2001) Characterisation of plant growth-promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. *Canadian Journal of Microbiology*, **47**, 642–652.

Belimov A.A., Safronova V.I., Mimura T. (2002) Response of spring rape to inoculation with plant growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase depends on nutrient status of the plant. *Canadian Journal of Microbiology*, **48**, 189–199.

Belimov A.A., Hontzeas N., Safronova V.I., Demchinskaya S.V., Piluzza G., Bullitta S., Glick B.R. (2005) Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biology and Biochemistry*, **37**, 241–250.

Belimov A.A., Dodd I.C., Safronova V.I., Hontzeas N., Davies W.J. (2007) *Pseudomonas brassicacearum* strain Am3 containing 1-aminocyclopropane-1-carboxylate deaminase can show both pathogenic and growth-promoting properties in its interaction with tomato. *Journal of Experimental Botany*, **58**, 1485–1495.

Belimov A.A., Dodd I.C., Hontzeas N., Theobald J.C., Safronova V.I., Davies W.J. (2009) Rhizosphere bacteria containing ACC deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. *New Phytologist*, **181**, 413–423.

Bent E., Tuzun S., Chanway C.P., Enebak S. (2001) Alterations in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. *Canadian Journal of Microbiology*, **47**, 793–800.

Bertell G., Eliasson L. (1992) Cytokinin effects on root growth and possible interactions with ethylene and indole-3-acetic acid. *Physiologia Plantarum*, **84**, 255–261.

Blaha D., Prigent-Combaret C., Mirza M.S., Moenne-Loccoz Y. (2006) Phylogeny of the 1-aminocyclopropane-1-carboxylic acid deaminase-encoding gene acdS in phytobeneficial and pathogenic Proteobacteria and relation with strain biogeography. *FEMS Microbiology Ecology*, **56**, 455–470.

Bomke C., Tudzynski B. (2009) Diversity, regulation, and evolution of the gibberellin biosynthetic pathway in fungi compared to plants and bacteria. *Phytochemistry*, **70**, 1876–1893.

Bottini R., Cassán F., Piccoli P. (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. *Applied Microbiology and Biotechnology*, **65**, 497–503.

Bottini R., Fulchieri M., Pearce D., Pharis R. (1989) Identification of gibberellins A1, A3, and Iso-A3 in cultures of *A. lipoferum*. *Plant Physiology*, **90**, 45–47.

Cary A.J., Liu W.N., Howell S.H. (1995) Cytokinin action is coupled to ethylene in its effects on the inhibition of root and hypocotyl elongation in *Arabidopsis thaliana* seedlings. *Plant Physiology*, **107**, 1075–1082.

Cassán F., Bottini R., Schneider G., Piccoli P. (2001) *Azospirillum brasilense* and *Azospirillum lipoferum* hydrolyze conjugates of GA(20) and metabolize the resultant aglycones to GA(1) in seedlings of rice dwarf mutants. *Plant Physiology*, **125**, 2053–2058.

Cassán F., Perrig D., Sgroy V., Masciarelli O., Penna C., Luna V. (2009) *Azospirillum brasilense* AZ39 and *Bradyrhizobium japonicum* E1009, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). *European Journal of Soil Biology*, **45**, 28–35.

Casson S.A., Lindsey K. (2003) Genes and signaling in root development. *New Phytologist*, **158**, 11–38.

Cattelan A.J., Hartel P.G., Fuhrmann J.J. (1992) Screening for plant growth-promoting rhizobacteria to promote early soybean growth. *Soil Science Society of America Journal*, **63**, 1670–1680.

Claus G., Kutzner H.J. (1983) Degradation of indole by *Alcaligenes* species. *Systematic and Applied Microbiology*, **4**, 169–180.

Cohen A.C., Bottini R., Piccoli P.N. (2008) *Azospirillum brasilense* Sp245 produces ABA in chemically-defined culture medium and increases ABA content in *Arabidopsis* plants. *Plant Growth Regulation*, **54**, 97–103.

Cohen A.C., Travaglia C.N., Bottini R., Piccoli P.N. (2009) Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. *Botany*, **87**, 455–462.

Cohen J.D., Slovin J.P., Hendrickson A.M. (2003) Two genetically discrete pathways convert tryptophan to auxin: more redundancy in auxin biosynthesis. *Trends in Plant Science*, **8**, 197–199.

Coleman N.V., Mattes T.E., Gossett J.M., Spain J.C. (2002) Phylogenetic and kinetic diversity of aerobic vinyl chloride-assimilating bacteria from contaminated sites. *Applied and Environmental Microbiology*, **68**, 6162–6171.

Contesto C., Desbrosses G., Lefoulon C., Béna G., Borel F., Galland M., Gamet L., Varoquaux F., Touraine B. (2008) Effects of rhizobacterial ACC deaminase activity on *Arabidopsis* indicate that ethylene mediates local root responses to plant growth-promoting rhizobacteria. *Plant Science*, **175**, 178–189.

Cornforth I.S. (1975) The persistence of ethylene in aerobic soils. *Plant and Soil*, **42**, 85–96.

Costacurta A., Vanderleyden J. (1995) Synthesis of phytohormones by plant-associated bacteria. *Critical Reviews in Microbiology*, **21**, 1–18.

Creus C.M., Sueldo R.J., Barassi C.A. (2004) Water relations and yield in *Azospirillum*-inoculated wheat exposed to drought in the field. *Canadian Journal of Botany*, **82**, 273–281.

Deak K.I., Malamy J. (2005) Osmotic regulation of root system architecture. *Plant Journal*, **43**, 17–28.

De Bont J.A.M. (1976) Oxidation of ethylene by soil bacteria. *Antonie van Leeuwenhoek*, **42**, 59–71.

De Bont J.A.M., Harder W. (1978) Metabolism of ethylene by *Mycobacterium* E20. *FEMS Microbiology Letters*, **3**, 89–93.

De Meyer G., Capieau K., Audenaert K., Buchala A., Métraux J.-P., Höfte M. (1999) Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. *Molecular Plant-Microbe Interactions*, **12**, 450–458.

De Salamone I.E.G., Hynes R.K., Nelson R.M. (2001) Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Canadian Journal of Microbiology*, **47**, 404–411.

Dey R., Pal K.K., Bhatt D.M., Chauhan S.M. (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. *Microbiological Research*, **159**, 371–394.

Dobbelaere S., Croonenborghs A., Thys A., Van de Broek A., Vanderleyden J. (1999) Phytostimulatory effect of *Azospirillum brasilense* wild type and mutant strains altered in IAA production on wheat. *Plant and Soil*, **212**, 155–164.

Dobbelaere S., Vanderleyden J., Okon Y. (2003) Plant growth-promoting effects of diazotrophs in the rhizosphere. *Critical Reviews in Plant Sciences*, **22**, 107–149.

Dodd I.C. (2005) Root-to-shoot signalling: assessing the roles of ''up'' in the up and down world of long-distance signalling *in planta*. *Plant and Soil*, **274**, 251–270.

Dodd I.C. (2007) Soil moisture heterogeneity during deficit irrigation alters root-to-shoot signalling of abscisic acid. *Functional Plant Biology*, **34**, 439–448.

Dodd I.C. (2009) Rhizosphere manipulations to maximise ''crop per drop'' during deficit irrigation. *Journal of Experimental Botany*, **60**, 2454–2459.

Dodd I.C., Theobald J.C., Richer S.K., Davies W.J. (2009*a*) Partial phenotypic reversion of ABA-deficient *flacca* tomato (*Solanum lycopersicum*) scions by a wild-type rootstock: normalising shoot ethylene relations promotes leaf area but does not diminish whole plant transpiration rate. *Journal of Experimental Botany*, **60**, 4029–4039.

Dodd I.C., Jiang F., Teijeiro R.G., Belimov A.A., Hartung W. (2009*b*) The rhizosphere bacterium *Variovorax paradoxus* 5C-2 containing ACC deaminase does not increase systemic ABA signaling in maize (*Zea mays* L.). *Plant Signaling and Behaviour*, **4**, 519–521.

Egebo L.A., Nielsen S.V.S., Jochimsen B.U. (1991) Oxygen-dependent catabolism of indole-3-acetic acid in *Bradyrhizobium japonicum*. *Journal of Bacteriology*, **173**, 4897–4901.

Ehman A. (1977) The van Urk–Salkowski reagent – a sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. *Journal of Chromatography*, **132**, 267–276.

El-Antably H.M.M., Larsen P. (1974) Distribution of gibberellins and abscisic acid in geotropically stimulated *Vicia faba* roots. *Physiologia Plantarum*, **32**, 322–329.

Eliasson L., Bertel G., Bolander E. (1989) Inhibitory action of auxin on root elongation not mediated by ethylene. *Plant Physiology*, **91**, 310–314.

Elsgaard E. (1998) Ethylene removal by a biofilter with immobilized bacteria. *Applied and Environmental Microbiology*, **64**, 4168–4173.

Elsgaard E., Andersen L. (1998) Microbial ethylene consumption in peat-soil during ethylene exposure of *Begonia elatior*. *Plant and Soil*, **202**, 231–239.

Forchetti G., Masciarelli O., Alemano S., Alvarez D., Abdala G. (2007) Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Applied Microbiology and Biotechnology*, **76**, 1145–1152.

Frankenberger W.T., Arshad M. (1995) *Phytohormones in Soils: Production and Function.* New York: Marcel Dekker, Inc. 504 pp.

Fukaki H., Tasaka M. (2009) Hormone interactions during lateral root formation. *Plant Molecular Biology*, **69**, 437–449.

Gahoonia T.S., Nielsen N.E. (2004) Barley genotypes with long root hairs sustain high grain yields in low-P field. *Plant and Soil*, **262**, 55–62.

Gamalero E., Berta G., Massa N., Glick B.R., Lingua G. (2008) Synergistic interactions between the ACC deaminase-producing bacterium *Pseudomonas putida* UW4 and the AM fungus *Gigaspora rosea* positively affect cucumber plant growth. *FEMS Microbiology Ecology*, **64**, 459–467.

Glick B.R., Jacobson C.B., Schwarze M.M.K., Pasternak J.J. (1994) 1-Aminocyclopropane-1-carboxylic acid deaminase mutants of the plant-growth promoting rhizobacterium *Pseudomonas putida* GR12-2 do not stimulate canola root elongation. *Canadian Journal of Microbiology*, **40**, 911–915.

Glick B.R., Karaturovic D.M., Newell P.C. (1995) A novel procedure for rapid isolation of plant-growth promoting pseudomonads. *Canadian Journal of Microbiology*, **41**, 533–536.

Glick B.R., Liu C., Ghosh S., Dumbroff E.B. (1997) Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. *Soil Biology and Biochemistry*, **29**, 1233–1239.

Glick B.R., Penrose D.M., Li J.P. (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *Journal of Theoretical Biology*, **190**, 63–68.

Glick B.R., Cheng Z., Czarny J., Duan J. (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. *European Journal of Plant Pathology*, **119**, 329–339.

Glickmann E., Dessaux Y. (1995) A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Applied and Environmental Microbiology*, **61**, 793–796.

Grishchenkov V.G., Shishmakov D.A., Kosheleva I.A., Boronin A.M. (2003) Growth of bacteria degrading naphthalene and salicylate at low temperatures. *Applied Biochemistry and Microbiology*, **39**, 282–288.

Guo D., Liang J., Li L. (2009) Abscisic acid (ABA) inhibition of lateral root formation involves endogenous ABA biosynthesis in *Arachis hypogaea* L. *Plant Growth Regulation*, **58**, 173–179.

Gutierrez-Manero F.J., Ramos-Solano B., Robanza A., Mehouachi J., Tadeo F.R., Talon M. (2001) The plant-growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. *Physiologia Plantarum*, **111**, 206–211.

Hartung W., Sauter A., Turner N.C., Fillery I., Heilmeier H. (1996) Abscisic acid in soils: What is its function and which factors and mechanisms influence its concentration? *Plant and Soil*, **184**, 105–110.

Hasegawa S., Poling S.M., Mayer V.P., Bennett R.D. (1984) Metabolism of abscisic acid: bacterial conversion to dehydrovomifoliol and vomifoliol dehydrogenase activity. *Phytochemistry*, **23**, 2769–2771.

Hewelt A., Prinsen E., Schell J., Van Onckelen H., Schmuelling T. (1994) Promoter tagging with a promoterless ipt gene leads to cytokinin-induced phenotypic variability in transgenic tobacco plants: implications of gene dosage effects. *Plant Journal*, **6**, 879–891.

Holland M.A. (1997) Occam's razor applied to hormonology. Are cytokinins produced by plants? *Plant Physiology*, **115**, 865–868.

Idriss E.E., Iglesias D.J., Talon M., Borriss R. (2007) Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. *Molecular Plant–Microbe Interactions*, **20**, 619–626.

Ilyas N., Bano A. (2010) *Azospirillum* strains isolated from roots and rhizosphere soil of wheat (*Triticum aestivum* L.) grown under different soil moisture conditions. *Biology and Fertility of Soils*, **46**, 393–406.

Islam M.R., Madhaiyan M., Boruah H.P.D., Yim W., Lee G., Saravanan V.S., Fu Q., Hu H., Sa T. (2009) Characterization of plant growth-promoting traits of free-living diazotrophic bacteria and their inoculation effects on growth and nitrogen uptake of crop plants. *Journal of Microbiology and Biotechnology*, **19**, 1213–1222.

Jaeger C.H., Lindow S.E., Miller S., Clark E., Firestone M.K. (1999) Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. *Applied and Environmental Microbiology*, **65**, 2685–2690.

Jaiswal P.K., Thakur I.S. (2007) Isolation and characterization of dibenzofuran-degrading *Serratia marcescens* from alkalophilic bacterial consortium of the chemostat. *Current Microbiology*, **55**, 447–454.

James E.K., Olivares F.L. (1998) Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs. *CRC Critical Reviews in Plant Sciences*, **17**, 77–119.

Janzen R.A., Rood S.B., Dormaar J.F., McGill W.B. (1992) *Azospirillum brasilense* produces gibberellin in pure culture on chemically-defined medium and in co-culture on straw. *Soil Biology and Biochemistry*, **24**, 1061–1064.

Jensen J.B., Egsgaard H., Vanonckelen H., Jochimsen B.U. (1995) Catabolism of indole-3-acetic-acid and 4-chloroindole-3-acetic and 5-chloroindole-3-acetic acid in *Bradyrhizobium japonicum*. *Journal of Bacteriology*, **177**, 5762–5766.

Joo G.-J., Kim Y.-M., Lee I.-J., Song K.-S., Rhee I.-K. (2004) Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus macroides* and *Bacillus pumilus*. *Biotechnology Letters*, **26**, 487–491.

Joo G.-J., Kang S.-M., Hamayun M., Kim S.-K., Na C.-I., Shin D.-H., Lee I.-J. (2009) *Burkholderia* sp. KCTC 11096BP as a newly isolated gibberellin producing bacterium. *The Journal of Microbiology*, **47**, 167–171.

Kang S.-M., Joo G.-J., Hamayun M., Na C.-I., Shin D.-H., Kim H.Y., Hong J.-K., Lee I.-J. (2009) Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. *Biotechnology Letters*, **31**, 277–281.

Kende H. (1993) Ethylene biosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **44**, 283–307.

Kesserü P., Kiss I., Bihari Z., Pál K., Portörö P., Polyák B. (2005) Nitrate-dependent salicylate degradation by *Pseudomonas butanovora* under anaerobic conditions. *Bioresource Technology*, **96**, 779–784.

Klee H.J., Hayford M.B., Kretzmer K.A., Barry G.F., Kishore G.M. (1991) Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. *Plant Cell*, **3**, 1187–1193.

Kravchenko L.V., Leonova E.I. (1993) Utilization of root metabolite tryptophan in biosynthesis of indole-acetic acid by associative bacteria. *Microbiologiya*, **62**, 453–459.

Kudo T., Kiba T., Sakakibara H. (2010) Metabolism and long-distance translocation of cytokinins. *Journal of Integrative Plant Biology*, **52**, 53–60.

Leveau J.H.J., Lindow S.E. (2005) Utilization of the plant hormone indole-3-acetic acid for growth by *Pseudomonas putida* strain 1290. *Applied and Environmental Microbiology*, **71**, 2365–2371.

Leveau J.H.J., Gerards S. (2008) Discovery of a bacterial gene cluster for catabolism of the plant hormone indole 3-acetic acid. *FEMS Microbiology Ecology*, **65**, 238–250.

Li J.P., Ovakim D.H., Charles T.C., Glick B.R. (2000) An ACC deaminase minus mutant of *Enterobacter cloacae* UW4 no longer promotes root elongation. *Current Microbiology*, **41**, 101–105.

Libbert E., Risch H. (1969) Interactions between plants and epiphytic bacteria regarding their auxin metabolism. V. Isolation and identification of the IAA-producing and destroying bacteria from pea plants. *Physiologia Plantarum*, **22**, 51–58.

López-Bucio J., Campos-Cuevas J.C., Hernández-Calderón E., Velásquez-Becerra C., Farías-Rodríguez R., Macías-Rodríguez L.I., Valencia-Cantero E. (2007) *Bacillus megaterium* rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in *Arabidopsis thaliana*. *Molecular Plant–Microbe Interactions*, **20**, 207–217.

Lorteau M.A., Ferguson B.J., Guinel F.C. (2001) Effects of cytokinin on ethylene production and nodulation in pea (*Pisum sativum*) cv. Sparkle. *Physiologia Plantarum*, **112**, 421–428.

Lugtenberg B., Kamilova F. (2009) Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology*, **63**, 541–556.

Lynch J.P. (2007) Roots of the second green revolution. *Australian Journal of Botany*, **55**, 493–512.

Madhaiyan M., Poonguzhali S., Ryu J., Sa T. (2006) Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carboxylate deaminase-containing *Methylobacterium fujisawaense*. *Planta*, **224**, 268–278.

Mantelin S., Touraine B. (2004) Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. *Journal of Experimental Botany*, **55**, 27–34.

Marasco E.K., Schmidt-Dannert C. (2008) Identification of bacterial carotenoid cleavage dioxygenase homologues that cleave the interphenyl α-β double bond of stilbene derivatives via a monooxygenase reaction. *Chembiochem*, **9**, 1450–1461.

Mayak S., Tirosh T., Glick B.R. (2004) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Science*, **166**, 525–530.

McDonnell L., Plett J.M., Andersson-Gunneras S., Kozela C., Dugardeyn J., Van Der Straeten D., Glick B.R., Sundberg B., Regan S. (2009) Ethylene levels are regulated by a plant encoded 1-aminocyclopropane-1-carboxylic acid deaminase. *Physiologia Plantarum*, **136**, 94–109.

Mino Y. (1970) Studies on the destruction of indole-3-acetic acid by a species of *Arthrobacter*. IV. Decomposition products. *Plant and Cell Physiology*, **11**, 129–138.

Morgan P.W., Drew M.C. (1997) Ethylene and plant responses to stress. *Physiologia Plantarum*, **100**, 620–630.

Munns R.E., Cramer G.R. (1996) Is co-ordination of leaf and root growth mediated by abscisic acid? Opinion. *Plant and Soil*, **185**, 33–49.

Negi S., Sukumar P., Liu X., Cohen J.D., Muday C.K. (2010) Genetic dissection of the role of ethylene in regulating auxin-dependent lateral and adventitious root formation in tomato. *Plant Journal*, **61**, 3–15.

Omer Z.S., Tombolini R., Broberg A., Gerhardson B. (2004*a*) Indole-3-acetic acid production by pink-pigmented facultative methylotrophic bacteria. *Plant Growth Regulation*, **43**, 93–96.

Omer Z.S., Bjorkman P.O., Nicander B., Tillberg E., Gerhardson B. (2004*b*) 5′-Deoxyisopentenyladenosine and other cytokinins in culture filtrates of the bacterium *Pantoea agglomerans*. *Physiologia Plantarum*, **121**, 439–447.

Patten C., Glick B.R. (2002) Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Applied and Environmental Microbiology*, **68**, 3795–3801.

Peiser G., Yang S.F. (1998) Evidence for 1-(malonylamino) cyclopropane-1-carboxylic acid being the major conjugate of aminocyclopropane-1-carboxylic acid in tomato fruit. *Plant Physiology*, **116**, 1527–1532.

Penrose D.M., Glick B.R. (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiologia Plantarum*, **118**, 10–15.

Penrose D.M., Moffatt B.A., Glick B.R. (2001) Determination of 1-aminocyclopropane-1-carboxylic acid (ACC) to assess the effects of ACC deaminase-containing bacteria on roots of canola seedlings. *Canadian Journal of Microbiology*, **47**, 77–80.

Perrig D., Boiero M.L., Masciarelli O.A., Penna C., Ruiz O.A., Cassan F.D., Luna M.V. (2007) Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. *Applied Microbiology and Biotechnology*, **75**, 1143–1150.

Persello-Cartieaux F., David P., Sarrobert C., Thibaud M.C., Achouak W., Robaglia C., Nussaume L. (2001) Utilization of mutants to analyze the interaction between *Arabidopsis thaliana* and its naturally root-associated Pseudomonas. *Planta*, **212**, 190–198.

Pierik R., Tholde D., Poorter H., Visser E.J.W., Voesenek L.A.C.J. (2006) The Janus face of ethylene: growth inhibition and stimulation. *Trends in Plant Science*, **11**, 176–183.

Pieterse C.M.J., Van Pelt J.A., Ton J., Parchmann S., Mueller M.J., Buchala A.J., Metraux J.P., Van Loon L.C. (2000) Rhizobacteria-mediated induced systemic

resistance (ISR) in *Arabidopsis* requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. *Physiological and Molecular Plant Pathology*, **57**, 123–134.

Pii Y., Crimi M., Cremonese G., Spena A., Pandolfini T. (2007) Auxin and nitric oxide control indeterminate nodule formation. *BMC Plant Biology*, **7**, 1–11.

Pirttilä A.M., Joensuu P., Pospiech H., Jalonen J., Hohtola A. (2004) Bud endophytes of Scots pine produce adenine derivatives and other compounds that affect morphology and mitigate browning of callus cultures. *Physiologia Plantarum*, **121**, 305–312.

Plotnikova E.G., Altyntseva O.V., Kosheleva I.A., Puntus I.F., Filonov A.E., Garvish E.Y., Demakov V.A., Boronin A.M. (2001) Bacterial degraders of polycyclic aromatic hydrocarbons isolated from salt-contaminated soils and bottom sediments in salt mining areas. *Microbiologiya*, **70**, 51–58.

Press C.M., Wilson M., Tuzun S., Kloepper J.W. (1997) Salicylic acid produced by *Serratia marcescens* 90-166 is not the primary determinant of induced systemic resistance in cucumber or tobacco. *Molecular Plant–Microbe Interactions*, **10**, 761–768.

Ran L.X., van Loon L.C., Bakker P.H.M. (2005) No role for bacterially produced salicylic acid in rhizobacterial induction of systemic resistance in *Arabidopsis*. *Phytopathology*, **95**, 1349–1355.

Rayle D.L., Ross C.W., Robinson N. (1982) Estimation of osmotic parameters accompanying zeatin induced growth of detached cucumber cotyledons. *Plant Physiology*, **70**, 1634–1636.

Remans R., Beebe S., Blair M., Manrique G., Tovar E., Rao I., Croonenborghs A., Torres-Gutierrez R., El-Howeity M., Michiels J., Vanderleyden J. (2008) Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L.). *Plant and Soil*, **302**, 149–161.

Ribaudo C.M., Krumpholz E.M., Cassan F.D., Bottini R., Cantore M.L., Cura J.A. (2006) *Azospirillum* sp. promotes root hair development in tomato plants through a mechanism that involves ethylene. *Journal of Plant Growth Regulation*, **24**, 175–185.

Roberto F.F., Klee H., White F., Nordeen R., Kosuge T. (1990) Expression of fine structure of the gene encoding NE-(indole-3-acetyl)-L-lysine synthetase from *Pseudomonas savastanoi*. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 5797–5801.

Rothballer M., Schmid M., Hartmann A. (2003) *In situ* localization and PGPR-effect of *Azospirillum brasilense* strains colonizing roots of different wheat varieties. *Symbiosis*, **34**, 261–279.

Royal Society. (2009) *Reaping the Benefits: Science and the Sustainable Intensification of Global Agriculture*. London: Marcel Dekker, Inc. 72 pp.

Ryu R.J., Patten C.L. (2008) Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by tyrR in *Enterobacter cloacae* UW5. *Journal of Bacteriology*, **190**, 7200–7208.

Sandhya V., Ask Z., Grover M., Reddy G., Venkateswarlu B. (2009) Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. *Biology and Fertility of Soils*, **46**, 17–26.

Sasonova O.I., Izmalkova T.Y., Kosheleva I.A., Boronin A.M. (2008) Salicylate degradation by *Pseudomonas putida* strains not involving the ''classical'' nah2 operon. *Microbiologiya*, **77**, 710–716.

Sgroy V., Cassán F., Masciarelli O., Del Papa M.F., Lagares A., Luna V. (2009) Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte *Prosopis strombulifera*. *Applied Microbiology and Biotechnology*, **85**, 371–381.

Sharp R.E., LeNoble M.E. (2002) ABA, ethylene and the control of shoot and root growth under water stress. *Journal of Experimental Botany*, **53**, 33–37.

Siewers V., Kokkelink L., Smedsgaard J., Tudzynski P. (2006) Identification of an abscisic acid gene cluster in the grey mold *Botrytis cinerea*. *Applied and Environmental Microbiology*, **72**, 4619–4626.

Silva T.R., Valdman E., Valdman B., Leite S.G.F. (2007) Salicylic acid degradation from aqueous solutions using *Pseudomonas fluorescens* HK44: parameters studies and application tools. *Brazilian Journal of Microbiology*, **38**, 39–44.

Smith K.A., Bremner J.M., Tabatabai M.A. (1973) Sorption of gaseous atmospheric pollutants by soil. *Soil Science*, **116**, 313–319.

Spaepen S., Vanderleyden J., Remans R. (2007) Indole-3-acetic acid in microbial and microorganism–plant signaling. *FEMS Microbiology Reviews*, **31**, 425–448.

Spaepen S., Dobbelaere S., Croonenborghs A., Vanderleyden J. (2008) Effects of *Azospirillum brasilense* indole-3-acetic acid production on inoculated wheat plants. *Plant and Soil*, **312**, 15–23.

Stenlid G. (1982) Cytokinins as inhibitors of root growth. *Physiologia Plantarum*, **56**, 500–506.

Swarup R., Perry P., Hagenbeek D., Van Der Straten D., Beemster G.T.S., Sandberg G., Bhalerao R., Liung K., Bennett M.J. (2007) Ethylene upregulates auxin biosynthesis in *Arabidopsis* seedlings to enhance inhibition of root cell elongation. *Plant Cell*, **19**, 2186–2196.

Takei K., Ueda N., Aoki K., Kuromori T., Hirayama T., Shinozaki K., Yamaya T., Sakakibara H. (2004) *AtIPT3* is a key determinant of nitrate-dependent cytokinin biosynthesis in *Arabidopsis*. *Plant and Cell Physiology*, **45**, 1053–1062.

Taylor I.B., Burbidge A., Thompson A.J. (2000) Control of abscisic acid biosynthesis. *Journal of Experimental Botany*, **51**, 1563–1574.

Taylor J.L., Zaharia L.I., Chen H., Anderson E., Abrams S.R. (2006) Biotransformation of adenine and cytokinins by the rhizobacterium *Serratia proteamaculans*. *Phytochemistry*, **67**, 1887–1894.

Tien T.M., Gaskins M.H., Hubbell M.H. (1979) Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied and Environmental Microbiology*, **37**, 1016–1024.

Timmusk S., Nicander B., Granhall U., Tillberg E. (1999) Cytokinin production by *Paenibacillus polymyxa*. *Soil Biology and Biochemistry*, **31**, 1847–1852.

Van der Ent S., Van Wees S.C.M., Pieterse C.M.J. (2009) Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. *Phytochemistry*, **70**, 1581–1588.

Vessey J.K. (2003) Plant-growth-promoting rhizobacteria as biofertilizers. *Plant and Soil*, **255**, 571–586.

Werner T., Motyka V., Strnad M., Schmülling T. (2001) Regulation of plant growth by cytokinin. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 10487–10492.

Woodward A.W., Bartel B. (2005) Auxin: regulation, action, and interaction. *Annals of Botany*, **95**, 707–735.

Xie H., Pasternak J.J., Glick B.R. (1996) Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* CR12-2 that overproduce indoleacetic acid. *Current Microbiology*, **32**, 67–71.

Yang S.F., Hoffman N.E. (1984) Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **35**, 155–189.

Yao L.X., Wu Z.S., Zheng Y.Y., Kaleem I., Li C. (2010) Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. *European Journal of Soil Biology*, **46**, 49–54.

Yaxley J.R., Ross J.J., Sherriff L.J., Reid J.B. (2001) Gibberellin biosynthesis mutations and root development in pea. *Plant Physiology*, **125**, 627–633.

Yen K.M., Serdar C.M. (1988) Genetics of naphthalene catabolism in Pseudomonads. *Critical Reviews in Microbiology*, **15**, 247–268.

Zhang H.M., Xie X.T., Kim M.S., Kornveyev D.A., Holaday S., Pare P.W. (2008) Soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels *in planta*. *Plant Journal*, **56**, 264–273.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Examples of rhizobacteria degrading the ethylene precursor ACC

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