BIOLOGICAL CONTROL OF SOIL-BORNE PATHOGENS BY FLUORESCENT PSEUDOMONADS

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Abstract | Particular bacterial strains in certain natural environments prevent infectious diseases of plant roots. How these bacteria achieve this protection from pathogenic fungi has been analysed in detail in biocontrol strains of fluorescent pseudomonads. During root colonization, these bacteria produce antifungal antibiotics, elicit induced systemic resistance in the host plant or interfere specifically with fungal pathogenicity factors. Before engaging in these activities, biocontrol bacteria go through several regulatory processes at the transcriptional and post-transcriptional levels.

SUPPRESSIVE SOIL
A soil in which plants do not suffer from certain diseases or where disease severity is reduced, although a pathogen might be present, and the host plant is susceptible to the disease; the opposite of a conducive soil. Suppressive soils occur worldwide.

CONDUCIVE SOIL
A soil that allows the development of disease; the opposite of a suppressive soil.

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Imagine a place on Earth where an organism does not suffer from infectious disease and is unlikely to become infected even though pathogens might be present. Such exceptional places exist and are known as natural SUPPRESSIVE SOILS[1–3]. They occur, for instance, in the Salinas Valley (California, United States), the Chateaurenard region near Cavaillon (France), the Canary Islands and the Broye Valley (Switzerland). In suppressive soils, the roots of crop plants are protected from diseases that would ordinarily be caused by soil-borne pathogenic microorganisms. Most of these pathogens are fungi, but some bacterial pathogens and plant-deleterious nematodes are also suppressed in certain soils (BOX 1). The main questions that interest us here are how disease suppression works and whether it is directed at specific pathogens or at pathogens in general.

The complexity of the disease-suppression phenomenon can be highlighted by four key observations. First, certain suppressive soils when pasteurized (for example, by wet heat at 60°C for 30 min) lose their suppressiveness, and other harsher antimicrobial treatments (for example, gamma radiation or autoclaving) have the same effect[4–7]. Second, suppressiveness can be transferable: an inoculum of 0.1–10% of a suppressive soil introduced into a CONDUCIVE SOIL can establish disease suppression[3,5–8]. Sensitivity to antimicrobial treatments and transferability indicate that disease suppression results from the activities of soil microorganisms that act as pathogen antagonists. There is evidence that each of the pathogens described in BOX 1 can be held in check by antagonistic microorganisms in suppressive soils[3,9]. Note, however, that the suppressiveness of some soils is not transferable; for a discussion of this observation, the reader is referred to specialized reviews[2,3]. Third, when the pH of a *Fusarium* wilt-suppressive soil was lowered from 8 to 6 by the addition of H_2SO_4, carnations were much less protected from wilting[6]. This loss of suppressiveness caused by a simple pH change illustrates the importance of the soil environment. Clay types and the mineral-ion content of soils, humidity, temperature and fertilizer input can all affect the success of disease suppression[10–13]. Fourth, several years of monoculture can induce disease suppression in some soils. The best-studied example is take-all decline, which has been observed, for instance, in soils in the northwestern United States, The Netherlands and Australia[3,5,14]. After 2 or more years of consecutive cultivation of wheat, the symptoms of take-all disease, which is caused by the fungus *Gaeumannomyces graminis* var. *tritici*, usually increase, but they decline in subsequent years of wheat monoculture[2,3]. The phenomenon of induced disease suppression shows that a host plant, when grown in monoculture, can have a profound influence on the interaction with a pathogen.

Box 1 | **Important root pathogens and suppressive soils**

Soil-borne pathogens belong to several different phyla: bacteria, fungi or nematodes. They reside in the soil for brief or extended periods, and survive on plant residues or as resting organisms until root exudates reach them and allow them to grow. They then escape competition with other microorganisms by penetrating the roots. They either remain inside the plants until host death, or move outside the plants to infect other parts of the root or other roots. Plants infected by soil-borne pathogens suffer from root rot, root blackening, wilt, stunting or seedling damping-off. Losses due to soil-borne pathogens can be prevented to some extent by planting the same crop only every 4–5 years and by using pathogen-free seeds. However, as this is not always possible for economic reasons, soil-borne pathogens can have devastating effects on field and greenhouse crops, in both industrialized and developing countries.

Natural suppressive soils have been described for the following pathogens (examples of associated diseases and symptoms are given in parenthesis): *Gaeumannomyces graminis* var. *tritici* (take-all of wheat, which causes blackening of the plant base, stunting and, in severe cases, white influorescence with shrivelled grains and no yield); *Fusarium oxysporum* (wilt diseases of tomato, radish, banana and others); *Phytophthora cinnamoni* (root rot of eucalyptus); *Pythium* spp. and *Rhizoctonia solani* (damping-off of seedlings of several crops, including sugar beet and radish); *Thielaviopsis basicola* (black root rot of tobacco, bean, cherry trees and others); *Streptomyces scabies* (bacterial potato scab; that is, lesions on potato tubers); *Ralstonia solanacearum* (bacterial wilt of tomato, tobacco and others); *Meloidogyne incognita* (root swelling and root-knot galls caused by this nematode on several crops, mostly in tropical and subtropical countries).

Soil-borne pathogens are notoriously difficult to control. Crop rotation, breeding for resistant plant varieties and the application of pesticides are insufficient to control root diseases of important crop plants. Since the earliest observations of antagonistic disease-suppressing soil microorganisms more than 70 years ago, plant pathologists have been fascinated by the idea that such microorganisms could be used as environmentally friendly biocontrol agents, both in the field and in greenhouses. However, as noted by Garrett 40 years ago, "there are no short cuts to biological control"[10]. In this review, we attempt to explain some of the scientific challenges that still exist in biocontrol research.

An early hypothesis

An intuitive, simple explanation of how the biological control of soil-borne pathogens could work was discussed at the 1963 international symposium entitled 'Ecology of soil-borne plant pathogens — prelude to biological control'[10]. The idea was that antagonistic microorganisms could compete with pathogens, particularly by producing antibiotic compounds. In soil, these antibiotics could interfere with pathogen development, for example, during spore germination and the onset of root infection[10]. This explanation was appealing, as many soil microorganisms (for example, *Streptomyces*, *Bacillus* and *Pseudomonas* spp.) were known to be excellent antibiotic producers *in vitro*[10]. However, there was little evidence 40 years ago that antibiotics were actually produced in soil and, if they were, that they could account for biocontrol activity. The observation made by Wright in 1956 (REF. 15) that the biocontrol fungus *Trichoderma viride* produced detectable amounts of the antifungal antibiotic gliotoxin in soil amended with straw remained unparalleled for many years and the crucial role of antibiotics in the biological control of root diseases remained speculative for three main reasons. First, researchers needed to realize that the chances of detecting environmentally relevant antibiotics are much better in the RHIZOSPHERE than in bulk soil, because microbial activities are 10–1,000-times higher in the vicinity of plant roots than in unplanted soil[16] and because antibiotics generally adsorb to soil particles[17]. Second, more sensitive techniques, such as high-performance liquid chromatography (HPLC), were required for the separation and detection of antibiotics[18]. Third, to establish a causal relationship between ANTIBIOSIS and biocontrol activity, it was necessary to construct mutants of biocontrol strains that specifically lacked antibiotic production and to confirm that these mutants were defective for biocontrol in MICROCOSMS[19–21]. These tools became available in the 1980s.

Plant growth-promoting rhizobacteria

Plant growth-promoting rhizobacteria (PGPR) competitively colonize plant roots, and stimulate plant growth and/or reduce the incidence of plant disease[22]. The PGPR concept has been vindicated by the isolation of many bacterial strains that fulfil at least two of the three criteria described above (aggressive colonization, plant growth stimulation and biocontrol)[3,13,21,23–26]. In some PGPR, termed biofertilizers, plant growth promotion dominates. The mechanisms that are involved in this process can include nitrogen fixation, phosphate solubilization, and the production of phytohormones (such as auxin and cytokinin) and volatile growth stimulants (such as ethylene and 2,3-butanediol)[26,27]. Biofertilizers are not discussed further in this review. In other PGPR, which are sometimes called biopesticides, the biocontrol aspect is most conspicuous. These PGPR, which mostly belong to *Pseudomonas* and *Bacillus* spp., are antagonists of recognized root pathogens. Some conceptual uncertainty was created by the early theory that PGPR might enhance plant growth by excluding so-called deleterious rhizobacteria, which are thought to inhibit plant growth without causing root invasion and classical disease[1]. In retrospect, the evidence for the existence of deleterious rhizobacteria in nature is not convincing[28].

Root-colonizing plant-beneficial fungi are also important in protecting plants from root pathogens. The principal groups consist of non-pathogenic *Fusarium* and *Trichoderma* spp., which have developed a symbiotic, instead of a parasitic, relationship with plants. These topics have been reviewed recently[29,30] and will not be covered here.

Finding effective biocontrol PGPR strains for fundamental research or practical applications requires a combination of ingenuity and hard work. The rhizosphere of soils that are characterized by transferable suppressiveness can be a good source of PGPR, although ordinary (conducive) soils also contain PGPR. The desirable trait of good root colonization can be selected by isolating bacteria that remain attached to the root surface, or have even penetrated into the intercellular spaces between the root epidermis and the cortex, after extensive washing of the roots[7,31]. Enrichment techniques can also be used to obtain competitive root colonizers. In one procedure,

RHIZOSPHERE
A nutrient-rich zone near (that is, a few millimetres from) the roots, where microbial growth is stimulated by root exudates (the rhizosphere effect).

ANTIBIOSIS
A condition in which one or several metabolites that are excreted by an organism have a harmful effect on other organisms. There is no evidence to indicate that antibiotic compounds that are produced in nature result in substantial killing of susceptible organisms.

MICROCOSM
A closed system that contains all the biotic and abiotic components of interest, and mimics environmental conditions in the laboratory.

AXENIC SYSTEM
Conditions in which the biological components to be studied, but no foreign organisms, are present.

Box 2 | ***Pseudomonas***

Members of the genus *Pseudomonas* are rod-shaped Gram-negative bacteria that are characterized by metabolic versatility, aerobic respiration (some strains also have anaerobic respiration with nitrate as the terminal electron acceptor and/or arginine fermentation), motility owing to one or several polar flagella, and a high genomic G+C content (59–68%).

The term pseudomonads (*Pseudomonas*-like bacteria) is often used to describe strains for which the taxonomic affiliation has not been established in detail. In recent years, a distinction has been made between *Pseudomonas sensu stricto* (in the γ-subclass of Proteobacteria) and the genera *Burkholderia, Ralstonia, Acidovorax* and *Comamonas* (all of which were formerly called *Pseudomonas* but belong to the β-subclass). Fluorescent pseudomonads produce the fluorescent pigment Pvd (also known as pseudobactin). This large and heterogeneous group comprises, most notably, *Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens* and *Pseudomonas syringae.*

seeds are inoculated with candidate strains. After germination and growth of the plant in an AXENIC SYSTEM, bacteria are isolated from the root tip. This cycle can be repeated and should be validated in natural soil[16,32]. In another procedure, which is carried out only in natural soil, biocontrol bacteria are added to soil in pots at the beginning of the experiment. Plants are grown for 3 weeks, then the shoots are cut, and the soil and roots are thoroughly mixed. In the following cycle, the soil is replanted with seeds. Root colonization by the introduced bacteria is evaluated after 5–10 cycles[33,34]. The next step — the *pièce de résistance* — is a plant growth-promotion and/or disease-suppression test under greenhouse conditions, which is performed in pots with and without added candidate strains. Typically, <5% of the candidates give a positive result[35,36]. Unfortunately, there are no *in vitro* diagnostic kits for identifying valuable PGPR more rapidly.

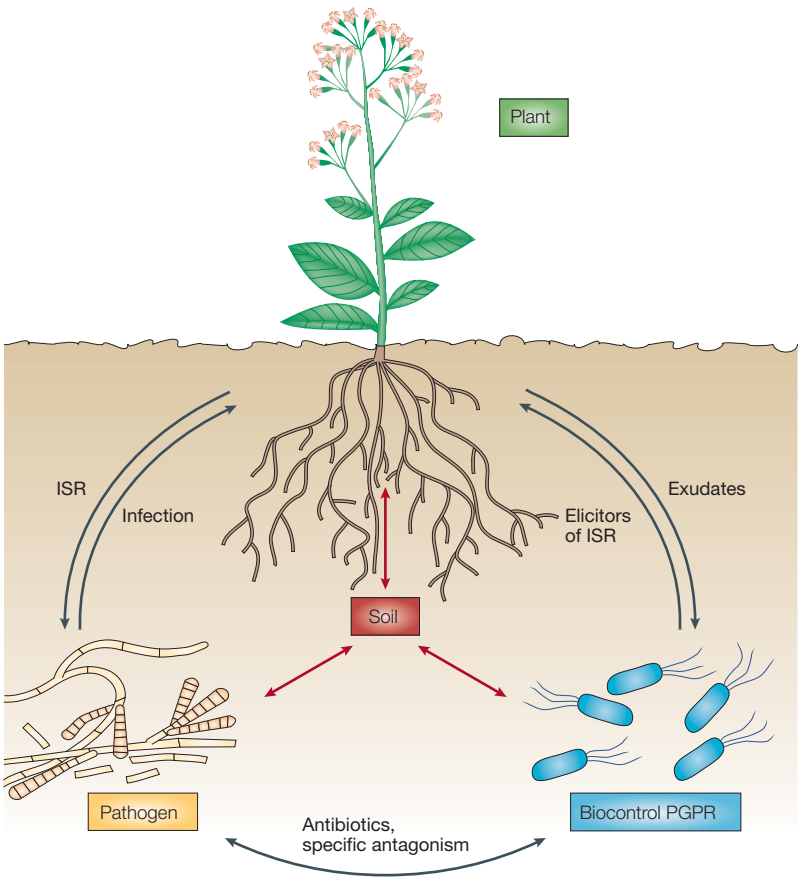

Figure 1 | **Interactions between biocontrol plant growth-promoting rhizobacteria (PGPR), plants, pathogens and soil.** These elements interact with one another through biotic and abiotic signals, many of which are still unknown. ISR, induced systemic resistance.

Biocontrol PGPR must be present on the roots in sufficient numbers to have a beneficial effect on the plant. The crucial colonization level that must be reached has been estimated at 10^5–10^6 CFU (colony-forming units) g^{-1} of root in the case of *Pseudomonas* spp., which protect plants from *G. tritici* or *Pythium* spp. Therefore, assuming that the roots are colonized by 10^8–10^9 culturable aerobic bacteria, it can be estimated that the biocontrol pseudomonads (BOX 2) usually represent 0.1–1% of the culturable aerobic rhizobacterial populations under natural conditions[37–41]. Artificially introduced PGPR can initially colonize roots at 10^7–10^8 CFU g^{-1}, but these levels always decline in a few weeks[33,42–44]. Many authors have discussed the idea that fast-growing rhizobacteria might out-compete fungal pathogens by competition for carbon and energy sources, which would provide a basis for biological control. Although there is evidence that rhizobacterial populations as a whole can cause fungistasis in soil[45], and effective biocontrol PGPR, by definition, must be able to compete for nutrients in the rhizosphere, it is difficult to imagine that 1% (or, at most, a few per cent) of the culturable rhizobacteria could prevent plant disease by 'mopping up' the carbon sources on the root[3]. Recent research points to three main modes of action: antibiosis; induced systemic resistance; and specific, often subtle, pathogen–antagonist interactions (FIG. 1). Here we focus on these mechanisms, with an emphasis on an extensively studied group of biocontrol PGPR consisting of certain fluorescent pseudomonads that protect a range of crop plants from important, mostly fungal, root pathogens.

Antibiotics made by biocontrol pseudomonads

Fluorescent pseudomonads owe their fluorescence to an extracellular diffusible pigment called pyoverdin (Pvd) or pseudobactin. This pigment has high affinity for Fe^{3+} ions (the association constant of the interaction (K_{ass}) is ~10^{24} at pH 7) and is a siderophore (iron-carrier) of the producer strain[46]. Ferripyoverdin (that is, Pvd complexed with Fe^{3+}) interacts with a specific outer-membrane receptor, which is present in the producer but might also occur in some non-producers. Subsequently, Fe^{3+} is transported into the cytoplasm and reduced to Fe^{2+} (REF. 47). In aerated, neutral or alkaline soils, Fe^{3+} is poorly soluble; the total soluble Fe^{3+} species represent ~10^{-10} M at equilibrium with soil iron[48]. In iron-depleted media *in vitro*, Pvd-producing *Pseudomonas* spp. inhibit the growth of bacteria and fungi with less potent siderophores[23,24]. On low-iron agar plates, Pvd that is deposited on a filter disc can produce a halo of inhibition on a susceptible microorganism. Therefore, under certain conditions, Pvd functions as a diffusible,

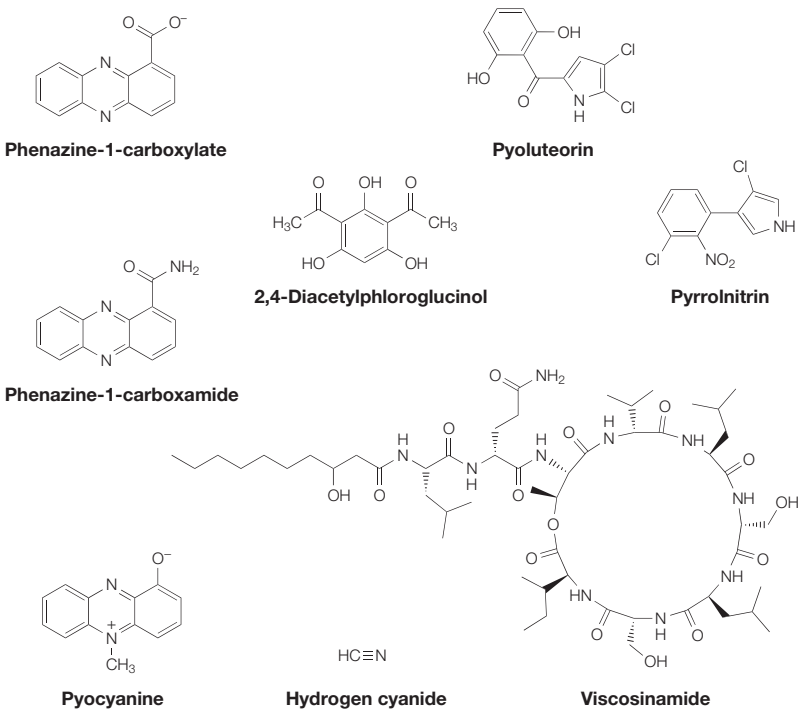

Figure 2 | **The antibiotic compounds produced by fluorescent pseudomonads that are relevant for biocontrol.** The phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin and cyclic lipopeptides are all diffusible, whereas hydrogen cyanide is volatile.

bacteriostatic or fungistatic antibiotic, whereas ferripyoverdin does not[23,24,49]. As a consequence, it seems that a sessile producer of a potent siderophore, such as Pvd, might compete at a distance with other microorganisms that have less avid iron-uptake systems.

The resulting siderophore hypothesis postulates that PGPR exert their plant growth-promotion activity by depriving pathogens of iron[1,24]. For example, under greenhouse conditions, *Pseudomonas putida* strain B10 suppressed *Fusarium* wilt and take-all, but this suppression was lost when the soil was amended with iron, which repressed siderophore production in this strain[23]. A critical assessment of the siderophore hypothesis shows that in some, but not all, plant–pathogen systems tested under various environmental conditions, Pvd-negative (Pvd^-) mutants of fluorescent pseudomonads protect plants less effectively than do the parental strains[50,51]. It is important to point out that Pvd-mediated iron deprivation is a contingent biocontrol mechanism, which works much better at pH 8 than at pH 6; this reflects the increasing solubility of Fe^{3+} species with decreasing pH[52,53]. Estimations of bioavailable iron in the rhizosphere using a biosensor have confirmed that soil pH is a principal factor influencing iron availability, and *Pseudomonas fluorescens* strain Pf-5 is not iron-limited in the rhizosphere of cotton after 1–2 days of growth[54]. Collectively, these data indicate that Pvd-type siderophores might contribute to disease suppression in some situations, but alone they are not sufficient to account for suppression; if they were, it would be difficult to explain why most fluorescent pseudomonads do not have biocontrol activity.

Another pseudomonad siderophore, pyochelin, has been identified as an antifungal antibiotic in a screening programme[55]. However, it has not yet been investigated whether iron deprivation is the antibiotic mechanism that is involved. As pyochelin is a relatively weak Fe^{3+} chelator, but a good Cu^{2+} and Zn^{2+} chelator[56,57], it might be able to deprive some fungi of copper and/or zinc. This example shows that the distinction between siderophores and typical antibiotics is blurred. Although siderophores are part of primary metabolism (because iron is an essential element), on occasion they also behave as antibiotics (which are commonly considered to be secondary metabolites).

Most biocontrol strains of *Pseudomonas* spp. with a proven effect in plant bioassays produce one or several antibiotic compounds that are unrelated to typical siderophores. *In vitro*, these antibiotics inhibit fungal pathogens, but they can also be active against many bacteria and, in some cases, against higher organisms. Comprehensive lists of antibiotics that are involved in biocontrol, producer strains, target pathogens and host plants have been compiled by Raaijmakers *et al.*[58] and Morrissey *et al.*[59] In this review, we focus on the six classes of antibiotic compounds for which the experimental evidence most clearly supports a function in the biocontrol of root diseases (FIG. 2): phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides (all of which are diffusible) and hydrogen cyanide (HCN; which is volatile).

The modes of action of these secondary metabolites are partly understood. The phenazines, which are analogues of flavin coenzymes, inhibit electron transport and are known to have various pharmacological effects on animal cells[60]. In the presence of ferripyochelin, phenazines catalyse the formation of hydroxyl radicals, which damage lipids and other macromolecules[61]. Interestingly, reduced phenazine-1-carboxamide can release soluble Fe^{2+} ions from insoluble $Fe^{3+}(OH^-)_3$ at neutral pH, which raises the possibility that phenazines might contribute to iron mobilization in soils[62]. 2,4-Diacetylphloroglucinol (Phl) is the best-known phloroglucinol compound in a family of related molecules that includes monoacetylphloroglucinol and uncharacterized condensation products of Phl and monoacetylphloroglucinol[63]. Phl causes membrane damage to *Pythium* spp. and is particularly inhibitory to zoospores of this oomycete[64]. At high concentrations, Phl is phytotoxic[65]. To our knowledge, no mode of action has been published for pyoluteorin, whereas pyrrolnitrin has been described as an inhibitor of fungal respiratory chains[66]. Pyrrolnitrin has been used as an antimycotic topical antibiotic in human medicine and synthetic analogues of pyrrolnitrin have been developed for use as agricultural fungicides[67]. Cyclic lipopeptides, which include biocontrol-active substances as well as toxins of phytopathogenic pseudomonads, have surfactant properties, and are able to insert into membranes and perturb their function, which results in broad antibacterial and antifungal activities. Some lipopeptides of *Bacillus* spp. chelate

cations (for example, Ca^{2+}), but this property has not been investigated for lipopeptides from *Pseudomonas* spp.[68] Finally, the cyanide ion derived from HCN is a potent inhibitor of many metalloenzymes, especially copper-containing cytochrome *c* oxidases[69]. In summary, with the exception of pyrrolnitrin, these antibiotic compounds show little selectivity towards fungi.

Role of antibiotics in disease suppression

The contribution of antibiotic compounds to the biological control of root diseases has been documented through five experimental steps, which are summarized below. For further details, the reader is directed to an extensively referenced review on this subject that was published recently[41].

In the first step, diffusible or volatile secondary metabolites that are produced by biocontrol strains *in vitro* are purified and chemically identified. The inhibition of sensitive microorganisms by the pure compounds is then confirmed and quantified *in vitro*. This identification step has been carried out with all of the compounds shown in FIG. 2 (REFS 19,65,67,70–75).

In the second step, the antibiotic compound of interest is detected and quantified in the rhizosphere, which has been inoculated with the producer strain, through extraction and HPLC purification. Small, but significant, amounts of phenazine-1-carboxylate, Phl, pyrrolnitrin, pyoluteorin and the lipopeptide viscosinamide have been found in rhizosphere samples[18,41]. To our knowledge, cyanide of microbial origin has not been measured in the rhizosphere. However, in one historical experiment, the effect of added cyanide was tested directly in the field. In this study, 'sick' soil was treated with $Ca(CN)_2$, which is a cheap water-soluble cyanide that is used by the mining industry and is known as 'cyanogas', at 500 pounds per acre. This treatment killed fungi *en masse*, significantly reduced 'grey speck' disease of oats, and tripled oat grain yields. No side effects on the fauna were recorded[76].

In the third step, the structural and principal regulatory genes controlling the expression of the antibiotic compounds are identified and characterized. Non-producing and over-producing strains are constructed using molecular genetic techniques, and tested in microcosms that contain a chosen plant–pathogen system, with appropriate controls (that is, the wild-type biocontrol strain or no added biocontrol agent). Mutants that are defective for the production of phenazine, Phl, pyrrolnitrin, pyoluteorin or HCN have all been shown to be less active in biocontrol. However, the complete loss of biocontrol activity by a single null mutation is exceptional, mainly because most biocontrol strains produce several antibiotics and rely on several mechanisms[19,20,65,71,77,78]. Does antibiotic overproduction result in improved biocontrol? In a few plant–pathogen systems, an enhancement of biocontrol efficacy or a broader target range has been observed; however, the phytotoxicity of some compounds, such as Phl and pyoluteorin, can impose a penalty on plant yields[79–82].

In the fourth step, intrinsically poor biocontrol strains can acquire biocontrol activity by the introduction of antibiotic biosynthetic genes that are not present in the original strains. This prediction has been verified experimentally, for example, for the transfer of the clustered HCN, Phl and phenazine biosynthetic genes[20,74,83]. Furthermore, a strain of *Pseudomonas* producing phenazine-1-carboxylate, which is a relatively poor biocontrol factor, has been rendered more effective by the introduction of the *phzH* gene, the product of which catalyses the conversion of phenazine-1-carboxylate to phenazine-1-carboxamide (a more potent biocontrol factor)[84]. However, it remains to be seen whether recombinant biocontrol strains can be constructed that surpass the performance of the best, naturally occurring biocontrol strains[85].

In the fifth and final step, the expression of antibiotic biosynthetic genes can be observed in the rhizosphere through the use of easily detectable reporter genes that are fused to structural genes for antibiotic biosynthesis[41,77,86–89]. *A priori*, microcolonies growing on roots are expected to have the potential to be good producers of secondary metabolites. This is because root-colonizing pseudomonads have maximal doubling times of approximately 3–6 h, as judged from seed- and root-colonization data[90,91]. This growth is about 10-times slower than that in rich laboratory media, which implies that bacterial growth in the rhizosphere is limited by some available nutrients. In general, conditions of restricted growth and high cell densities favour secondary metabolism, whereas optimal nutrient conditions tend to be exploited for primary metabolism and rapid multiplication[92]. Rhizobacteria that are equipped with specific biosensor constructs have shown that the nutritional status of the rhizosphere is often nitrogen-limited (because exudates have a high C:N ratio) or oxygen-limited (because roots and rhizosphere organisms both consume oxygen for respiration). By contrast, phosphate and iron limitation seem to be less common[54,93,94]. Pseudomonads cope well with limitations of all kinds. These bacteria have sophisticated regulatory systems that can adapt to variable C:N ratios[95], and have respiratory chains with high affinity for oxygen and/or use nitrate as an alternative electron acceptor[93,96].

Does antibiotic production confer a selective advantage on the producer strain? In the case of the siderophores, which are viewed as contingent antibiotics, the selective advantage is easily verified by placing a bacterial culture in a medium that contains an iron chelator: only those bacteria that produce a more avid iron chelator grow. In natural soil, fluorescent pseudomonads that produce phenazines have a competitive survival advantage over non-producing mutants[97]. If phenazines mobilize iron in soil[62], they could be considered as auxiliary siderophores and this might explain the greater ecological fitness of the phenazine producers. Although it might be assumed that antibiotics help the producers to defend their ecological niches against antibiotic-sensitive competitors in the rhizosphere, there is little experimental evidence to support this assumption[41].

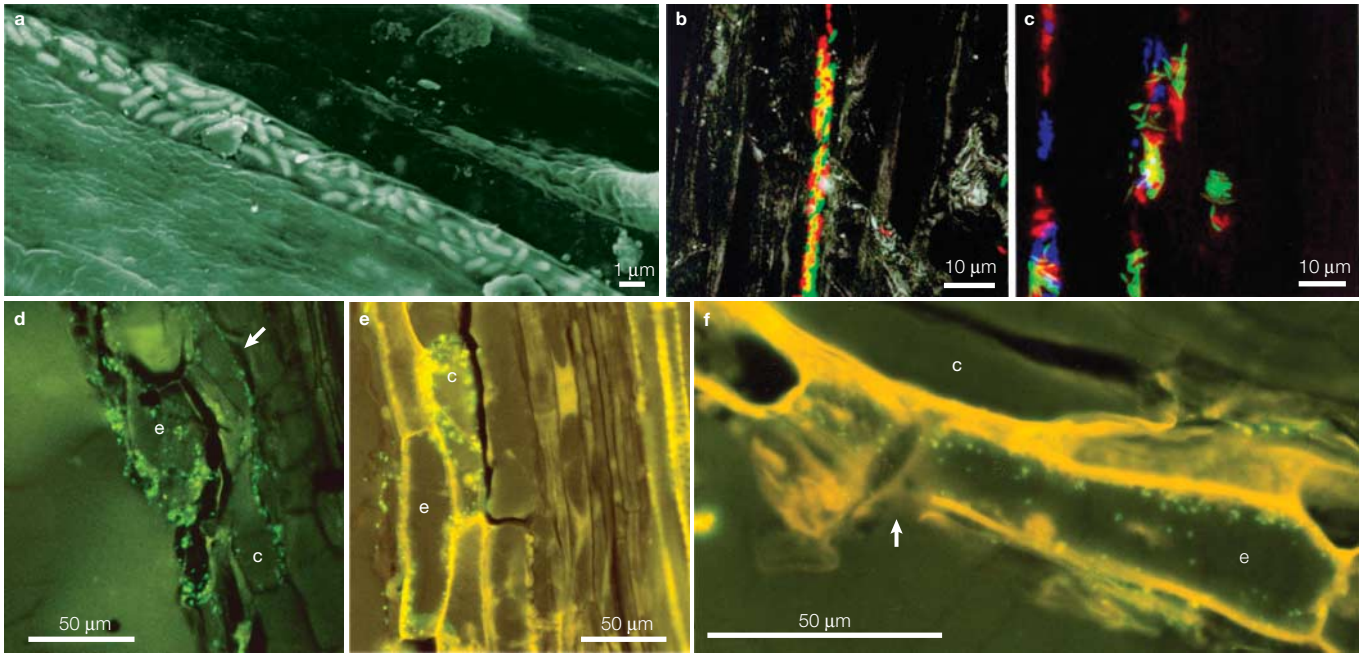

Figure 3 | **Root colonization by fluorescent pseudomonads. a** | Scanning electron micrograph of a microcolony of *Pseudomonas fluorescens* strain WCS365 on tomato root[90]. **b** | Confocal scanning microscopy analysis of tomato root colonization by *P. fluorescens* WCS365 expressing autofluorescent proteins. A mixture of two WCS365 derivatives expressing either cyan fluorescent protein (red cells) or yellow fluorescent protein (green cells) is shown; the overlap of the red and green colours results in yellow. **c** | A mixture of three WCS365 derivatives; the red and green cells are the same as those in panel **b**, and the blue cells express red fluorescent protein[105]. **d–f** | Immunofluorescence microscopy of *P. fluorescens* strain CHA0 cells associated with the epidermis (**e**) and the cortex (**c**) of tobacco roots. Bacterial cells are present between and inside the epidermal and cortical cells. The arrow in **d** points to bacteria present between cortical cells. The arrow in **f** indicates the damaged cell wall of an epidermal cell[107]. Panel **a** reproduced, with permission, from REF. 90 © (1997) American Phytopathological Society. Panels **b** and **c** reproduced, with permission, from REF. 105 © (2000) American Phytopathological Society. Panels **d–f** reproduced, with permission, from REF. 107 © (1997) Blackwell Publishing.

Interactions between plants and rhizobacteria

The structure of rhizobacterial communities is determined by the plant species[98–101], and differences in the composition and amounts of root exudates probably account for the differences in microbial populations. Root exudates offer a carbon-rich diet to the rhizosphere microorganisms: organic acids (such as citrate, malate, succinate, pyruvate, fumarate, oxalate and acetate) and sugars (such as glucose, xylose, fructose, maltose, sucrose, galactose and ribose) constitute the 'main course', whereas variable amounts of α-amino acids, nucleobases and vitamins (such as thiamin and biotin) provide the 'entrée' or 'dessert'[10,16,102]. The ability of rhizobacteria to use organic acids as carbon sources correlates with rhizosphere competence[99].

Assuming that specific interactions take place when rhizobacteria make initial contacts with roots, evidence for this might be found in some bacterial mutants that are impaired in root colonization, but not during growth in ordinary laboratory media. Extensive searches for colonization-defective mutants of *Pseudomonas* spp. have shown that the ability to use malate and succinate is more crucial than the ability to use glucose and fructose, in terms of rhizosphere competence[16]. Chemotaxis, flagellar mobility, lipopolysaccharide (LPS) structure, the outer membrane protein OprF and, to a lesser extent, pili are all important for competitive root colonization[16]. A root glycoprotein complex known as agglutinin can be involved in the short-term adherence of pseudomonads[103]. So far, no genetic trait has been identified in biocontrol pseudomonads that would point to a mechanism allowing the bacteria to recognize specific plant surface receptors or to interact with specific plant signals. This is in line with the observation that many biocontrol PGPR strains colonize a range of different plant species, and apparently contrasts with the well-known, specific dialogue that occurs between symbiotic rhizobia and leguminous plants; in this case, specific flavonoid compounds that are secreted by the plants instruct the bacteria to start the nodulation process[104]. Nonetheless, several colonization-defective mutants of *Pseudomonas* spp. are affected in genes with unknown functions[16], so specific interactions remain possible.

Once biocontrol pseudomonads have moved and attached to a root zone, microcolonies form in a few days (FIG. 3a). These have been observed mainly in the grooves between epidermal cells. Other bacteria can reach the same site at a later time and intermingle with pre-existing microcolonies. This development has been followed using differentially fluorescence-tagged *P. fluorescens* cells[105] (FIG. 3b). Typical cell densities range from 10^3 to 10^7 CFU cm^{-1} of root, depending on the age and location of the microcolonies[10,90]. The root collar — where the root

joins the main stem — is a site of intense exudation and is more strongly colonized by bacteria than is the root tip[90,106]. Some biocontrol pseudomonads penetrate into intercellular spaces in the epidermis and cortex; damaged root cells can be invaded by these bacteria[107] (FIG. 3c). This kind of endophytic growth might be an important attribute of biocontrol rhizobacteria, which allows them to communicate better with the host plant[31,108].

Role of induced systemic resistance

It has been proposed that, in suppressive soils, plant roots are associated with microbial communities that have an overall beneficial (PROBIOTIC) effect on plant health. The loss of disease suppression as a result of soil pasteurization supports this idea. Indeed, some biocontrol PGPR elicit a phenomenon that is known as induced systemic resistance (ISR) in the host plant. ISR allows plants to withstand pathogen attack to the leaves or roots, without offering total protection (reviewed in REF. 30). Many effective biocontrol PGPR elicit ISR, irrespective of antibiotic production[109–111]. The effects of three different strains of *Pseudomonas* spp. mediating ISR in *Arabidopsis thaliana* have been investigated through transcriptome analysis of plants with roots that were colonized by one of these strains (*P. fluorescens* WCS417r, *Pseudomonas thivervalensis* or *P. fluorescens* CHA0). In each instance, the transcript levels in the leaves were not markedly changed (that is, they varied by less than a factor of three) compared with the uninoculated control, and systemic responses that are typically seen after attack by necrotizing pathogens did not occur during ISR[112,113] (E. Boutet and J.-P. Métraux, personal communication). In one study[112], substantial changes in the expression of several genes were found in roots; however, it remains unclear by which mechanism the plants react to ISR-eliciting bacteria. Challenge inoculation of plants with a leaf pathogen (such as *Pseudomonas syringae* pv. *tomato*) shows that ISR-positive plants are 'primed'; that is, they react faster and more strongly to pathogen attack by inducing defence mechanisms[112]. Studies with *A. thaliana* mutants indicate that the jasmonate/ethylene-inducible defence pathway is important for ISR, whereas the salicylate-inducible pathway mediating systemic acquired resistance (SAR) seems to be less important. The evidence has come mainly from *A. thaliana* mutants that are non-responsive to jasmonate and/or ethylene and are impaired in ISR[111,114]. For a description of these defence pathways, the reader is referred to a recent review[30]. In bean, ISR elicited by a *P. putida* strain was associated with elevated levels of hexenal, which is a volatile antifungal compound, and with enhanced expression of enzymes that are involved in hexenal synthesis[110].

Which bacterial signals elicit ISR? Phl^- mutants of *P. fluorescens* CHA0 are less effective than the wild-type bacteria in protecting *Arabidopsis* from the leaf pathogen *Peronospora parasitica* and application of Phl to the roots triggers ISR to this pathogen[115]. A salicylate-overproducing recombinant of *P. fluorescens* strain P3 affords enhanced protection of tobacco against tobacco necrosis virus compared with the wild-type P3, which indicates that salicylate might also stimulate defence[116]. In another *Pseudomonas* biocontrol strain, a combination of pyocyanin and pyochelin seems to be most effective for inducing resistance in tomato[117]. The plant-growth-stimulating volatile 2,3-butanediol that is found in *Bacillus* spp. can also initiate ISR[118]. In several ISR-competent strains of fluorescent pseudomonads, it has been difficult to isolate specific ISR elicitors; it has therefore been proposed that a combination of siderophores, O-antigens and flagella might account for the ISR effect[114,119]. Generalizations about the signal-transduction pathways that are involved in ISR are further complicated by the fact that the ISR response to a given PGPR strain depends on the plant species and cultivar[119].

How plants interact with beneficial rhizobacteria is not known, although studies on take-all decline and other cases of induced disease suppression might provide a clue. The most effective *Pseudomonas* biocontrol strains, which were isolated from wheat grown in monoculture, were Phl producers[75]; in suppressing soils, these bacteria colonized wheat roots at 10^5–10^6 CFU g^{-1} of root, which was above the threshold required for the control of take-all. In conducive soils, Phl^+ strains were found on roots below the threshold[38]. Successive cultivation of wheat increased the population densities of Phl^+ pseudomonads, whereas cultivation of oats, which eliminates the suppression of take-all, had a negative effect on the population densities of Phl^+ pseudomonads[3]. Similar observations that point to a positive correlation between Phl producers and suppressiveness have been made in a Dutch take-all decline soil[120], in a US soil suppressive to *Fusarium* wilt after extensive pea monoculture[121], and in a Swiss soil suppressive to *Thielaviopsis basicola*[122]. The roots of several plants, including wheat and maize, are stimulated for efflux of amino acids by Phl. This effect might result from an inhibition of competing amino-acid uptake by Phl. By producing Phl, pseudomonads might therefore directly benefit from an enhanced availability of amino acids as carbon and nitrogen sources[123]. With rare exceptions, Phl producers are also HCN producers[124], which implies a potential synergy between Phl and HCN in biocontrol. It would be interesting to determine whether monoculture leads to the modification of soil properties and, therefore, to biochemical changes in the crop plants and their exudates over time. Such changes might explain why the cell densities of Phl and HCN producers are so high on these plant roots.

Subtle pathogen–biocontrol interactions

Antibiosis and ISR do not target specific pathogens. Introduced biocontrol PGPR strains are usually effective against a range of pathogens, whereas natural suppressive soils seem to be antagonistic to specific pathogens. Therefore, specific pathogen–biocontrol strain interactions should be considered. Unfortunately, although some examples have been reported, a coherent picture is yet to emerge. First, fusaric acid, which is a toxin for plants and a pathogenicity factor of *Fusarium oxysporum*, can be degraded by biocontrol strains of *Burkholderia* (formerly *Pseudomonas*) spp., which results in the

PROBIOTICS
Microorganisms that have beneficial effects on their host. This term is commonly used for microorganisms that survive passage through the gastrointestinal tract and might prevent, or even cure, diarrhoea.

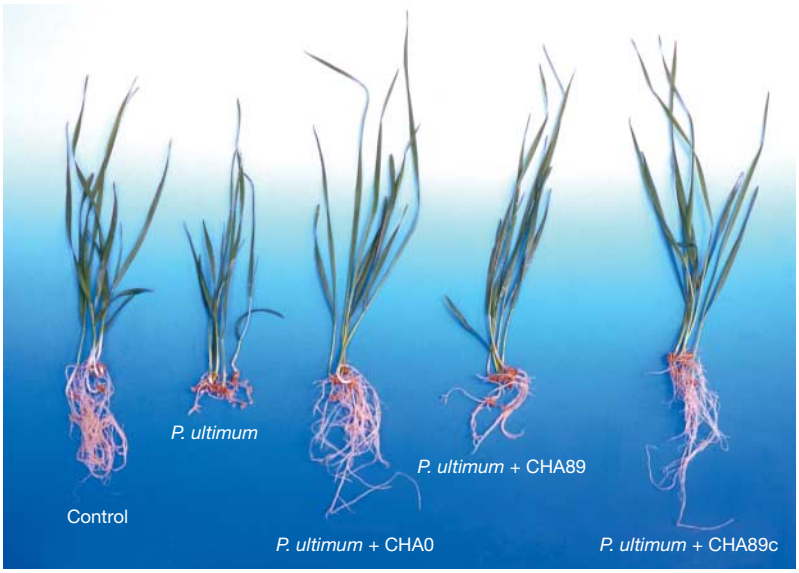

Figure 4 | **Protection of wheat from *Pythium ultimum* by *Pseudomonas fluorescens*.** The GacS/GacA two-component system of *P. fluorescens* strain CHA0 makes an essential contribution to biocontrol. Treatments from left to right: control plant without added microorganisms; diseased plant in the presence of *P. ultimum*; plant protected by the wild type CHA0 from *P. ultimum*; absence of plant protection from *P. ultimum* by the *gacA* mutant CHA89; plant protected from *P. ultimum* by the complemented *gacA* mutant CHA89c. Images courtesy of Christoph Keel.

protection of the host plant[125,126]. Second, the suppression of *Pythium* damping-off involves the degradation of linolenic acid by suppressive bacterial consortia; linolenic acid is an exudate component that stimulates the germination of *Pythium ultimum* sporangia[127]. Similarly, *P. putida* strain N1R provides biocontrol of *P. ultimum* by degrading volatile seed exudates, which would otherwise stimulate the pathogen to cause seed rot[128]. Moreover, glycolipids that are produced by *Pseudomonas* spp. can damage the zoospores that are released from the sporangia of *Pythium* spp.[129] Third, *P. putida* strain KD colonizes *P. ultimum* hyphae and uses type III secretion of unknown compounds to stop the production of pectinase, which is a pathogenicity factor (F. Rezzonico and G.D., unpublished observations).

Soil amendments can tip the balance in favour of biocontrol. For instance, the addition of Zn^{2+} ions results in the repression of fusaric acid production by *F. oxysporum*. As fusaric acid represses Phl biosynthesis in the antagonist *P. fluorescens* CHA0, the net result of the Zn amendment is an overall improvement of biocontrol[130]. Many studies have shown that chitin or chitosan (deacetylated chitin) applied together with biocontrol organisms improves biocontrol efficacy[109,131–133]. Chitin probably induces chitinolytic enzymes, which are produced, for instance, by biocontrol strains of *Pseudomonas* spp.[134] and *Trichoderma* spp.[30], and might damage fungal cell walls. However, chitin and chitosan also selectively bind Cu^{2+} ions and, for this reason, have been used to remove toxic copper from waste water[135]. It is therefore possible that chitin amendments might enhance biocontrol by influencing copper bioavailability in the rhizosphere.

Pathogenic fungi have subtle ways in which to evade biocontrol microorganisms. For example, as mentioned above, fusaric acid of fungal origin represses Phl biosynthesis in *P. fluorescens* CHA0 (REF. 136). Various *G. tritici* isolates show differential sensitivities towards Phl and phenazine-1-carboxylate. Introduced Phl- or phenazine-producing pseudomonads have a higher probability of suppressing take-all disease in soils that contain the more sensitive *G. tritici* varieties[137]. Some pathogenic fungi can inactivate biocontrol factors, for example, using enzymes that are able to metabolize Phl or HCN[138,139]. These examples show that complex interactions between pathogens and antagonists can determine the balance between plant disease and health.

Transcriptional regulation of biocontrol activity

In natural suppressive soils, disease incidence is consistently low. By contrast, when biocontrol PGPR are introduced into conducive soils under field conditions, their effects are variable[2,140]. This inconsistent performance might stem from a lack of expression of biocontrol traits. Although the level of root colonization is clearly important for biocontrol, the production of extracellular antibiotic compounds can also be a key determinant in many biocontrol strains of fluorescent pseudomonads[41]. Some important aspects of the metabolic regulation of biocontrol factor expression are discussed below.

At the transcriptional level, a common feature stands out: siderophores and antibiotics positively autoregulate their own biosyntheses. Both Pvd and pyochelin have a positive effect on the expression of their biosynthetic genes; this regulation involves the sigma factor PvdS and the AraC-like transcription factor PchR, respectively, in *Pseudomonas aeruginosa*[141,142]. Phl and pyoluteorin positively control the expression of their biosynthetic genes through the transcriptional regulators PhlF and PltR, respectively, in *P. fluorescens*[136,143,144]. In some plant-beneficial *Pseudomonas* spp. that use *N*-acyl-homoserine lactone (AHL) autoinducers for cell–cell communication, the phenazine- and HCN-biosynthetic genes are expressed through a positive autoregulation loop that is dependent on autoinducer-responsive LuxR-type regulators[89,145–147]. What benefits do the bacteria derive from positive autoregulation? Above a certain threshold population density, the bacterial cells mutually reinforce the production of the extracellular metabolites, whereas below a threshold density, the cells are more reluctant to engage substantial cellular resources in secondary metabolism.

AHL-dependent cell–cell communication operates in the rhizosphere over a distance of up to 60 μm. *N*-hexanoyl-homoserine lactone, as well as being a signal of biocontrol strains of *Pseudomonas chlororaphis* and *Pseudomonas aureofaciens*, has been observed to elicit resistance of tomato to the leaf pathogen *Alternaria alternata*; this plant response seems to differ from ISR and SAR[148]. AHL-signalling can be disrupted by microorganisms that enzymatically degrade AHLs.

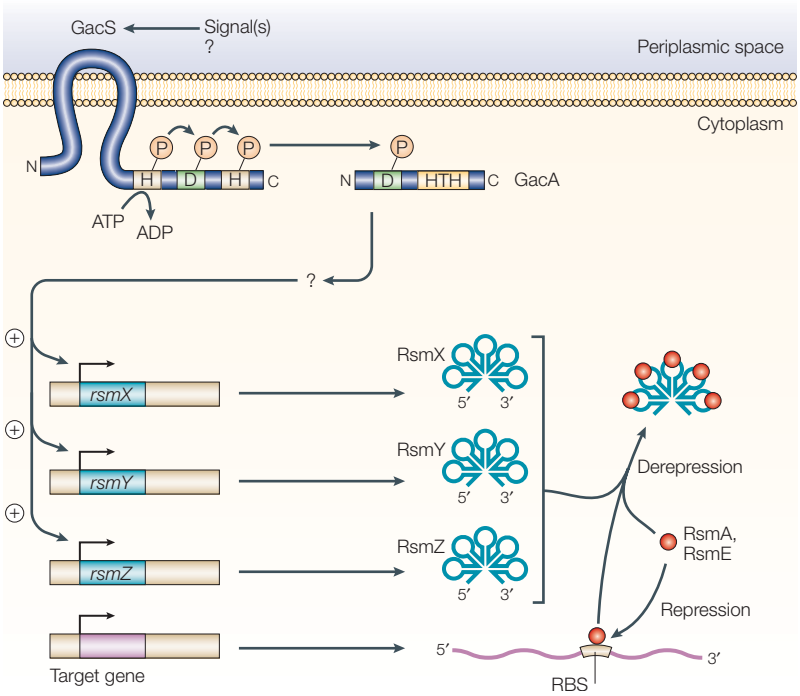

Figure 5 | **Model of the GacS/GacA signal-transduction pathway in *Pseudomonas fluorescens* strain CHA0.** The GacS sensor kinase has an autophosphorylation domain around His294 (denoted H), a phosphoacceptor domain around Asp717 (denoted D) and a histidine phosphotransfer domain around His863 (denoted H). On interaction with bacterial signal molecules, GacS is autophosphorylated and a phospho-relay mechanism transfers a phosphate residue to the acceptor domain (D) of the response regulator GacA[159,160]; this then activates — directly or indirectly — the transcription of the three small RNA genes *rsmX*, *rsmY* and *rsmZ*. Titration of these RNAs by the RsmA and RsmE proteins relieves the translational repression exerted by these proteins at, or near, the ribosome binding site (RBS) of the target mRNAs (for example, *hcn* for HCN synthase, *apr* for exoprotease and *phl* for Phl synthase).

For instance, in *P. chlororaphis* strain PCL1391, the expression of phenazine-1-carboxamide depends on AHLs[146]; the biocontrol activity of this strain is strongly reduced when AHL-degrading bacteria are applied simultaneously to roots[149]. Whether AHL-degraders, which are common among rhizobacteria[150], have an important role in rhizosphere ecology remains to be seen. On the one hand, AHL-degraders can be antagonists of AHL-dependent biocontrol strains, but on the other hand, these degraders can also be antagonists of AHL-dependent bacterial plant pathogens, such as *Erwinia carotovora* and *Agrobacterium tumefaciens*[151].

Biotic and abiotic environmental signals can have an important input into the regulation of biocontrol genes in pseudomonads. The best-known example is the repression of Pvd and pyochelin biosynthesis by excess iron, which is mediated by the Fur repressor[47]. Low oxygen concentrations are a prerequisite for the activity of the transcription factor ANR, which positively regulates HCN biosynthesis[147]. Carbon and nitrogen sources that are present in exudates have a considerable influence on the expression of *phl*, *plt* and *phz* genes[77,152], although the molecular mechanisms that are involved are mostly unknown.

ANR
A transcription factor in the FNR family, responsive to low oxygen concentrations.

GACS/GACA
A two-component system controlling the global activation of exoproduct (for example, antibiotics and cyanide) synthesis. GacS/GacA is found in many Gram-negative bacteria.

Post-transcriptional regulation of biocontrol

At a post-transcriptional level, the GACS/GACA two-component system has an important effect, in that mutations in *gacS* or *gacA* abolish biocontrol activity in different biocontrol pseudomonads[87,145,146,153–157] as well as in *Serratia plymuthica* (previously *Enterobacter agglomerans*)[158] (FIG. 4). In *P. fluorescens* CHA0, where this system tightly controls the expression of several biocontrol factors — including Phl, HCN, pyoluteorin, pyrrolnitrin and exoprotease[41] — it functions as follows (FIG. 5). An as-yet-unidentified bacterial signal stimulates autophosphorylation of the GacS sensor. The phosphate group is then transferred to the response regulator GacA by a phospho-relay mechanism[159,160]. Three genes that encode the small non-coding RNAs RsmX, RsmY and RsmZ are expressed well in a *gacA*$^+$ background, but not in *gacS* or *gacA* mutants. A crude preparation of the inducing signal stimulates the transcription of *rsmX*, *rsmY* and *rsmZ* by about threefold. A deletion mutation of *gacS*, which removes the linker region between the second transmembrane segment and the autophosphorylation domain (FIG. 5), causes enhanced signal-independent expression of biocontrol genes[156,160–164]. A conserved palindromic upstream-activating sequence occurs in the *rsmX*, *rsmY* and *rsmZ* promoters; whether phosphorylated GacA binds directly to, or interacts indirectly with, this sequence remains to be established. A triple *rsmX rsmY rsmZ* mutant mimics *gacS* and *gacA* mutants in that it is essentially defective for exoproduct formation and biocontrol activity (E. Kay and D.H, unpublished observations).

The three small RNAs each specifically bind two small proteins, RsmA and RsmE, with similar affinities. RsmA and RsmE belong to a protein family in which the carbon-storage regulator, CsrA, of enteric bacteria is a prominent member[156]. In *P. fluorescens*, RsmA and RsmE function as post-transcriptional repressors of typical biocontrol genes — for example, those that encode Phl-, HCN- or pyoluteorin-biosynthetic enzymes — by interfering with the function of the ribosome-binding site[161,163,165]. Both RsmA and RsmE recognize a GGA motif that is repeated between five and seven times in unpaired regions of RsmX, RsmY and RsmZ; the deletion of five GGA repeats in RsmY results in the loss of recognition[166]. What constitutes an RsmA/E binding site on target mRNAs is not clear at present; a ANGGA motif (where N is any nucleotide), which can be part of the ribosome binding site, seems to be necessary, but not sufficient, for recognition (K. Starke and D.H., unpublished observations). Extensive studies on the mode of action of the GacS/GacA and RsmA homologues in enteric bacteria support this model[167]. However, in *P. fluorescens*, the Gac/Rsm signal-transduction pathway has a more decisive influence (with induction factors of 50 at the level of target gene expression) than it has in enteric bacteria. A similar decisive role of small RNAs has been found in *Vibrio* spp. in the post-transcriptional regulation of bioluminescence and virulence[168].

Box 3 | **Commercially available biocontrol strains**

Although biocontrol strains of fluorescent pseudomonads have contributed greatly to the understanding of the mechanisms that are involved in disease suppression, these strains have a disadvantage from an application point of view; they generally lose viability when stored for a period of several weeks. Spore-forming biocontrol strains of *Bacillus* spp. have a much better shelf-life, which facilitates the development of commercial products. Similar to fluorescent pseudomonads, specific strains of *Bacillus* spp. can provide plant protection by antibiosis and induced systemic resistance[172]. Commercially available biocontrol rhizobacteria include *Bacillus subtilis* strains GB03 (Kodiak; Gustafson), MBI 600 (Subtilex; Becker Underwood) and QST 713 (Serenade; AgraQuest), *Bacillus pumilus* strain GB34 (YieldShield; Gustafson), *Bacillus licheniformis* strain SB3086 (EcoGuard; Novozymes), a mixture of *B. subtilis* strain GB122 and *Bacillus amyloliquefaciens* strain GB99 (BioYield; Gustafson), several *Bacillus* spp. (yield-increasing bacteria in China), *Streptomyces griseoviridis* K61 (Mycostop; AgBio development), and a few strains of *Pseudomonas fluorescens, Pseudomonas putida* and *Pseudomonas chlororaphis* (Cedomon; BioAgri)[170,173]. These biocontrol bacteria can be applied as dry products (granules or powders), cell suspensions (with or without microencapsulation) or seed coatings[173].

VERMICULITE CLAY
A hydrous aluminium silicate clay mineral that is rich in magnesium and iron.

ILLITE CLAY
A hydrous aluminium silicate clay mineral that is rich in potassium.

Outlook

The natural disease-suppressive characteristic of soils is consistent over years and seems to be pathogen-specific. By contrast, when biocontrol PGPR are added to conducive soils, the degree of suppression can vary considerably over time and the spectrum of diseases that are suppressed can be broad. One possible explanation for this apparent discrepancy is that natural suppressive soils might contain several PGPR strains with different biocontrol properties, and suppressiveness could be brought about by a consortium of PGPR strains that are specifically adapted to the roots of the host plant and to the stress caused by a specific root pathogen. Another explanation might be that some as-yet-unrecognized soil properties, in particular the bioavailability of minerals, are important in the success of biocontrol. For instance, *P. fluorescens* CHA0 has been shown to protect plant roots from fungal pathogens in VERMICULITE but not ILLITE clay, and iron availability is not involved in this phenomenon[11,50]. The availability of other clay minerals might cause this effect. In soils at pH 7, the concentration of soluble Cu^{2+} is below that of Fe^{3+} and some PGPR might have a competitive advantage over pathogens owing to a superior ability to acquire copper or to proliferate with relatively little copper. Finally, in the case of take-all decline, the aggressiveness of the pathogen (*G. tritici*) might decrease during wheat monoculture[169].

Under greenhouse conditions, the application of biocontrol PGPR strains has given promising results in vegetable, fruit and ornamental plant production[170]. Mixtures of PGPR strains combining antibiosis and ISR might be most effective in practice[171]. Under field conditions, the efficacy and consistency of biocontrol PGPR still needs to be improved. The market for biocontrol PGPR is relatively small at present (BOX 3), but has the potential to grow as they provide an environmentally friendly means to control pathogens.

At present, there are many scientific challenges for research in the field of biocontrol pseudomonads. It will be important to exploit molecular techniques to study the genome expression of plant-beneficial and plant-pathogenic microorganisms *in situ*, and to obtain a fuller picture of rhizosphere biodiversity. In addition, many specific questions remain to be addressed. Which signal molecules of microbial and plant origin turn on the expression of biocontrol traits? Which microbial signals are most effective in eliciting pathogen resistance in plants through ISR, SAR or alternative pathways? Are there plant-specific root-colonization mechanisms? Does the type III-secretion system in certain biocontrol PGPR have a role in root colonization and does this system act directly on pathogens? How do clay minerals and other abiotic factors influence biocontrol mechanisms? What determines the apparent specificity of natural suppressive soils? How are pathogenicity factors of fungal pathogens regulated in the rhizosphere? The answers to these questions will help us to understand how biocontrol pseudomonads and other plant-beneficial microorganisms manage to appease the virulent inhabitants of the rhizosphere.

1. Schroth, M. N. & Hancock, J. G. Disease-suppressive soil and root-colonizing bacteria. *Science* **216**, 1376–1381 (1982).
2. Hornby, D. Suppressive soils. *Annu. Rev. Phytopathol.* **21**, 65–85 (1983).
3. Weller, D. M., Raaijmakers, J. M., Gardener, B. B. M. & Thomashow, L. S. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol.* **40**, 309–348 (2002).
A comprehensive review on suppressive soils, which is recommended for the specialist reader.
4. Shipton, P. J., Cook, R. J. & Sitton, J. W. Occurrence and transfer of a biological factor in soil that suppresses take-all of wheat in eastern Washington. *Phytopathology* **63**, 511–517 (1973).
5. Cook, R. J. & Rovira, A. D. The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. *Soil Biol. Biochem.* **8**, 269–273 (1976).
The authors of this paper formulated a coherent and influential hypothesis on the role of fluorescent pseudomonads in disease suppression.
6. Scher, F. M. & Baker, R. Mechanism of biological control in a *Fusarium*-suppressive soil. *Phytopathology* **70**, 412–417 (1980).
This study emphasizes soil pH as a crucial parameter that determines disease suppression.
7. Stutz, E. W., Défago, G. & Kern, H. Naturally occurring fluorescent pseudomonads involved in suppression of black root rot of tobacco. *Phytopathology* **76**, 181–185 (1986).
8. Menzies, J. D. Occurrence and transfer of a biological factor in soil that suppresses potato scab. *Phytopathology* **49**, 648–652 (1959).
9. Mazzola, M. Mechanisms of natural soil suppressiveness to soilborne diseases. *Antonie van Leeuwenhoek* **81**, 557–564 (2002).
10. Baker, K. F. & Snyder, W. C. (eds) *Ecology of Soil-Borne Plant Pathogens: Prelude to Biological Control* (Univ. of California Press, Berkeley, 1965).
This symposium shaped early research on the biocontrol mechanisms that operate in the rhizosphere. Recommended chapters include those by Garrett, S. D., Jackson, R. M., Katznelson, H. and Rovira, A. D.
11. Stutz, E., Kahr, G. & Défago, G. Clays involved in suppression of tobacco black root rot by a strain of *Pseudomonas fluorescens*. *Soil Biol. Biochem.* **21**, 361–366 (1989).
12. Ownley, B. H., Duffy, B. K. & Weller, D. M. Identification and manipulation of soil properties to improve the biological control performance of phenazine-producing *Pseudomonas fluorescens*. *Appl. Environ. Microbiol.* **69**, 3333–3343 (2003).
13. Lucy, M., Reed, E. & Glick, B. R. Applications of free living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek* **86**, 1–25 (2004).
14. Cook, R. J. Making greater use of introduced microorganisms for biological control of plant pathogens. *Annu. Rev. Phytopathol.* **31**, 53–80 (1993).
15. Wright, J. M. The production of antibiotics in soil. III. Production of gliotoxin in wheat straw buried in soil. *Ann. Appl. Biol.* **44**, 461–466 (1956).
16. Lugtenberg, B. J. J. & Bloemberg, G. V. in *Pseudomonas* Vol. 1 (ed. Ramos, J.-L.) 403–430 (Kluwer Academic/Plenum Publishers, New York, 2004).
17. Gottlieb, D. The production and role of antibiotics in soil. *J. Antibiot.* **29**, 987–1000 (1976).
18. Thomashow, L. S., Bonsall, R. F. & Weller, D. M. in *Manual of Environmental Microbiology* (eds Hurst, C. J., Knudsen, G. R., McInervey, M. J., Stetzenbach, L. D. & Walter, M. V.) 493–499 (ASM Press, Washington DC, 1997).
19. Thomashow, L. S. & Weller, D. M. Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. *J. Bacteriol.* **170**, 3499–3508 (1988).
In this study, genetic manipulation of a pseudomonad was used for the first time to show the involvement of an antibiotic in biocontrol.
20. Voisard, C., Keel, C., Haas, D. & Défago, G. Cyanide production by *Pseudomonas fluorescens* helps suppress

black root rot of tobacco under gnotobiotic conditions. *EMBO J.* **8**, 351–358 (1989).
This study provides the first experimental evidence of HCN being involved in biocontrol.
21. Défago, G. & Haas. D. in *Soil Biochemistry* Vol. 6 (eds Bollag, J. M. & Stotzky, G.) 249–291 (Marcel Dekker, New York, 1990).
22. Kloepper, J. W. & Schroth, M. N. in *Proceedings of the IVth International Conference on Plant Pathogenic Bacteria* Vol. 2 879–882 (Station de Pathologie Végétale et Phytobactériologie, INRA, Angers, France, 1978).
23. Kloepper, J. W., Leong, J., Teintze, M. & Schroth, M. N. *Pseudomonas* siderophores: a mechanism explaining disease-suppressive soils. *Curr. Microbiol.* **4**, 317–320 (1980).
24. Kloepper, J. W., Leong, J., Teintze, M. & Schroth, M. N. Enhancing plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **286**, 885–886 (1980).
This work stimulated interest in siderophores as a biocontrol mechanism and promoted the PGPR concept.
25. Preston, G. M. Plant perceptions of plant growth-promoting *Pseudomonas*. *Phil. Trans. R. Soc. Lond. B* **359**, 907–918 (2004).
26. Vessey, K. J. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* **255**, 571–586 (2003).
27. Ryu, C.-M. *et al*. Bacterial volatiles promote growth in *Arabidopsis*. *Proc. Natl Acad. Sci. USA* **100**, 4927–4932 (2003).
28. Kloepper, J. W. in *6th International PGPR Workshop* 81–92 (Indian Institute of Spices Research, Calicut, India, 2003).
29. Fravel, D., Olivain, C. & Alabouvette, C. *Fusarium oxysporum* and its biocontrol. *New Phytologist* **157**, 493–502 (2003).
30. Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. & Lorito, M. *Trichoderma* species: opportunistic, avirulent plant symbionts. *Nature Rev. Microbiol.* **2**, 43–56 (2004).
This review on fungal biocontrol strains provides a useful complement to our present paper.
31. Hallmann, J., Quadt-Hallmann, A., Mahaffee, W. F. & Kloepper, J. W. Bacterial endophytes in agricultural crops. *Can. J. Microbiol*. **43**, 895–914 (1997).
32. Kuiper, I., Kravchenkov, L., Bloemberg, G. V. & Lugtenberg, B. J. J. *Pseudomonas putida* strain PCL1444, selected for efficient root colonization and naphthalene degradation, effectively utilizes root exudates components. *Mol. Plant Microbe Interact.* **15**, 734–741 (2002).
33. Landa, B. B., Mavrodi, D. M., Thomashow, L. S. & Weller, D. M. Interactions between strains of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* in the rhizosphere of wheat. *Phytopathology* **93**, 982–994 (2003).
34. Raaijmakers, J. M. & Weller D. M. Exploiting genotypic diversity of 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp.: characterization of superior root-colonizing *P. fluorescens* strain Q8r1-96. *Appl. Environ. Microbiol*. **67**, 2545–2554 (2001).
35. Burr, T. J. & Caesar, A. Beneficial plant bacteria. *CRC Crit. Rev. Plant Sci.* **2**, 1–20 (1984).
36. Stephens, P. M., Crowley, J. J. & O'Connell, C. Selection of pseudomonad strains inhibiting *Pythium ultimum* on sugarbeet seeds in soil. *Soil Biol. Biochem.* **25**, 1283–1288 (1993).
37. Bull, C. T., Weller, D. M. & Thomashow, L. S. Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2-79. *Phytopathology* **81**, 954–959 (1991).
38. Raaijmakers, J. M., Weller, D. M. & Thomashow, L. S. Frequency of antibiotic-producing *Pseudomonas* spp. in natural environments. *Appl. Environ. Microbiol.* **63**, 881–887 (1997).
39. Raaijmakers, J. M., Bonsall, R. E. & Weller, D. M. Effect of population density of *Pseudomonas fluorescens* on production of 2,4-diacetylphloroglucinol in the rhizosphere of wheat. *Phytopathology* **89**, 470–475 (1999).
This work, which is part of a series of important studies by the same authors, determines the key parameters for effective biocontrol.
40. Chatterton, S., Sutton, J. C. & Boland, G. J. Timing *Pseudomonas chlororaphis* applications to control *Pythium aphanidermatum*, *Pythium dissotocum*, and root rot in hydroponic peppers. *Biol. Control* **30**, 360–373 (2004).
41. Haas, D. & Keel, C. Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annu. Rev. Phytopathol.* **41**, 117–153 (2003).
42. Weller, D. M. Colonization of wheat roots by a fluorescent pseudomonad suppressive to take-all. *Phytopathology* **73**, 1548–1553 (1983).
43. Bahme, J. B. & Schroth, M. N. Spatial-temporal colonization of a rhizobacterium on underground organs of potato. *Phytopathology* **77**, 1093–1100 (1987).
44. Tang, W., Pasternak, J. J. & Glick, B. R. Persistence in soil of the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2 and genetically manipulated derived strains. *Can. J. Microbiol.* **41**, 445–451 (1995).
45. Lockwood, J. L. Evolution of concepts associated with soilborne plant pathogens. *Annu. Rev. Phytopathol.* **26**, 93–121 (1988).
46. Meyer, J. M. & Abdallah, M. A. The fluorescent pigment of *Pseudomonas fluorescens*: biosynthesis, purification and physicochemical properties. *J. Gen. Microbiol.* **107**, 319–328 (1978).
47. Visca, P. in *Pseudomonas* Vol. 2 (ed. Ramos, J.-L.) 69–123 (Kluwer Academic/Plenum Publishers, New York, 2004).
48. Simeoni, L. A., Lindsay, W. L. & Baker, R. Critical iron level associated with biological control of *Fusarium* wilt. *Phytopathology* **77**, 1057–1061 (1987).
49. Scher, F. M. & Baker, R. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium* wilt pathogens. *Phytopathology* **72**, 1567–1573 (1982).
50. Keel, C., Voisard, C., Berling, C. H., Kahr, G. & Défago, G. Iron sufficiency, a prerequisite for suppression of tobacco black root rot by *Pseudomonas fluorescens* strain CHA0 under gnotobiotic conditions. *Phytopathology* **79**, 584–589 (1989).
51. Loper, J. E. & Buyer, J. S. Siderophores in microbial interactions on plant surfaces. *Mol. Plant Microbe Interact.* **4**, 5–13 (1991).
This paper presents a critical appraisal of the siderophore hypothesis.
52. Elad, Y. & Baker, R. Influence of trace amounts of cations and siderophore-producing pseudomonads on chlamydospore germination of *Fusarium oxysporum*. *Phytopathology* **75**, 1047–1052 (1985).
53. Misaghi, I. J., Olsen, M. W., Cotty, P. J. & Donndelinger, C. R. Fluorescent siderophore-mediated iron deprivation: a contingent biological control mechanism. *Soil Biol. Biochem.* **20**, 573–574 (1988).
54. Loper, J. E. & Henkels, M. D. Availability of iron to *Pseudomonas fluorescens* in rhizosphere and bulk soil evaluated with an ice nucleation reporter gene. *Appl. Environ. Microbiol.* **63**, 99–105 (1997).
55. Phoebe, C. H. Jr *et al*. Extremophilic organisms as an unexplored source of antifungal compounds. *J. Antibiot.* **54**, 56–65 (2001).
56. Cuppels, D. A., Stipanovic, R. D., Stoessl, A. & Stothers, J. B. The constitution and properties of a pyochelin–zinc complex. *Can. J. Chem.* **65**, 2126–2130 (1987).
57. Visca, P. *et al*. Metal regulation of siderophore synthesis in *Pseudomonas aeruginosa* and functional effects of siderophore–metal complexes. *Appl. Environ. Microbiol.* **58**, 2886–2893 (1992).
58. Raaijmakers, J. M., Vlami, M. & de Souza, J. T. Antibiotic production by bacterial biocontrol agents. *Antonie van Leeuwenhoek* **81**, 537–547 (2002).
59. Morrissey, J. P., Cullinane, M., Abbas, A., Mark, G. L. & O'Gara, F. in *Pseudomonas* Vol. 3 (ed. Ramos, J.-L.) 637–670 (Kluwer Academic/Plenum Publishers, New York, 2004).
60. Ran, H., Hassett, D. J. & Lau, G. W. Human targets of *Pseudomonas aeruginosa* pyocyanin. *Proc. Natl Acad. Sci. USA* **100**, 14315–14320 (2003).
61. Britigan, B. E. *et al*. Interaction of the *Pseudomonas aeruginosa* secretory products pyocyanin and pyochelin generates hydroxyl radical and causes synergistic damage to endothelial cells. Implications for *Pseudomonas*-associated tissue injury. *J. Clin. Invest.* **90**, 2187–2196 (1992).
62. Hernandez, M. E., Kappler, A. & Newman, D. K. Phenazines and other redox-active antibiotics promote microbial mineral reduction. *Appl. Environ. Microbiol*. **70**, 921–928 (2004).
63. Mavrodi, O. V. *et al.* Genetic diversity of *phlD* from 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. *Phytopathology* **91**, 35–43 (2001).
64. de Souza, J. T. *et al*. Effect of 2,4-diacetylphloroglucinol on *Pythium*: cellular responses and variation in sensitivity among propagules and species. *Phytopathology* **93**, 966–975 (2003).
65. Keel, C. *et al*. Suppression of root diseases by *Pseudomonas fluorescens* CHA0: importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. *Mol. Plant Microbe Interact.* **5**, 4–13 (1992).
This study, together with references 74 and 75, shows the importance of Phl in biocontrol.
66. Tripathi, R. K. & Gottlieb, D. Mechanism of action of the antifungal antibiotic pyrrolnitrin. *J. Bacteriol.* **100**, 310–318 (1969).
67. Ligon, J. M. *et al*. Natural products with antifungal activity from *Pseudomonas* biocontrol bacteria. *Pest Manag. Sci.* **56**, 688–695 (2000).
68. Nybroe, O. & Sørensen, J. in *Pseudomonas* Vol. 3 (ed. Ramos, J-L.) 147–172 (Kluwer Academic/Plenum Publishers, New York, 2004).
69. Blumer, C. & Haas, D. Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. *Arch. Microbiol.* **173**, 170–177 (2000).
70. Ahl, P., Voisard, C. & Défago, G. Iron bound-siderophores, cyanic acid, and antibiotics involved in suppression of *Thielaviopsis basicola* by a *Pseudomonas fluorescens* strain. *J. Phytopathol.* **116**, 121–134 (1986).
71. Chin-A-Woeng, T. F. C. *et al*. Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Mol. Plant Microbe Interact.* **11**, 1069–1077 (1998).
72. Howell, C. R. & Stipanovic, R. D. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. *Phytopathology* **69**, 480–482 (1979).
In this study, pyrrolnitrin, which is an antibiotic that is produced by *P. fluorescens*, is shown to be effective against fungal infection of a plant.
73. Howell, C. R. & Stipanovic, R. D. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings *by Pseudomonas fluorescens* and its antibiotic, pyoluteorin. *Phytopathology* **70**, 712–715 (1980).
74. Fenton, A. M., Stephens, P. M., Crowley, J., O'Callaghan, M. & O'Gara, F. Exploitation of gene(s) involved in 2,4-diacetylphloroglucinol biosynthesis to confer a new biocontrol capability to a *Pseudomonas* strain. *Appl. Environ. Microbiol.* **58**, 3873–3878 (1992).
75. Vincent, M. N. *et al*. Genetic analysis of the antifungal activity of a soilborne *Pseudomonas aureofaciens* strain. *Appl. Environ. Microbiol.* **57**, 2928–2934 (1991).
76. Timonin, M. I. Microflora of the rhizosphere in relation to the manganese-deficiency disease of oats. *Soil Sci. Soc. Am. Proc.* **11**, 284–292 (1947).
77. Kraus, J. & Loper, J. E. Characterization of a genomic region required for production of the antibiotic pyoluteorin by the biological control agent *Pseudomonas fluorescens* Pf-5. *Appl. Environ. Microbiol.* **61**, 849–854 (1995).
78. Maurhofer, M., Keel, C., Haas, D. & Défago, G. Pyoluteorin production by *Pseudomonas fluorescens* strain CHA0 is involved in the suppression of *Pythium* damping-off of cress but not of cucumber. *Eur. J. Plant Pathol.* **100**, 221–232 (1994).
79. Maurhofer, M., Keel, C., Haas, D. & Défago, G. Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHA0 with enhanced antibiotic production. *Plant Pathol.* **44**, 40–50 (1995).
80. Schnider, U. *et al*. Amplification of the housekeeping sigma factor in *Pseudomonas fluorescens* CHA0 enhances antibiotic production and improves biocontrol abilities. *J. Bacteriol.* **177**, 5387–5392 (1995).
81. Delany, I. R. *et al*. Enhancing the biocontrol efficacy of *Pseudomonas fluorescens* F113 by altering the regulation and production of 2,4-diacetylphloroglucinol. *Plant Soil* **232**, 195–205 (2001).
82. Huang, Z., Bonsall, R. F., Mavrodi, D. V., Weller, D. M. & Thomashow, L. S. Transformation of *Pseudomonas fluorescens* with genes for biosynthesis of penazine-1-carboxylic acid improves biocontrol of rhizoctonia root rot and *in situ* antibiotic production. *FEMS Microbiol. Ecol*. **49**, 243–251 (2004).
83. Timms-Wilson, T. M. *et al*. Chromosomal insertion of phenazine-1-carboxylic acid biosynthetic pathway enhances efficacy of damping-off disease control by *Pseudomonas fluorescens*. *Mol. Plant Microbe Interact.* **13**, 1293–1300 (2000).
84. Chin-A-Woeng, T. F. C., Thomas-Oates, J. E., Lugtenberg, B. J. J. & Bloemberg, G. V. Introduction of the *phzH* gene of *Pseudomonas chlororaphis* PCL1391 extends the range of biocontrol ability of phenazine-1-carboxylic acid-producing *Pseudomonas* spp. strains. *Mol. Plant Microbe Interact.* **14**, 1006–1015 (2001).
85. Viebahn, M. *et al*. Repeated introduction of genetically modified *Pseudomonas putida* WCS358r without intensified effects on the indigenous microflora of field-grown wheat. *Appl. Environ. Microbiol.* **69**, 3110–3118 (2003).
86. Georgakopoulos, D. G., Hendson, M., Panopoulos, N. J. & Schroth, M. N. Cloning of a phenazine biosynthetic locus of *Pseudomonas aureofaciens* PGS12 and analysis of its expression *in vitro* with the ice nucleation reporter gene. *Appl. Environ. Microbiol.* **60**, 2931–2938 (1994).

87. Koch, B. *et al*. Lipopeptide production in *Pseudomonas* sp. strain DSS73 is regulated by components of sugar beet seed exudate via the Gac two-component regulatory system. *Appl. Environ. Microbiol.* **68**, 4509–4516 (2002).
88. Notz, R. *et al*. Biotic factors affecting expression of the 2,4-diacetylphloroglucinol biosynthesis gene *phlA* in *Pseudomonas fluorescens* biocontrol strain CHA0 in the rhizosphere. *Phytopathology* **91**, 873–881 (2001).
89. Pierson, L. S., Wood, D. W. & Pierson, E. A. Homoserine lactone-mediated gene regulation in plant-associated bacteria. *Annu. Rev. Phytopathol.* **36**, 207–225 (1998).
90. Chin-A-Woeng, T. F. C., de Priester, W., van der Bij, A. & Lugtenberg, B. J. J. Description of the colonization of a gnotobiotic tomato rhizosphere by *Pseudomonas fluorescens* biocontrol strain WCS365, using scanning electron microscopy. *Mol. Plant Microbe Interact.* **10**, 79–86 (1997).
91. Osburn, R. M., Schroth, M. N., Hancock, J. G. & Hendson, M. Dynamics of sugar beet seed colonization by *Pythium ultimum* and *Pseudomonas* species: effects on seed rot and damping-off. *Phytopathology* **79**, 709–716 (1989).
92. Haas, D., Blumer, C. & Keel, C. Biocontrol ability of fluorescent pseudomonads genetically dissected: importance of positive feedback regulation. *Curr. Opin. Biotechnol.* **11**, 290–297 (2000).
93. Højberg, O., Schnider, U., Winteler, H., Sørensen, J. & Haas, D. Oxygen-sensing reporter strain of *Pseudomonas fluorescens* for monitoring the distribution of low-oxygen habitats in soil. *Appl. Environ. Microbiol.* **65**, 4085–4093 (1999).
94. Sørensen, J., Jensen, L. E. & Nybroe, O. Soil and rhizosphere as habitats for *Pseudomonas* inoculants: new knowledge on distribution, activity and physiological state derived from micro-scale and single-cell studies. *Plant Soil* **232**, 97–108 (2001).
95. Nishijyo, T., Haas, D. & Itoh, Y. The CbrA–CbrB two-component regulatory system controls the utilization of multiple carbon and nitrogen sources in *Pseudomonas aeruginosa*. *Mol. Microbiol.* **40**, 917–931 (2001).
96. Ghiglione, J.-F., Gourbiere, F., Potier, P., Philippot, L. & Lensi, R. Role of respiratory nitrate reductase in ability of *Pseudomonas fluorescens* YT101 to colonize the rhizosphere of maize. *Appl. Environ. Microbiol.* **66**, 4012–4016 (2000).
97. Mazzola, M., Cook, R. J., Thomashow, L. S., Weller, D. M. & Pierson, L. S. Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. *Appl. Environ. Microbiol.* **58**, 2616–2624 (1992).
98. Lemanceau, P. *et al*. Effect of two plant species, flax (*Linum usitatissimum* L.) and tomato (*Lycopersicon esculentum* Mill.), on the diversity of soilborne populations of fluorescent pseudomonads. *Appl. Environ. Microbiol.* **61**, 1004–1012 (1995).
99. Goddard, V. J., Bailey, M. J., Darrah, P., Lilley, A. K. & Thompson, I. P. Monitoring temporal and spatial variation in rhizosphere bacterial population diversity: a community approach for the improved selection of rhizosphere competent bacteria. *Plant Soil* **232**, 181–193 (2001).
100. Smalla, K. *et al*. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. *Appl. Environ. Microbiol.* **67**, 4742–4751 (2001).
101. Marschner, P., Crowley, D. & Yang, C. H. Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. *Plant Soil* **261**, 199–208 (2004).
102. Rovira, A. D. Plant root exudates. *Bot. Rev.* **35**, 35–57 (1969).
103. Glandorf, D. C. M., van der Sluis, I., Anderson, A. J., Bakker, P. A. H. M. & Schippers, B. Agglutination, adherence, and root colonization by fluorescent pseudomonads. *Appl. Environ. Microbiol*. **60**, 1726–1733 (1994).
104. Bais, H. P., Sang-Wook, P., Weir, T. L., Callaway, R. M. & Vivanco, J. M. How plants communicate using the underground information superhighway. *Trends Plant Sci*. **9**, 26–32 (2004).
105. Bloemberg, G. V., Wijfjes, A. H. M., Lamers, G. E. M., Stuurman, N. & Lugtenberg, B. J. J. Simultaneous imaging of *Pseudomonas fluorescens* WCS365 populations expressing three different autofluorescent proteins in the rhizosphere: new perspectives for studying microbial communities. *Mol. Plant Microbe Interact.* **13**, 1170–1176 (2000).
A nice example of how rhizobacteria can be labelled with fluorescent tags for *in situ* detection.
106. Gamalero, E. *et al*. Colonization pattern of primary tomato roots by *Pseudomonas fluorescens* A6RI characterized by dilution plating, flow cytometry, fluorescence, confocal and scanning electron microscropy. *FEMS Microbiol. Ecol.* **48**, 79–87 (2004).
107. Troxler, J., Berling, C.-H., Moënne-Loccoz, Y., Keel, C. & Défago, G. Interactions between the biocontrol agent *Pseudomonas fluorescens* CHA0 and *Thielaviopsis basicola* in tobacco roots observed by immunofluorescence microscopy. *Plant Pathol.* **46**, 62–71 (1997).
108. Moënne-Loccoz, Y. & Défago, G. in *Pseudomonas* Vol. 1 (ed. Ramos, J.-L.) 457–476 (Kluwer Academic/Plenum Publishers, New York, 2004).
109. Zehnder, G. W., Murphy, J. F., Sikora, E. J. & Kloepper, J. W. Application of rhizobacteria for induced resistance. *Eur. J. Plant Pathol.* **107**, 39–50 (2001).
110. Ongena, M. *et al*. Stimulation of the lipoxygenase pathway is associated with systemic resistance induced in bean by a nonpathogenic *Pseudomonas* strain. *Mol. Plant Microbe Interact.* **17**, 1009–1018 (2004).
111. Ton, J. *et al*. Characterization of *Arabidopsis* enhanced disease susceptibility mutants that are affected in systemically induced resistance. *Plant J*. **29**, 11–21 (2002).
112. Verhagen, B. W. M. *et al*. The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Mol. Plant Microbe Interact.* **17**, 895–908 (2003).
The genomic approach used in this study presents evidence for a priming mechanism in ISR.
113. Cartieaux, F. *et al*. Transcriptome analysis of *Arabidopsis* colonized by a plant-growth promoting rhizobacterium reveals a general effect on disease resistance. *Plant J.* **36**, 177–188 (2003).
114. Bakker, P. A. H. M., Ran, L. X., Pieterse, C. M. J. & van Loon, L. C. Understanding the involvement of rhizobacteria-mediated induction of systemic resistance in biocontrol of plant diseases. *Can. J. Plant Pathol.* **25**, 5–9 (2003).
115. Iavicoli, A., Boutet, E., Buchala, A. & Métraux, J.-P. Induced systemic resistance in *Arabidopsis thaliana* in response to root inoculation with *Pseudomonas fluorescens* CHA0. *Mol. Plant Microbe Interact*. **16**, 851–858 (2003).
116. Maurhofer, M. *et al*. Salicylic acid biosynthetic genes expressed in *Pseudomonas fluorescens* strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. *Phytopathology* **88**, 678–684 (1998).
117. Audenaert, K., Pattery, T., Cornelis, P. & Höfte, M. Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: role of salicylic acid, pyochelin, and pyocyanin. *Mol. Plant Microbe Interact*. **15**, 1147–1156 (2002).
118. Ryu, C.-M. *et al*. Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol.* **134**, 1017–1026 (2004).
119. Van Loon, L. C. & Bakker, P. A. H. M. in *Root Ecology* (eds de Kroon, H. & Visser, W. J. W.) 297–330 (Springer-Verlag, Berlin, 2003).
120. De Souza, J. T., Weller, D. M. & Raaijmakers, J. M. Frequency, diversity, and activity of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in Dutch take-all decline soils. *Phytopathology* **93**, 54–63 (2003).
121. Landa, B. B. *et al*. Differential ability of genotypes of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* strains to colonize the roots of pea plants. *Appl. Environ. Microbiol.* **68**, 3226–3237 (2002).
122. Ramette, A., Moënne-Loccoz, Y. & Défago, G. Prevalence of fluorescent pseudomonads producing antifungal phloroglucinols and/or hydrogen cyanide in soils naturally suppressive or conducive to tobacco black root rot. *FEMS Microbiol. Ecol.* **44**, 35–43 (2003).
123. Phillips, D. A., Fox, T. C., King, M. D., Bhuvaneswari, T. V. & Teuber, L. R. Microbial products trigger amino acid exudation from plant roots. *Plant Physiol*. **136**, 2887–2894 (2004).
124. Wang, C. *et al*. Cosmopolitan distribution of *phlD*-containing dicotyledonous crop-associated biocontrol pseudomonads of worldwide origin. *FEMS Microbiol. Ecol.* **37**, 105–116 (2001).
125. Toyoda, H., Hashimoto, H., Utsumi, R., Kobayashi, H. & Ouchi, S. Detoxification of fusaric acid by a fusaric acid-resistant mutant of *Pseudomonas solanacearum* and its application to biological control of *Fusarium* wilt of tomato. *Phytopathology* **78**, 1307–1311 (1988).
126. Utsumi, R. *et al*. Molecular cloning and characterization of the fusaric acid-resistance gene from *Pseudomonas cepacia*. *Agric. Biol. Chem.* **55**, 1913–1918 (1991).
127. McKellar, M. E. & Nelson, E. B. Compost-induced suppression of *Pythium* damping-off is mediated by fatty-acid-metabolizing seed-colonizing microbial communities. *Appl. Environ. Microbiol.* **69**, 452–460 (2003).
128. Paulitz, T. C. Effect of *Pseudomonas putida* on the stimulation of *Pythium ultimum* by seed volatiles of pea and soybean. *Phytopathology* **81**, 1282–1287 (1991).
129. Stanghellini, M. E. & Miller, R. M. Biosurfactants. Their identity and potential efficacy in the biological control of zoosporic plant pathogens. *Plant Disease* **81**, 4–12 (1997).
130. Duffy, B. & Défago, G. Zinc improves biocontrol of *Fusarium* crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. *Phytopathology* **87**, 1250–1257 (1997).
This paper provides a good example of how a mineral ion can influence the interaction between a pathogen and a biocontrol agent.
131. Mitchell, R. & Alexander, M. The mycolytic phenomenon and biological control of *Fusarium* in soil. *Nature* **190**, 109–110 (1961).
132. Benhamou, N., Kloepper, J. W. & Tuzun, S. Induction of resistance against *Fusarium* wilt of tomato by combination of chitosan with an endophytic bacterial strain: ultrastructure and cytochemistry of the host response. *Planta* **204**, 153–168 (1998).
133. Hallmann, J., Rodríguez-Kábana, R. & Kloepper, J. W. Chitin-mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. *Soil Biol. Biochem.* **31**, 551–560 (1999).
134. Nielsen, M. N. & Sørensen, J. Chitinolytic activity of *Pseudomonas fluorescens* isolates from barley and sugar beet rhizosphere. *FEMS Microbiol. Ecol.* **30**, 217–227 (1999).
135. Shahidi, F., Arachchi, J. K. V. & Jeon, Y.-J. Food application of chitin and chitosans. *Trends Food Sci. Technol.* **10**, 37–51 (1999).
136. Schnider-Keel, U. *et al*. Autoinduction of 2,4-diacetylphloroglucinol biosynthesis in the biocontrol agent *Pseudomonas fluorescens* CHA0 and repression by the bacterial metabolites salicylate and pyoluteorin. *J. Bacteriol.* **182**, 1215–1225 (2000).
In this study, Phl, similar to several other extracellular metabolites of bacteria, is shown to be an autoinducer.
137. Mazzola, M., Fujimoto, D. K., Thomashow, L. S. & Cook, J. Variation in sensitivity of *Gaeumannomyces graminis* to antibiotics produced by fluorescent *Pseudomonas* spp. and effect on biological control of take-all of wheat. *Appl. Environ. Microbiol.* **61**, 2554–2559 (1995).
138. Duffy, B., Schouten, A. & Raaijmakers, J. M. Pathogen self-defense: mechanisms to counteract microbial antagonism. *Annu. Rev. Phytopathol.* **41**, 501–538 (2003).
139. Schouten, A. *et al*. Defense responses of *Fusarium oxysporum* to 2,4-diacetylphloroglucinol, a broad-spectrum antibiotic produced by *Pseudomonas fluorescens*. *Mol. Plant Microbe Interact.* **17**, 1201–1211 (2004).
The response of a pathogen to a typical biocontrol factor is analysed in molecular detail in this paper.
140. Weller, D. M. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol.* **26**, 379–407 (1988).
141. Lamont, I. L., Beare, P. A., Ochsner, U., Vasil, A. I. & Vasil, M. L. Siderophore-mediated signaling regulates virulence factor production in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA* **99**, 7072–7077 (2002).
142. Reimmann, C., Serino, L., Beyeler, M. & Haas, D. Dihydroaeruginoic acid synthetase and pyochelin synthetase, products of the *pchEF* genes, are induced by extracellular pyochelin in *Pseudomonas aeruginosa*. *Microbiology* **144**, 3135–3148 (1998).
143. Brodhagen, M., Henkels, M. D. & Loper, J. E. Positive autoregulation and signaling properties of pyoluteorin, an antibiotic produced by the biological control organism *Pseudomonas fluorescens* Pf-5. *Appl. Environ. Microbiol*. **70**, 1758–1766 (2004).
144. Maurhofer, M., Baehler, E., Notz, R., Martinez, V. & Keel, C. Cross-talk between 2,4-diacetylphloroglucinol-producing biocontrol pseudomonads on wheat roots. *Appl. Environ. Microbiology* **70**, 1990–1998 (2004).
145. Chancey, S. T., Wood, D. W. & Pierson, L. S. Two-component transcriptional regulation of *N*-acyl-homoserine lactone production in *Pseudomonas aureofaciens*. *Appl. Environ. Microbiol.* **65**, 2294–2299 (1999).
146. Chin-A-Woeng, T. F. C. *et al*. Phenazine-1-carboxamide production in the biocontrol strain *Pseudomonas chlororaphis* PCL1391 is regulated by multiple factors secreted into the growth medium. *Mol. Plant Microbe Interact.* **14**, 969–979 (2001).
147. Pessi, G. & Haas, D. in *Pseudomonas* Vol. 3 (ed. Ramos, J.-L.) 671–687 (Kluwer Academic/Plenum Publishers, New York, 2004).
148. Hartmann, A. *et al*. in *Biology of Plant–Microbe Interactions* Vol. 4 (eds Tikhonovich, I., Lugtenberg, B. & Provorov, N.) 554–556 (ISMPMI, St. Paul, 2004).

149. Molina, L. *et al*. Degradation of pathogen quorum-sensing molecules by soil bacteria: a preventive and curative biological control mechanism. *FEMS Microbiol. Ecol.* **45**, 71–81 (2003).
150. Uroz, S. *et al*. Y. Novel bacteria degrading *N*-acylhomoserine lactones and their use as quenchers of quorum-sensing-regulated functions of plant-pathogenic bacteria. *Microbiology* **149**, 1981–1989 (2003).
151. Zhang, L-H. & Dong, Y.-H. Quorum sensing and signal interference: diverse implications. *Mol. Microbiol.* **53**, 1563–1571 (2004).
152. Van Rij, E. T., Wesselink, M., Chin-A-Woeng, T. F. C., Bloemberg, G. V. & Lugtenberg, B. J. J. Influence of environmental conditions on the production of phenazine-1-carboxamide by *Pseudomonas chlororaphis* PCL1391. *Mol. Plant Microbe Interact.* **17**, 557–566 (2004).
153. Laville, J. *et al*. Global control in *Pseudomonas fluorescens* mediating antibiotic synthesis and suppression of black root rot of tobacco. *Proc. Natl Acad. Sci. USA* **89**, 1562–1566 (1992).
In this study, the GacA response regulator was found to be a key determinant in biocontrol efficacy.
154. Gaffney, T. D. *et al*. Global regulation of expression of antifungal factors by a *Pseudomonas fluorescens* biological control strain. *Mol. Plant Microbe Interact.* **7**, 455–463 (1994).
155. Whistler, C. A., Corbell, N. A., Sarniguet, A., Ream, W. & Loper, J. E. The two-component regulators GacS and GacA influence accumulation of the stationary phase sigma factor RpoS and the stress response in *Pseudomonas fluorescens* Pf-5. *J. Bacteriol.* **180**, 6635–6641 (1998).
156. Heeb, S. & Haas, D. Regulatory roles of the GacS/GacA two-component system in plant-associated and other Gram-negative bacteria. *Mol. Plant Microbe Interact.* **14**, 1351–1363 (2001).
157. Van den Broek, D. *et al*. Biocontrol traits of *Pseudomonas* spp. are regulated by phase variation. *Mol. Plant Microbe Interact*. **16**, 1003–1012 (2003).
158. Ovadis, M. *et al*. The global regulator genes from biocontrol strain *Serratia plymuthica* IC1270: cloning, sequencing, and functional studies. *J. Bacteriol.* **186**, 4986–4993 (2004).
159. Pernestig, A.-K., Melefors, O. & Georgellis, D. Identification of UvrY as the cognate response regulator for the BarA sensor kinase in *Escherichia coli*. *J. Biol. Chem.* **276**, 225–231 (2001).
160. Zuber, S. *et al*. GacS sensor domains pertinent to the regulation of exoproduct formation and to the biocontrol potential of *Pseudomonas fluorescens* CHA0. *Mol. Plant Microbe Interact.* **61**, 634–644 (2003).
161. Blumer, C., Heeb, S., Pessi, G. & Haas, D. Global GacA-steered control of cyanide and exoprotease production in *Pseudomonas fluorescens* involves specific ribosome binding sites. *Proc. Natl Acad. Sci. USA* **96**, 14073–14078 (1999).
A link between biocontrol activity and a post-transcriptional regulatory mechanism involving small RNAs is proposed in this paper.
162. Aarons, S., Abbas, A., Adams, C., Fenton, A. & O'Gara, F. A regulatory RNA (PrrB RNA) modulates expression of secondary metabolite genes in *Pseudomonas fluorescens* F113. *J. Bacteriol.* **182**, 3913–3919 (2000).
163. Heeb, S., Blumer, C. & Haas. D. Regulatory RNA as mediator in GacA/RsmA-dependent global control of exoproduct formation in *Pseudomonas fluorescens* CHA0. *J. Bacteriol.* **184**, 1046–1056 (2002).
164. Valverde, C., Heeb, S., Keel, C. & Haas, D. RsmY, a small regulatory RNA, is required in concert with RsmZ for GacA-dependent expression of biocontrol traits in *Pseudomonas fluorescens* CHA0. *Mol. Microbiol.* **50**, 1361–1379 (2003).
165. Reimmann, C., Valverde, C., Kay, E. & Haas, D. Posttranscriptional repression of GacS/GacA-controlled genes by the RNA-binding protein RsmE acting together with RsmA in the biocontrol strain *Pseudomonas fluorescens* CHA0. *J. Bacteriol.* **187**, 276–285 (2005).
166. Valverde, C., Lindell, M., Wagner, E. G. H. & Haas, D. A repeated GGA motif is critical for the activity and stability of the riboregulator RsmY of *Pseudomonas fluorescens*. *J. Biol. Chem.* **279**, 25066–25074 (2004).
167. Baker, C. S., Morozov, I., Suzuki, K., Romeo, T. & Babitzke, P. CsrA regulates glycogen biosynthesis by preventing translation of *glgC* in *Escherichia coli*. *Mol. Microbiol.* **44**, 1599–1610 (2002).
168. Lenz, D. H. *et al*. The small RNA chaperone Hfq and multiple small RNAs control quorum sensing in *Vibrio harveyi* and *Vibrio cholerae*. *Cell* **118**, 69–82 (2004).
169. Lebreton, L. *et al*. Changes in population structure of the soil-borne fungus *Gaeumannomyces graminis* var. *tritici* during continuous wheat cropping. *Environ. Microbiol*. **6**, 1174–1185 (2004).
170. Paulitz, T. C. & Bélanger, R. R. Biological control in greenhouse systems. *Annu. Rev. Phytopathol.* **39**, 103–133 (2001).
171. de Boer, M. *et al*. Control of *Fusarium* wilt of radish by combining *Pseudomonas putida* strains that have different disease-suppressive mechanisms. *Phytopathology* **93**, 626–632 (2003).
172. Kloepper, J. W., Ryu, C.-M. & Zhang, S. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* **94**, 1259–1266 (2004).
This review on biocontrol strains of *Bacillus* spp. emphasizes their applications.
173. Schisler, D. A., Slininger, P. J., Behle, R. W. & Jackson, M. A. Formulation of *Bacillus* spp. for biological control of plant diseases. *Phytopathology* **94**, 1267–1271 (2004).

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Competing interests statement
The authors declare no competing financial interests.

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