Chapter 8
Phytostimulation and Biocontrol by the Plant-Associated *Bacillus amyloliquefaciens* FZB42: An Update

Rainer Borriss

Abstract *Bacillus amyloliquefaciens* FZB42, the type strain for representatives of the plant-associated subspecies *plantarum*, stimulates plant growth and suppresses soilborne plant pathogens. The strain has been sequenced in 2007 (Chen et al. (2007); *National Biotechnology 25*, 1007–1014). The *B. amyloliquefaciens* FZB42 genome reveals an unexpected potential to produce secondary metabolites. In total, 11 gene clusters representing nearly 10 % of the genome are devoted to synthesizing antimicrobial metabolites and/or to confer immunity against them. Ability to synthesize non-ribosomally the antibacterial polyketides macrolactin and difficidin and the antifungal lipopeptide bacillomycin D is a unique feature of the subspecies *plantarum*. However, according to the latest research, most of the secondary metabolites are not expressed in plant rhizosphere suggesting that the antibiome expressed during the plant-associated state of PGPR *Bacilli* does not reflect the vast genetic arsenal devoted to the formation of secondary metabolites. There is now strong evidence that plant-associated *Bacilli* trigger pathways of induced systemic resistance, which protect plants against attacks of pathogenic microbes, viruses, and nematodes.

8.1 Introduction

Environmental-friendly biotechnological approaches, such as the use of microbial biopesticides, offer alternatives to chemical control of plant diseases and pests. Among these alternatives, the use of bioformulations, which are manufactured from plant growth-promoting rhizobacteria (PGPR) with biocontrol activity (BC) (Lugtenberg et al. 2013), is steadily increasing. At present, due to the long-term shelf life of their endospores, bacilli are the most widely used bacteria on the

R. Borriss (✉)
Humboldt-Universität zu Berlin, Institut für Agar- und Gartenbauwissenschaften, Fachgebiet Phytomedizin, 14195 Berlin, Germany

Nord Reet UG, 17489 Greifswald, Germany
e-mail: rainer.borriss@rz.hu-berlin.de

M.T. Islam et al. (eds.), *Bacilli and Agrobiotechnology*,
DOI 10.1007/978-3-319-44409-3_8

biopesticide market. Their use in agriculture has been previously reviewed (Borriss 2011). An update of *Bacillus*-based bioformulations, currently available for the farmer interested on sustainable agriculture, is presented in Table 8.1.

Plant rhizosphere is a highly competitive environment in which bacteria are abundantly present due to availability of nutrients actively secreted by the plant root and mucilage. Some of these bacteria which are living within or in the vicinity of plant rootsand supporting plant growth are generally referred as being "PGPR" (Kloepper et al. 1980). In many cases, their plant growth-promoting activity is linked with their ability to suppress soilborne plant pathogens (bacteria and microfungi), occurring in the competing microflora. Different mechanisms are discussed in this context. Besides production of antimicrobial ("antibiotics") and nematicidal compounds, also stimulation of plant-induced systemic resistance (ISR, Doornbos et al. 2012) and a beneficial effect on the composition of the host-plant microbiome might contribute to their suppressive effect (Erlacher et al. 2014). In other PGPR, termed "biofertilizer," plant growth promotion by hormone-like compounds and increased accessibility of nutrients dominate. The mechanisms that are involved in this process can include nitrogen fixation, phosphate and mineral solubilization, and the production of macromolecule-degrading enzymes (amylases, proteases, hemicellulases), phytohormones (auxin, cytokinin, and gibberellins), and volatile growth stimulants (such as acetoin and 2,3-butanediol) (Borriss 2011).

Bacillus amyloliquefaciens FZB42 is the type strain for a group of plant-associated *Bacillus* spp. classified as *B. amyloliquefaciens* subsp. *plantarum* (Borriss et al. 2011). Its 3918-kb genome, containing an estimated 3693 protein-coding sequences, lacks extended phage insertions, which occur ubiquitously in the closely related but non-plant-associated *Bacillus subtilis* 168 genome. Further analysis revealed that FZB42 is a bacterium with impressive capacity to produce metabolites with antimicrobial activity (Chen et al. 2007). Its antifungal activity is due to non-ribosomal synthesis of the cyclic lipopeptides bacillomycin D and fengycin (Koumoutsi et al. 2004), while its antibacterial activity is mainly due to non-ribosomally synthesized polyketides (Chen et al. 2006), bacilysin (Chen et al. 2009a), and ribosomally synthesized bacteriocins (Scholz et al. 2011, 2014). Recent proteome and transcriptome studies revealed that plant root exudates stimulate expression of genes involved in root colonization and plant-bacteria interactions (Borriss 2015a, b; Fan et al. 2012a, b; 2015; Kierul et al. 2015). Its plant-colonizing ability was demonstrated with a GFP-labeled FZB42 strain on maize and *Arabidopsis* using confocal laser scanning microscopy (Fan et al. 2011). Beneficial effects on plant growth and disease suppression were documented for *B. amyloliquefaciens* FZB42 on tomato, cucumber, cotton, tobacco, and lettuce, for example (Grosch et al. 1999; Idriss et al. 2004; Yao et al. 2006; Guel et al. 2008; Wang et al. 2009; Chowdhury et al. 2013). Two review articles published in open access journals in 2015 (Chowdhury et al. 2015b; Wu et al. 2015b) cover the aspects stressed in this contribution in more detail and are recommended for further reading.

Table 8.1 Examples for commercial use of *Bacillus*-based bioformulations in agriculture. Note: the US governmental EPA registration does not depend on successful field trials; it is only necessary to demonstrate that no negative effects are connected with the use of the biofungicide (The table is taken from Borriss (2015b))

Trade name	*Bacillus* strain	Known properties	Company
Kodiak™	*Bacillus subtilis* GB03	EPA-registered (71065–2) biological and seed treatment fungicide	Bayer Crop Science, former Gustafson LLC
Companion	*Bacillus subtilis* GB03	EPA-registered (71065–2) biofungicide, prevent and control plant diseases. It produces a broad-spectrum iturin antibiotic that disrupts the cell wall formation of pathogens, and it triggers an advantageous induced systemic resistance (ISR) in plants, whereby a plant's natural immune system is activated to fight plant diseases	Growth Products Ltd., White Plains, NY 10603
Yield Shield	*Bacillus pumilus* GB34 (=INR7)	EPA-registered biofungicide (264–985), suppression of root diseases caused by *Rhizoctonia* and *Fusarium*	Bayer Crop Science, previously Gustafson
BioYield™	*B. amyloliquefaciens* GB99 + *Bacillus subtilis* GB122	Combination of strong ISR activity (GB99) with phytostimulation (GB122)	Bayer Crop Science, previously Gustafson
Subtilex®, INTEGRAL®	*Bacillus subtilis* MBI600	EPA-registered (71840–8) biofungicide provides protection against soilborne pathogens such as *Rhizoctonia solani*, *Pythium* spp., and *Fusarium* spp. to help prevent damping-off and other root diseases	Becker Underwood, Saskatoon, Canada acquired by BASF
VAULT®	*Bacillus subtilis* MBI600	Produced by "BioStacked®" technology, enhancing growth of soy beans and pea nuts	Becker Underwood, Saskatoon, Canada
	Bacillus pumilus BU F-33	EPA-registered (71840-RG, -RE, 2013) plant growth stimulator, induced systemic resistance	Becker Underwood, Saskatoon, Canada
SERENADE Max	*Bacillus subtilis* QST713	EPA-registered (69592–11) biofungicide, Annex 1 listing of the EU agrochemical registration directive (91/414)	Bayer Crop Science, previously AgraQuest

(continued)

Table 8.1 (continued)

Trade name	*Bacillus* strain	Known properties	Company
SERENADE SOIL(R)	*Bacillus subtilis* QST713	EPA-registered (69592-EI, 2012) biofungicide for food crops	Bayer Crop Science, previously AgraQuest
SERENADE Optimum®	*Bacillus subtilis* QST713	EPA-registered (2013) biofungicide/bactericide for prevention. It works by stopping spore germination, disrupting cell membrane and inhibiting attachment of the pathogen to leaves. For use in leafy and fruiting vegetables, strawberries, and potatoes. Active against fungal (*Botrytis*, *Sclerotinia*), and bacterial pathogens (*Xanthomonas* and *Erwinia*)	Bayer Crop Science, previously AgraQuest
CEASE(R)	*Bacillus subtilis* QST713	Aqueous suspension biofungicide, recommended for leafy and fruiting vegetables, herbs and spices, and ornamentals	BioWorks, Inc., Victor, New York, USA
SONATA®	*Bacillus pumilus* QST2808	EPA-registered (69592–13) biofungicide, powdery mildew control	Bayer Crop Science, previously AgraQuest Inc
RhizoVital®	*Bacillus amyloliquefaciens* FZB42	Biofertilizer, plant growth-promoting activity, provides protection against various soilborne diseases, stimulation of ISR	ABiTEP GmbH, Berlin
RhizoPlus®	*Bacillus subtilis*	Plant growth-promoting rhizobacterium and biocontrol agent. It can be used for potatoes, corn, vegetables, fruits and also turf	ABiTEP GmbH, Berlin
Taegro®	*Bacillus subtilis* FZB24	EPA-registered biofungicide. FZB24 has been originally isolated by FZB Berlin, the forerunner of ABiTEP GmbH. Registration as a biofungicide for the USA was performed by Taegro Inc. and then sold to Novozymes without agreement with ABiTEP GmbH where the product is still offered	Syngenta, Basel, previously Novozyme, Davis, California and Earth Biosciences

(continued)

Table 8.1 (continued)

Trade name	*Bacillus* strain	Known properties	Company
POMEX	*Bacillus subtilis* CMB26	Microbial fungicide, control and inhibition germination effect on powdery mildew, *Cladosporium fulvum* and *Botrytis cinerea*	NIN Co. Ltd.
	Bacillus subtilis CX9060	EPA-registered 71840-RG,-RE (2012) fungicide, bactericide for food crops, turf and ornamentals	Certis Columbia, MD USA
Easy Start® TE-Max	*Bacillus subtilis E4-CDX*	Rhizosphere bacterium that competes with harmful pathogens for space around the roots of the grass plant. Once established this unique strain physically protects the roots and inhibits the advance of soilborne fungi	COMPO Expert GmbH, Münster, Germany
Double Nickel 55™	*B. amyloliquefaciens* D747	EPA-registered (70051-RNI, 2011), a broad-spectrum preventive biofungicide for control or suppression of fungal and bacterial plant diseases (powdery mildew, *Sclerotinia*, *Botrytis*, *Alternaria*, bacterial leaf spot, bacterial spot and speck, fire blight, *Xanthomonas*, *Monilinia*)	Certis Columbia, MD USA
Amylo-X®	*B. amyloliquefaciens* D747	Annex 1 listing of the EU agrochemical registration directive. Launched to Italy by Intrachem Bio Italia SpA for control of *Botrytis* and other fungal diseases of grapes, strawberries, and vegetables and bacterial diseases such as fire blight in pome fruit and PSA in kiwi fruit	Certis Columbia, MD USA/Intrachem Bio Italia SpA
BmJ WG	*Bacillus mycoides* BmJ	It works entirely as a microbial SAR activator with no direct effect on the plant pathogen itself. Under development	Certis Columbia, MD USA
	Bacillus pumilus GHA 181	EPA-registered fungicide (2012), food crops, seeds, ground cover, and ornamentals	Premier Horticulture
BioNem	*Bacillus firmus* GB-126	EPA-registered (2008), suppressing plant pathogenic nematodes; *Bacillus firmus* creates a living barrier that prevents nematodes from reaching the roots	Agrogreen, Israel acquired by Bayer Crop Science

8.2 Root Colonization by FZB42 and Its Impact on the Host Plant Microbiome

The ability of FZB42 to colonize the rhizoplane is a precondition for plant growth promotion. Using a GFP-tagged derivative (Fan et al. 2011, 2012a, b), the fate of bacterial root colonization was recently studied. It ruled out that the bacterium behaves distinctly in colonizing root surfaces of different plants. In contrast to maize, FZB42 colonized preferentially root tips when colonizing *Arabidopsis thaliana* (Dietel et al. 2013). On duckweed, *Lemna minor*, FZB42 accumulated preferably along the grooves between epidermal cells of roots and in the concave spaces on ventral sides of fronds. In vitro studies performed with maize seedlings revealed that the segment within 2–8 cm distant from the basal site of the primary root was a most colonized region by FZB42. On the contrary, few bacterial cells could be observed within the range of 2 cm of root tip. In general, the green fluorescent FZB42 cells were decreasingly observed from the upper part of a root down to the root tip. Scanning electron microscopy confirmed the presence of FZB42 on root hairs, where the bacterial cells were usually associated with a wealth of presumed root exudates (Fan et al. 2012b). In lettuce, *Lactuca sativa*, seedlings, bacterial colonization occurred mainly on primary roots and root hairs as well as on root tips and adjacent border cells. Occurrence of labeled bacteria decreased toward the root tips of the lateral roots, and no colonization of the finer roots could be observed (Chowdhury et al. 2015a).

The rhizosphere competence of FZB42 was recently studied using a combination of field and greenhouse trials. FZB42 is able to effectively colonize the rhizosphere (7.45–6.61 Log_{10} CFU g^{-1} root dry mass) within the growth period of lettuce in the field. Our results demonstrated that FZB42 is able to effectively reduce the disease severity of bottom rot caused by soilborne pathogen *Rhizoctonia solani* on lettuce (Chowdhury et al. 2013).

From the practical point of view, it is interesting to note that the application mode of the biocontrol agent is a key factor for efficacy of FZB42. An effective suppression of *R. solani* was found only after two times application of FZB42 before and after transplanting. For the settlement of the inoculated strain in the rhizosphere in a sufficient high number, it might be important that the microflora in the rhizosphere of young plants is not yet stabilized (Berendsen et al. 2012).

As revealed by T-RFLP, application of FZB42, independent of its mode of application, did not shift the composition of rhizosphere bacterial community in a measurable extent – as also shown for *B. amyloliquefaciens* BNM122 on soybean (Correa et al. 2009). By contrast, inoculation with the pathogen did change the rhizosphere microbial community structure. In complementing that study, the effect of FZB42 and the pathogen *R. solani* on the microbial community of lettuce was more deeply analyzed by 454-amplicon sequencing focusing on presence of gamma-proteobacteria (Erlacher et al. 2014). Clear differences between plants infected by *R. solani* compared to non-inoculated healthy plants were found, corroborating the results obtained by T-RFLP. A significant increase in gamma-proteobacterial diver-

sity was detected in samples inoculated with the pathogen. However, together with FZB42, this increase was less distinct, suggesting a selective compensation of the impact of a pathogen on the indigenous plant-associated microbiome by FZB42. The number of DNA fragments corresponding to FZB42 in samples taken in vicinity of plant roots was steadily decreasing. After 5 weeks, still 55 % of the initial number of FZB42 DNA was traceable (Kröber et al. 2014).

8.3 Plant Growth Promotion

Although the ability of FZB42 to support growth of potatoes, maize, cotton, tobacco, leafy and fruiting vegetables, and ornamentals is well documented (Bochow et al. 2001; Yao et al. 2006; Guel et al. 2008; Burkett-Cadena et al. 2008; Chowdhury et al. 2013), the molecular reasons for the "biofertilizer" effect of beneficial plant-associated *Bacilli* are still not completely understood. However, we know that several factors are involved in the complex interplay between root-colonizing bacteria and plant:

8.3.1 Ability to Colonize and to Persist at Plant Roots

Ability to colonize and to persist at plant roots (see previous section). Their ability to suppress soilborne pathogens might positively affect the indigenous microbiome of the rhizosphere.

8.3.2 Stimulation of Plant Growth

Stimulation of plant growth by tryptophan-dependent synthesis of indole-3-acetic acid. Inactivation of genes involved in tryptophan biosynthesis and in a putative tryptophan-dependent IAA biosynthesis pathway led to reduction of both IAA concentration and plant growth-promoting activity in the respective mutant strains (Idris et al. 2007).

8.3.3 Volatiles

Volatiles, as 2,3-butanediol and 3-hydroxy-2-butanone (acetoin), released by *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a, were reported as enhancing plant growth (Ryu et al. 2003). To synthesize 2,3-butanediol, pyruvate is converted to acetolactate by the acetolactate synthase (AlsS), which is subsequently

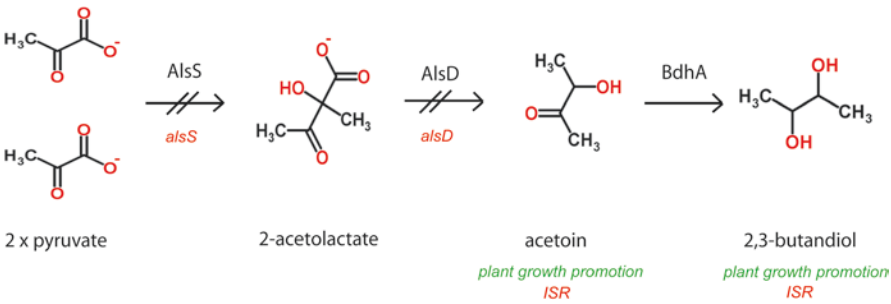

Fig. 8.1 Anaerobic and aerobic formation of 2,3-butanediol via acetoin involves acetolactate synthase and decarboxylase encoded by the *alsSD* operon. The *alsS* insertion mutation abolishes synthesis of 2,3-butandiol (Renna et al. 1993; Cruz-Ramos et al. 2000) (The figure is taken from Chowdhury et al. 2015b)

converted to acetoin by the acetolactate decarboxylase (AlsD) (Fig. 8.1). FZB42 mutant strains, deficient in synthesis of volatiles due to mutations interrupting the *alsD* and *alsS* genes, were found impaired in plant growth promotion (Borriss 2011).

8.3.4 Enhancement of Nutrient Availability

Enhancement of nutrient availability by phytase-producing bacteria. Soil phosphorous is an important macronutrient for plants. Improved phosphorous nutrition is achievable by "mobilization" of phosphorous fixed as insoluble organic phosphate in phytate (myo-inositol hexakisphosphate) by soil bacteria (Singh and Satyanarayana 2011). The extracellular 3(1)-phytase of the plant growth-promoting *B. amyloliquefaciens* FZB45 hydrolyzed phytate to D/L-Ins (1,2,4,5,6)P5 in vitro. A phytase-negative mutant strain, whose *phyA* gene was disrupted, did not stimulate plant growth under phosphate limitation (Idriss et al. 2002). Further experiments under field conditions revealed that FZB45 can only stimulate plant growth when phytate is present in soils, which are poor in soluble phosphate, suggesting that phytase acts only under certain conditions as plant growth stimulator (Ramirez and Kloepper 2010).

8.4 Biocontrol

Genome analysis revealed that nearly 10 % of the genome is devoted to synthesizing antimicrobial metabolites and their corresponding immunity genes (Chen et al. 2009b). FZB42 harbors 11 gene clusters involved in synthesis of antimicrobial compounds. Nine of them are involved in non-ribosomal synthesis of lipopeptides and

polyketides and two in conventional synthesis and modification of bacteriocin peptides. In addition, three further gene clusters contain genes mediating immunity against antimicrobial compounds produced by other related *Bacillus* strains (Table 8.2). This antibiotic arsenal makes *B. amyloliquefaciens* FZB42 and related *B. amyloliquefaciens plantarum* strains to an efficient microbial biopesticides, developed to control plant diseases (Borriss 2011).

For a long time, the plant protective activity of PGPR has been correlated with the potential to secrete a wide array of antibiotic compounds upon growth as planktonic cells in isolated cultures under laboratory conditions. We determined expression of the corresponding secondary metabolites by MALDI TOF mass spectrometry from FZB42 cultures grown in liquid Landy medium under laboratory conditions. Except the orphan *nrs* gene cluster, all expected bioactive compounds were synthesized in reasonable amounts, but the iron siderophore bacillibactin was detected only under iron-deprived conditions. In recent years, it became doubtful that synthesis of metabolites by the planktonic cells grown under laboratory conditions does correspond to their capability to produce those compounds also when grown in biofilm-related structures on the surface of plant tissues.

8.4.1 Lipopeptides, Bacillibactin, and Antifungal Activity

Five-gene cluster involved in non-ribosomal synthesis of cyclic lipopeptides and the iron siderophore bacillibactin were identified in the genome of FZB42 (Table 8.2). Three of the respective gene clusters were assigned for synthesis of surfactin, fengycin, and bacillomycin D. Bacillomycin D was identified as being the most powerful antifungal metabolite produced by FZB42 (Fig. 8.2). The heptapeptide moiety of bacillomycin D, belonging to the iturin family of cyclic lipopeptides (LP), is attached to a β-amino fatty acid chain of variable length (C_{14}–C_{17}). The peptide moiety of the heptapeptide surfactin is linked to a β-hydroxyl fatty acid (C_{12}–C_{16}), while the fengycin decapeptides are linked to a β-hydroxyl fatty acid chain (C_{14}–C_{18}). Their synthesis is performed by multimodular peptide synthetases and depends on a functional phospho-panthenyl transferase (Sfp) which transfers 4′-phosphopantheteine from coenzyme A to the carrier proteins during non-ribosomal synthesis.

Within the last few years, Ongena and coworkers performed pioneering work for elucidating antibiotic production *in planta* using matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI). They investigated antibiotic production in a gnotobiotic system in which the plantlet and the associated *B. amyloliquefaciens* S499, a close relative of FZB42, were growing on a gelified medium covering the MALDI target plate. Under these conditions, S499 grows as biofilm on the surface of the plant roots, allowing exact assays of secondary metabolites in the vicinity of root surface. Surfactins were detected in the root environment in much higher relative amounts, which are representing more than 90 % of the whole LP production, and their synthesis is rapidly progressing during early biofilm formation.

Table 8.2 Genes and gene cluster encoding for secondary metabolites and immunity against bacteriocin in FZB42. The table is taken from Chowdhury et al. (2015b) with modifications

Gene cluster	From	To	Size	Metabolite	Effect against	Reference
Sfp-dependent non-ribosomal synthesis of lipopeptides						
srfABCD, aat,334,ycx,CycxD,sfp,yczE	342,618	368,776	32.0 kb	Surfactin	Virus	Koumoutsi et al. (2004)
bmyCBAD	1,871,172	1,908,422	39.7 kb	Bacillomycin D	Fungi	Koumoutsi et al. (2004)
fenABCDE	1,931,328	1,968,997	38.2 kb	Fengycin	Fungi	Koumoutsi et al. (2004)
nrsABCDEF	2,868,410	2,885,927	15.0 kb	Orphan NRP1	Unknown, siderophore?	Chen et al. (2007)
dhbABCDEF	3,021,250	3,032,970	12.8 kb	Bacillibactin	Iron deficiency, siderophore	Chen et al. (2007)
Sfp-dependent non-ribosomal synthesis of polyketides						
mlnABCDEFGHI	1,391,841	1,445,094	53.9 kb	Macrolactin	Bacteria	Schneider et al. (2007)
baeBCDE,acpK,baeGHIJLMNRS	1,700,345	1,701,022	74.3 kb	Bacillaene	Bacteria	Chen et al. (2006)
dfnAYXBCDEFGHIJKLM	2,276,743	2,346,266	71.1 kb	Difficidin	Bacteria	Chen et al. (2006)
Sfp-independent non-ribosomal synthesis						
bacABCDE,ywfG	3,593,877	3,599,784	6.9 kb	Bacilysin	Bacteria	Chen et al. (2009a)
Ribosomal synthesis of modified peptides (bacteriocins)						
pznFKGHIAJCDBEL	726,469	736,360	9.96 kb	Plantazolicin	Gram-positive bacteria	Scholz et al. (2011)
acnBACDEF	3,044,506	3,048,678	4.49 kb	Amylocyclicin	Closely related bacteria	Scholz et al. (2014)
Immunity, but no synthesis genes						
mrsK2R2FGE	3,769,734	3,774,552	4.82 kb	Mersacidin	Resistance against Y2	He et al. (2012)
bceBASR	2,856,835	2,861,322	4.49 kb	Bacitracin	Resistance against *B. cereus*	Unpubl. results
spaKREF	3,210,423	3,214,712	4.29 kb	Subtilin	Resistance against *B. subtilis*	Unpubl. results

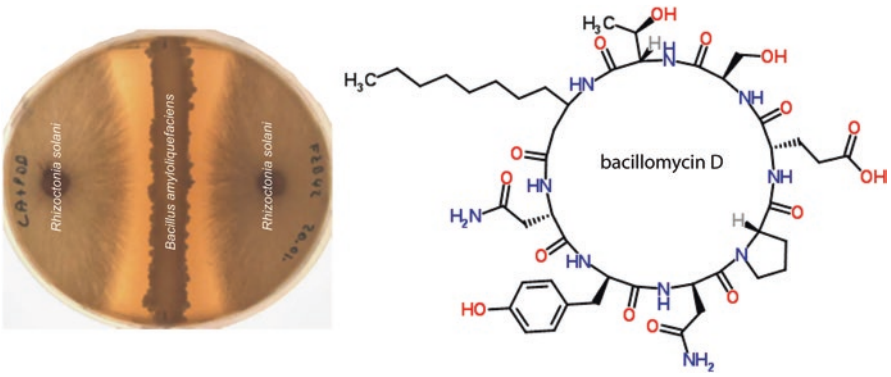

Fig. 8.2 Effect of FZB42 on *Rhizoctonia solani*. A clear inhibition zone indicating growth suppression of the fungal pathogen is visible on agar plates simultaneously inoculated with both microbes. Bacillomycin D was detected as the only prominent compound by MALDI TOF mass spectrometry of samples taken from the surface of the agar plate within the inhibition zone (The figure is taken from Chowdhury et al. 2015b with slight modifications)

By contrast, synthesis of iturin and fengycin was delayed until the end of the aggressive phase of colonization (Nihorimbere et al. 2012; Debois et al. 2014). Earlier experiments performed with FZB42 colonizing duckweed (*Lemna minor*) plantlets corroborated that surfactin is the most prominent compound which could be detected by MALDI TOF MS in the plant-bacteria system (Idris et al. 2007). Using a gnotobiotic quartz sand system consisting of lettuce plants, the beneficial bacterium FZB42, and the pathogen *R. solani*, it was demonstrated by using alternative techniques (e.g., Fourier transform ion cyclotron mass spectrometry) that lipopeptides were detectable in the order surfactin > bacillomycin D > fengycin at the plant-bacteria interface (Chowdhury et al. 2015a).

An early surfactin secretion could be of biological relevance since this lipopeptide, although less fungitoxic then iturins and fengycins, is essential for moving on tissues (Kinsinger et al. 2003) and for matrix formation in biofilms (Hofemeister et al. 2004; Lopez et al. 2009a, b). Considering the relative low amounts of the fungitoxic iturins and fengycins in vicinity of plant roots, it might be concluded that their biocontrol effect is possibly less important than expected. The same is true for the iron siderophore bacillibactin, which could not be detected under the conditions of the artificial plant-bacteria associations applied in these studies.

8.4.2 Polyketides, Bacilysin, and Bacteriocins Direct Antibacterial Activity

The polyketides, non-ribosomally synthesized by FZB42 (Chen et al. 2006; Schneider et al. 2007), have been extensively reviewed previously (Chen et al. 2009b, c; Borriss 2013). The three gene clusters encoding the modularly

organized polyketide synthases (PKS) for synthesis of bacillaene, macrolactin, and difficidin cover nearly 200 kb and are the largest ones, which are occurring in the FZB42 genome (Table 8.2). Difficidin is the most effective antibacterial compound produced by FZB42^T, but also macrolactin and bacillaene possess antibacterial activity. Difficidin is efficient in suppressing plant pathogenic bacterium *Erwinia amylovora*, which causes fire blight disease in orchard trees (Chen et al. 2009a).

Another product of non-ribosomal synthesis, the dipeptide bacilysin consisting of anticapsin and alanine moieties, was found as also being involved in suppression of *Erwinia amylovora*. By contrast to the lipopeptides and polyketides mentioned above, bacilysin synthesis is not dependent on the Sfp PP-transferase. A mutant strain CH3, with a disruption of the *sfp* gene and unable to produce any polyketide or lipopeptide, was still able to synthesize bacilysin and to suppress *E. amylovora* (Chen et al. 2009a). Recent experiments, performed by the group of Xuewen Gao, Nanjing Agriculture University, demonstrated that bacilysin, despite difficidin, is efficient in suppressing *Microcystis aeruginosa*, the main causative agent of cyanobacterial bloom in lakes and rivers (Wu et al. 2015a). However, corroborating these results in field trials has to be done. Until now, polyketides and bacilysin have not been detected in plants colonized by *B. amyloliquefaciens* (Debois et al. 2014).

Antimicrobial peptides, ribosomally synthesized as linear precursor peptides, remained unknown in *B. amyloliquefaciens plantarum* for a long time with one remarkable exception: mersacidin, a B-type lantibiotic, was detected in *Bacillus* sp HIL Y85 (Chatterjee et al. 1992). The strain HIL Y85 was later classified as being *B. amyloliquefaciens plantarum* (Herzner et al. 2011). Nowadays, mersacidin production was also detected in *B. amyloliquefaciens* B9601-Y2 (He et al. 2012). Genes involved in mersacidin self-protection reside also in the genome of FZB42. Transfer of mersacidin biosynthesis genes from HIL Y85 resulted in efficient mersacidin production by the surrogate strain constructed from the FZB42 host (Herzner et al. 2011).

Another representative of the type B lantibiotics, amylolysin from *B. amyloliquefaciens* GA1, was recently described. These lantibiotics are active on an array of Gram-positive bacteria, including *Listeria* spp. and methicillin-resistant *S. aureus* by interacting with the membrane lipid II (Arguelles Arias et al. 2013).

The driving force in our search for ribosomally synthesized peptides in FZB42 was the finding that the FZB42 mutant RS06, which is deficient in the Sfp-dependent synthesis of lipopeptides and polyketides and in the Sfp-independent bacilysin production (Chen et al. 2009a), still produced an antibacterial substance active against *Bacillus subtilis* HB0042. In fact, a metabolite (cpd1335) with a molecular mass of $[M+H]^+ = 1336$ Da was assigned by MALDI TOF MS in FZB42 and in RS06, as well. The compound was named plantazolicin, and the respective gene cluster pzn consisting of 12 genes was identified by cassette mutagenesis. Plantazolicin was characterized as a highly modified peptide undergoing several steps of modification

after synthesis. It ruled out that it is a thiazole/oxazole-modified microcin (TOMM) resembling microcin B17 and streptolysin S. Plantazolicin displayed antibacterial activity toward closely related Gram-positive bacteria. Due to its extensive degree of modification, Pzn is highly protected from premature degradation by peptidases within the plant rhizosphere (Scholz et al. 2011). Remarkably, human pathogen *Bacillus anthracis* was found sensitive against PZN and underwent massive lysis at 4 μg mL^{-1} (Molohon et al. 2011). The exact structures of plantazolicin A and B were elucidated, unveiling a hitherto unusual number of thiazoles and oxazoles formed from a linear 14mer precursor peptide (Kalyon et al. 2011).

By transposon mutagenesis of the FZB42 mutant strain RS06, we identified a hitherto unknown gene cluster involved in synthesis and posttranslational processing of a novel circular bacteriocin, named amylocyclicin (Fig. 8.3). It ruled out that amylocyclicin inhibits growth of bacterial strains closely related to FZB42 suggesting that this bacteriocin might have a function in competing with other *Bacillus* strains attracted to the plant rhizosphere (Scholz et al. 2014).

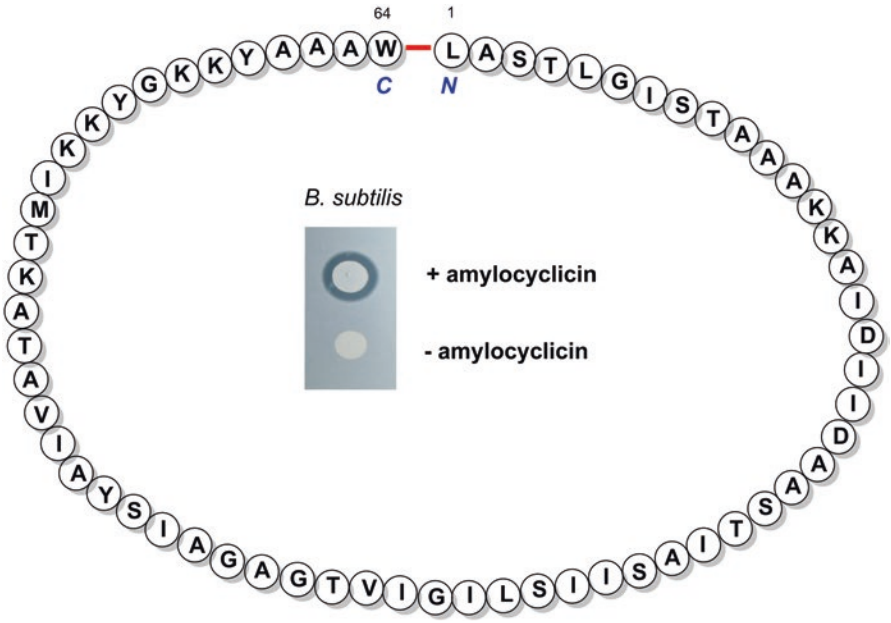

Fig. 8.3 The structure of the mature bacteriocin amylocyclicin bearing a head-to-tail cyclization of L_1 and W_{64}. Amylocyclicin effect on a related *B. subtilis* strain without immunity against the bacteriocin was demonstrated by a spot-on-lawn test performed with a amylocyclicin producing (*top*) and nonproducing strain (*bottom*) (The figure is taken from Chowdhury et al. 2015b)

8.4.2.1 Nematicidal Activity Is Directed by Plantazolicin

Parasitic nematodes of plants are important plant pathogens that represent a significant financial burden on agriculture. The annual losses in agriculture resulting from this pest amounted to $125 billion worldwide in past years (Sasser and Freckman 1987; Oka et al. 2000). Chemical insecticides of nonselective nature possessing rapid nematicidal effects are widely used as control measures against these pathogens. However, the potential negative impact on the environment and ineffectiveness after prolonged use have led to banning or restricting of the use of most chemical nematicides. Therefore, identification of safe and effective nematicides is urgently needed, and biocontrol measures have recently been given much attention as viable options (Xia et al. 2011). BioNem® prepared from *Bacillus firmus* GB-126 (Table 8.1) was proven for its efficiency in greenhouse and field trials. The numbers of nematode females, eggs, and vermiform life stages at the end of the growing season decreased in the presence of the biocontrol agent, and the cotton yields were similar to those from aldicarb, the chemical nematicide standard; however, the molecular reason for this effect remained unknown (Castillo et al. 2013).

FZB42 has been shown to reduce nematode eggs in roots, juvenile worms in soil, and plant galls on tomato (Burkett-Cadena et al. 2008). In order to identify specific nematicide-related genes, a random transposon insertion library of FZB42 was screened for relevant genes involved in nematicidal activity, and – surprisingly – a gene within the *pzn* gene cluster was identified as a pathogenic factor against nematodes. Further experiments revealed that PZN displayed a moderate nematicidal activity (Liu et al. 2013).

8.4.3 *Induced Systemic Resistance Is Triggered by Plant Growth-Promoting* Bacilli

Except surfactin, concentration of antifungal lipopeptides determined *in planta* was found relatively low. Moreover, antibacterial polyketides were not detected so far in vicinity of plant roots colonized by PGPR *Bacilli* (Debois et al. 2014). Therefore, it is tempting to speculate that induced systemic resistance (ISR) is a main factor for suppressing plant pathogens by PGPR *Bacilli*. ISR occurs when the plant's defense mechanisms are stimulated and primed to resist infection by pathogens (Van Loon 1997). This activation is distinct from systemic acquired resistance (SAR) in which the response is triggered by pathogenic microorganisms associated with the aerial portions of the plant. Selected *Bacillus* PGPR strains emit volatiles (VOCs) that can elicit plant defenses. Exposure to VOCs consisting of 2,3-butanediol and acetoin (3-hydroxy-2-butanone) from PGPR *Bacillus amyloliquefaciens* activates ISR in *Arabidopsis* seedlings (Ryu et al. 2004). *Arabidopsis thaliana* plants exposed to *Bacillus subtilis* strain FB17 result in reduced disease severity against *Pseudomonas syringae* compared to plants without FB17 treatment. Exogenous application of

acetoin triggers ISR and protects plants against the pathogen in the aerial parts, while 2,3-butanediol did not (Rudrappa et al. 2010). In this context, it is worth to mention that expression of AlsS of FZB42 involved in synthesis of acetoin (Fig. 8.1) was triggered in presence of maize root exudate (Kierul et al. unpublished), suggesting that root exudates play a role in eliciting of acetoin biosynthesis in FZB42. It is known that some of the plant metabolites present in root exudates, such as organic acids, trigger the *alsSD* operon (Rudrappa et al. 2010). *B. amyloliquefaciens* FZB24 and FZB42 applied to tobacco roots led to a reduction of tobacco mosaic virus symptoms visible on tobacco leaves and to decreasing amounts of virus proteins present in leaf tissues. Due to spatial distance between beneficial bacterium and the pathogen, plant ISR, stimulated by the rhizobacterium, might be responsible for this effect (Wang et al. 2009).

The induction of ISR when treated with PGPRs is mediated primarily through plant signaling molecules such as jasmonic acid (JA), a lipoxygenase pathway product, and ethylene (ET). Salicylic acid (SA) appears to be a critical plant messenger of pathogen exposure and disease resistance in systemic acquired resistance (SAR) (Durner et al. 1997). ISR restricts pathogen multiplication and disease progression through a SA/ET and NPR1-dependent mechanism. In order to determine the signaling pathways triggered by FZB42, the expression of several marker genes in lettuce plants, exposed to FZB42 and the pathogenic fungus *Rhizoctonia solani*, was analyzed by quantitative real time (RT)-PCR (Chowdhury et al. 2015a). In absence of the pathogen, FZB42 increased expression of PR1 (pathogenesis protein 1, SA marker gene) and PDF1.2 (defensin, JA/ET marker gene), suggesting that SA and ET pathways are involved in upregulating defense response by ISR in lettuce. A similar result was obtained previously, when *Arabidopsis* plantlets were inoculated with *Bacillus subtilis* FB17 and acetoin (Rudrappa et al. 2010). In simultaneous presence of FZB42 and the pathogen *R. solani*, PDF1.2 expression was dramatically enhanced, suggesting a synergistic activation of the JA/ET pathway, while the SA pathway – as indicated by a decreased expression of PR-1 – was suppressed in presence of both antagonists.

It was found that the circular lipopeptides surfactin and fengycin can act as elicitors of host plant immunity and contribute to increased resistance toward further pathogenesis ingress in bean and tomato plants (Raaijmakers et al. 2010). In bean, purified fengycins and surfactins provided a significant ISR-mediated protective effect on bean plants against the fungal pathogen *Botrytis cinerea*, similar to the one induced by living cells of the producing strain *B. amyloliquefaciens* S499 (Ongena et al. 2007).

We found (Chowdhury et al. 2015a) that the dramatic increase of the defensin 1.2 gene (PDF1.2) expression in simultaneous presence of both antagonists occurred only when wild-type cells of FZB42 were applied. Mutant strains deficient in synthesis of surfactin, fengycin, or acetoin did not stimulate expression of the JA/ET pathway, suggesting that cyclic lipopeptides and acetoin contribute together to the ISR plant response triggered by FZB42.

8.5 Conclusion

An increasing amount of data has been accumulated in course of the last years, suggesting that the antibiome expressed during the plant-associated state of PGPR *Bacilli* does not necessarily reflect the vast genetic arsenal devoted to the formation of lipopeptides, polyketides, and bacteriocins, which has been elucidated, for example, in the *B. amyloliquefaciens plantarum* FZB42 genome. Obviously, there is a large discrepancy in gene expression of the planktonic cells growing in liquid laboratory cultures and cells growing as biofilms on plant tissue surfaces. Except cyclic lipopeptides, no other bioactive compounds such as polyketides were detected in samples taken from the vicinity of plant roots colonized by PGPR *B. amyloliquefaciens* (Debois et al. 2014). Interestingly, surfactin has multiple biological functions in motility, biofilm formation, and cell to cell signaling, but is less efficient in direct suppressing of other competing microbes than other lipopeptides or polyketides, and was by far the most prominent compound occurring in the plant rhizosphere, previously being inoculated by PGPR *B. amyloliquefaciens*. For this reason, I conclude that the direct effects exerted by the array of secondary metabolites encoded by the *Bacillus* genome might not be as important and that the biocontrol effects exerted by that Gram-positive bacteria are mainly due to other more indirect effects. I assume that under field conditions, the stimulating effects on plant ISR are more important than direct biocontrol by secreted secondary metabolites. In case of *Bacilli*, it is very likely that ISR stimulation is a multifactorial process dependent on several compounds produced by the rhizobacteria. Candidate compounds are surfactin, and volatiles, especially acetoin and 2,3-butanediol (Fig. 8.4), since mutants of FZB42, deficient in synthesis of these compounds, were found unable to protect plants from pathogens. Moreover, high expression of defensin, indicating the JA/ET pathway in ISR, was not found when the mutant strains were applied to the plant.

These findings are important for future strategies for screening of powerful PGPR and BC strains. It is known for long time that high efficiency in suppressing fungal or bacterial pathogens do not necessarily reflect the potential of these selected strains for their performance under field conditions. Novel screening procedures have to be developed for functional tests under more appropriate conditions, either directly on plants or at least under conditions allowing biofilm formation on artificial surfaces. However, performance under field conditions remains the most important criterion.

Taken together, the beneficial effect of *Bacillus* PGPR depends – besides their rhizosphere competence – on at least three main factors:

1. Stimulation of plant ISR by bacterial metabolites produced in vicinity of plant roots. Volatiles, such as acetoin and 2,3-butanediol, contribute not only to ISR but have a direct plant growth-promoting effect, while surfactin is important in the early stage of colonization and biofilm formation. In addition, surfactin strengthens the plant ISR response, which suppresses growth of fungal, bacterial, viral, and other plant pathogens.

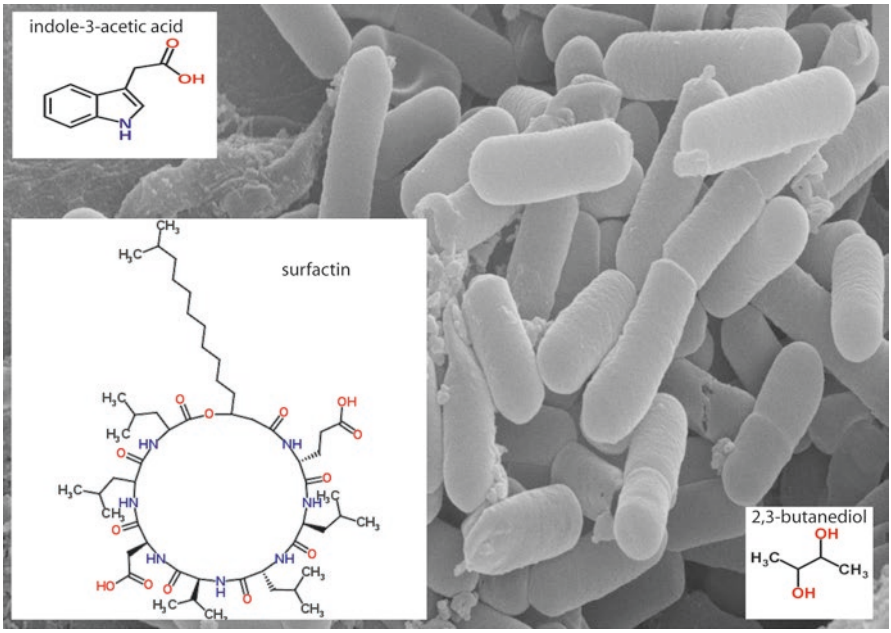

Fig. 8.4 Scanning electron microscopy of FZB42 cells colonizing *Arabidopsis thaliana* roots. Important compounds as surfactin, indole-3-acetic acid, and 2,3-butanediol, which are formed when growing on root surfaces (*in planta*), are indicated

2. Direct antifungal action by secondary metabolites, such as iturins (e.g., bacillomycin D) and fengycins, secreted into the rhizosphere. However, the suppressing effect exerted by such compounds might be relatively weak, since the amount of such compounds in vicinity of plant root was found relatively low. Until now, antibacterial compounds, such as polyketides, were not detected in this environment.
3. Application of PGPR *Bacilli*, as FZB42, might compensate, at least in part, undesired changes in the composition of the plant microbiome, caused by the presence of pathogens, as *R. solani*.

Without doubt, other features of PGPR, as production of plant hormones, and making available fixed macro- and micronutrients for plant nutrition, contribute also to the beneficial effect exerted by these microbes, but could not be appropriately treated in this review due to space limitation.

Acknowledgments Many of the recent data, reported in this review, have been obtained in close collaboration with the Helmholtz Center in Munich in frame of the PATHCONTROL project and the laboratory of Yuewen Gao, Nanjing Agricultural University, China, in frame of a Chinese Collaborative project, financially supported by the BMBF, the German Ministry of Education and Research. I thank especially Soumitra Paul Chowdhury, Anton Hartmann, Joachim Vater, Liming Wu, Xuewen Gao, and Ben Fan (Nanjing Forestry University) for fruitful collaboration during the last years.

References

Arguelles Arias, A., Ongena, M., Devreese, B., Terrak, M., Joris, B., & Fickers, P. (2013). Characterization of amylolysin, a novel lantibiotic from *Bacillus amyloliquefaciens* GA1. *PLoS ONE, 8*(12), e83037. doi:10.1371/journal.pone.0083037. 9.

Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science, 17*, 478–486.

Bochow, H., El Sayed, S. F., Junge, H., Stavropoulos, A., & Schmiedeknecht, G. (2001). Use of *Bacillus subtilis* as biocontrol agent. IV. Salt-stress tolerance induction by *Bacillus subtilis* FZB24 seed treatment in tropical field crops, and its mode of action. *Journal Plant Disease Protection, 108*, 21–30 (in German).

Borriss, R. (2011). Use of plant-associated *Bacillus* strains as biofertilizers and biocontrol agents. In D. K. Maheshwari (Ed.), *Bacteria in agrobiology: Plant growth responses* (pp. 41–76). Heidelberg: Springer.

Borriss, R. (2013). Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium Bacillus amyloliquefaciens FZB42 p. In F. J. de Bruijn (Ed.), *Molecular microbial ecology of the rhizosphere* (pp. 883–898). New Jersey: Wiley Blackwell Hoboken.

Borriss, R. (2015a). Transcriptome and proteome profiling for analyzing fates of global gene expression in plant-beneficial Bacilli. *Transcriptomics, 3*, e110. doi:10.4172/2329-8936.1000e110.

Borriss, R. (2015b). Bacillus, a plant beneficial bacterium. In B. Lugtenberg (Ed.), *Principles of plant-microbe interactions*. Cham: Springer.

Borriss, R., Chen, X. H., Rueckert, C., Blom, J., Becker, A., Baumgarth, B., Fan, B., Pukall, R., Schumann, P., Sproer, C., Junge, H., Vater, J., Pühler, A., & Klenk, H. P. (2011). Relationship of *Bacillus amyloliquefaciens* clades associated with strains DSM 7^{T} and *Bacillus amyloliquefaciens* subsp. *plantarum* subsp. nov. based on their discriminating complete genome sequences. *International Journal of Systematic and Evolutionary Microbiology, 61*, 1786–1801.

Burkett-Cadena, M., Kokalis-Burelle, N., Lawrence, K. S., van Santen, E., & Kloepper, J. W. (2008). Suppressiveness of root-knot nematodes mediated by rhizobacteria. *Biological Control, 47*, 55–59.

Castillo, J. D., Lawrence, K. S., & Kloepper, J. W. (2013). Biocontrol of the reniform nematode by *Bacillus firmus* GB-126 and *Paecilomyces lilacinus* 251 on cotton. *Plant Disease, 97*, 967–976.

Chatterjee, S., Chatterjee, D. K., Lad, S. J., Phansalkar, M. S., Rupp, R. H., Ganguli, B. N., Fehlhaber, H. W., & Kogler, H. (1992). Mersacidin, a new antibiotic from *Bacillus*: Fermentation, isolation, purification and chemical characterization. *Journal of Antibiotics, 45*, 832–838.

Chen, X. H., Vater, J., Piel, J., Franke, P., Scholz, R., Schneider, K., Koumoutsi, A., Hitzeroth, G., Grammel, N., Strittmatter, A. W., Gottschalk, G., Süssmuth, R. D., & Borriss, R. (2006). Structural and functional characterization of three polyketide synthase gene clusters in *Bacillus amyloliquefaciens* FZB 42. *Journal of Bacteriology, 188*, 4024–4036.

Chen, X. H., Koumoutsi, A., Scholz, R., Eisenreich, A., Schneider, K., Heinemeyer, I., Morgenstern, B., Voss, B., Hess, W. R., Reva, O., Junge, H., Voigt, B., Jungblut, P. R., Vater, J., Süssmuth, R., Liesegang, H., Strittmatter, A., Gottschalk, G., & Borriss, R. (2007). Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Nature Biotechnology, 25*, 1007–1014.

Chen, X. H., Scholz, R., Borriss, M., Junge, H., Mögel, G., Kunz, S., & Borriss, R. (2009a). Difficidin and bacilysin produced by plant-associated *Bacillus amyloliquefaciens* are efficient in controlling fire blight disease. *Journal of Biotechnology, 140*, 38–44.

Chen, X. H., Koumoutsi, A., Scholz, R., & Borriss, R. (2009b). More than anticipated – production of antibiotics and other secondary metabolites by *Bacillus amyloliquefaciens* FZB42. *Journal of Molecular Microbiology and Biotechnology, 16*, 14–24.

Chen, X. H., Koumoutsi, A., Scholz, R., Schneider, K., Vater, J., Suessmuth, R., Piel, J., & Borriss, R. (2009c). Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens. *Journal of Biotechnology, 140*, 27–37.

Chowdhury, S. P., Dietel, K., Rändler, M., Schmid, M., Junge, H., Borriss, R., Hartmann, A., & Grosch, R. (2013). Effects of *Bacillus amyloliquefaciens* FZB42 on lettuce growth and health under pathogen pressure and its impact on the rhizosphere bacterial community. *PLoS ONE, 8*(7), e68818. doi: 10.1371.

Chowdhury, S. P., Uhl, J., Grosch, R., Alquéres, S., Pittroff, S., Dietel, K., Schmitt-Kopplin, P., Borriss, R., & Hartmann, A. (2015a). Cyclic lipopeptides of *Bacillus amyloliquefaciens* subsp. *plantarum* colonizing the lettuce rhizosphere enhance plant defense responses toward the bottom rot pathogen *Rhizoctonia solani. Molecular Plant-Microbe Interactions, 28*(9), 984–995. doi:10.1094/MPMI-03-15-0066-R.

Chowdhury, S. P., Gao, X., Hartmann, A., & Borriss, R. (2015b). Biocontrol by root-associated Bacillus amyloliquefaciens FZB42 – review. *Frontiers in Microbiology, 6*, 780. doi:10.3389/fmicb.2015.00780.

Correa, O. S., Montecchia, M. S., Berti, M. F., Ferrari, M. C. F., Pucheu, N. L., et al. (2009). *Bacillus amyloliquefaciens* BNM122, a potential microbial biocontrol agent applied on soybean seeds, causes a minor impact on rhizosphere and soil microbial communities. *Applied Soil Ecology, 41*, 185–194.

Cruz Ramos, H., Hoffmann, T., Marino, M., Nedjari, H., Presecan-Siedel, E., Dreesen, O., Glaser, P., & Jahn, D. (2000). Fermentative metabolism of Bacillus subtilis: Physiology and regulation of gene expression. *Journal of Bacteriology, 182*, 3072–3080.

Debois, D., Jourdan, E., Smargiasso, N., Thonart, P., de Pauw, E., & Ongena, M. (2014). Spatiotemporal monitoring of the antibiome secreted by *Bacillus* biofilms on plant roots using MALDI mass spectrometry imaging. *Analytical Chemistry*. doi:10.1021/ac500290s.

Dietel, K., Beator, B., Budiharjo, A., Fan, B., & Borriss, R. (2013). Bacterial traits involved in colonization of *Arabidopsis thaliana* roots by *Bacillus amyloliquefaciens* FZB42. *Plant Pathology Journal, 29*, 59–66.

Doornbos, R. F., van Loon, L. C., & Bakker, P. A. (2012). Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. *Agronomy for Sustainable Development, 32*, 227–243.

Durner, J., Shah, J., & Klessig, D. F. (1997). Salicylic acid and disease resistance in plants. *Trends in Plant Science, 2*, 266–274.

Erlacher, A., Cardinale, M., Grosch, R., Grube, M., & Berg, G. (2014). The impact of the pathogen *Rhizoctonia solani* and its beneficial counterpart *Bacillus amyloliquefaciens* on the indigenous lettuce microbiome. *Frontiers in Microbiology*. doi:10.3389/fmicb.2014.00175. publ ahead of print.

Fan, B., Chen, X. H., Budiharjo, A., Bleiss, W., Vater, J., & Borriss, R. (2011). Efficient colonization of plant roots by the plant growth promoting bacterium *Bacillus amyloliquefaciens* FZB42, engineered to express green fluorescent protein. *Journal of Biotechnology, 151*, 303–311.

Fan, B., Carvalhais, L. C., Becker, A., Fedoseyenko, D., von Wirén, N., & Borriss, R. (2012a). Transcriptomic profiling of Bacillus amyloliquefaciens FZB42 in response to maize root exudates. *BMC Microbiology, 12*, 116. doi:10.1186/1471-2180-12-116.

Fan, B., Borriss, R., Bleiss, W., & Wu, X. Q. (2012b). Gram-positive rhizobacterium Bacillus amyloliquefaciens FZB42 colonizes three types of plants in different patterns. *Journal of Microbiology, 50*, 38–44.

Fan, B., Li, L., Chao, Y., Förstner, K., Vogel, J., Borriss, R., & Wu, X. Q. (2015). dRNA-Seq reveals genomewide TSSs and noncoding RNAs of plant beneficial rhizobacterium *Bacillus amyloliquefaciens* FZB42. *PLoS ONE, 10*(11), e0142002. doi:10.1371/journal.pone.0142002.

Grosch, R., Junge, H., Krebs, B., & Bochow, H. (1999). Use of *Bacillus subtilis* as a biocontrol agent. III. Influence of *Bacillus subtilis* on fungal root diseases and yield in soilless culture. *Journal Plant Disease Protection, 106*, 568–580.

Guel, A., Kidoglu, F., Tuzel, Y., & Tuzel, I. H. (2008). Effects of nutrition and Bacillus amyloliquefaciens on tomato (Solarium lycopersicum L.) growing in perlite. *Spanish Agriculture Journal Research, 6*, 422–429.

He, P., Hao, K., Blom, J., Rückert, C., Vater, J., Mao, Z., Wu, Y., Hou, M., He, P., He, Y., & Borriss, R. (2012). Genome sequence of the plant growth promoting strain *Bacillus amyloliquefaciens* subsp. *plantarum* B9601-Y2 and expression of mersacidin and other secondary metabolites. *Journal of Biotechnology, 15*, 281–291.

Herzner, A. M., Dischinger, J., Szekat, C., Josten, M., Schmitz, S., Yakéléba, A., Reinartz, R., Jansen, A., Sahl, H. G., Piel, J., & Bierbaum, G. (2011). Expression of the lantibiotic mersacidin in *Bacillus amyloliquefaciens* FZB42. *PLoS ONE, 6*(7), e22389. doi:10.1371/journal.pone.0022389.

Hofemeister, J., Conrad, B., Adler, B., Hofemeister, B., Feesche, J., Kucheryava, N., Steinborn, G., Franke, P., Grammel, N., Zwintscher, A., Leenders, F., Hitzeroth, G., & Vater. (2004). Genetic analysis of the biosynthesis of non-ribosomal peptide- and polyketide like antibiotics, iron uptake and biofilm formation by Bacillus subtilis A1/3. *Molecular Genetics and Genomics, 272*, 363–378.

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

Idriss, E. E. S., Makarewicz, O., Farouk, A., Rosner, K., Greiner, R., Bochow, H., Richter, T., & Borriss, R. (2002). Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB 45 contributes to its plant growth-promoting effect. *Microbiology, 148*, 2097–2109.

Idriss, E. E., Bochow, H., Ross, H., & Borriss, R. (2004). Use of *Bacillus subtilis* and *Bacillus subtilis* FZB37 as biocontrol agent. VI. Phytohormone-like action of culture filtrates prepared from plant growth-promoting *Bacillus amyloliquefaciens* FZB24, FZB42, FZB45. *Journal Plant Disease Protection, 111*, 583–597.

Kalyon, B., Helaly, S. E., Scholz, R., Nachtigall, J., Vater, J., Borriss, R., & Süssmuth, R. D. (2011). Plantazolicin A and B: Structure of ribosomally synthesized thiazole/oxazole peptides from *Bacillus amyloliquefaciens* FZB42. *Organic Letters, 13*, 2996–2999.

Kierul, K., Voigt, B., Albrecht, D., Chen, X. H., Carvalhais, L. C., & Borriss, R. (2015). Influence of root exudates on the extracellular proteome of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Microbiology, 161*(Pt 1), 131–147. doi:10.1099/mic.0.083576-0.

Kinsinger, R. F., Shirk, M. C., & Fall, R. (2003). Rapid surface motility in *Bacillus subtilis* is dependent on extracellular surfactin and potassium ion. *Journal of Bacteriology, 185*(18), 5627–5631.

Kloepper, J. W., Leong, J., Teintze, M., & Schroth, M. (1980). Enhancing plant growth by siderophores produces by plant-growth-promoting rhizobacteria. *Nature, 286*, 885–886.

Koumoutsi, A., Chen, X. H., Henne, A., Liesegang, H., Hitzeroth, G., Franke, P., Vater, J., & Borriss, R. (2004). Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in *Bacillus amyloliquefaciens* strain FZB42. *Journal of Bacteriology, 186*, 1084–1096.

Kröber, M., Wibberg, D., Grosch, R., Eikmeyer, F., Verwaaijen, B., Chowdhury S. P., et al. (2014). Effect of the strain Bacillus amyloliquefaciens FZB42 on the microbial community in the rhizosphere of lettuce under field conditions analyzed by whole metagenome sequencing. *Frontiers in Microbiology, 5*, 636. doi:103389/fmicb.2014.00636.

Liu, Z., Budiharjo, A., Wang, P., Shi, H., Fang, J., Borriss, R., et al. (2013). The highly modified microcin peptide plantazolicin is associated with nematicidal activity of Bacillus amyloliquefaciens FZB42. *Applied Microbiology and Biotechnology, 97*, 10081–10090.

Lopez, D., Fischbach, M. A., Chu, F., Losick, R., & Kolter, R. (2009a). Structurally diverse natural products that cause leakage trigger multicellularity in Bacillus subtilis. *Proceedings National Science USA, 106*, 280–285.

Lopez, D., Vlamakis, H., Losick, R., & Kolter, R. (2009b). Cannibalism enhances biofilm development in Bacillus subtilis. *Molecular Microbiology, 74*, 609–618.

Lugtenberg, B. J. J., Malfanova, N., Kamilova, F., & Berg, G. (2013). Plant growth promotion by microbes. In F. J. de Brujn (Ed.), *Molecular microbial ecology of the rhizosphere* (Vol. 2, pp. 561–573). Hoboken: Wiley Blackwell.

Molohon, K. J., Melby, J. O., Lee, J., Evans, B. S., Dunbar, K. L., Bumpus, S. B., Kelleher, N. L., & Mitchell, D. A. (2011). Structure determination and interception of biosynthetic intermediates for the plantazolicin class of highly discriminating antibiotics. *ACS Chemical Biology, 6*(12), 1307–1313.

Nihorimbere, V., Cawoy, H., Seyer, A., Brunelle, A., Thonart, P., & Ongena, M. (2012). Impact of rhizosphere factors on cyclic lipopeptide signature from the plant beneficial strain amyloliquefaciens S499. *FEMS Microbiology Ecology, 79*, 176–191.

Oka, Y., Koltai, H., Bar-Eyal, M., Mor, M., Sharon, E., Chet, I., & Spiegel, Y. (2000). New strategies for the control of plant-parasitic nematodes. *Pest Management Science, 56*, 983–988.

Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., Arpigny, J. L., & Thonart, P. (2007). Surfactin and fengycin lipopeptdes of *Bacilllus subtilis* as elicitors of induced systemic resistance in plants. *Environmental Microbiology, 9*, 1084–1090.

Raaijmakers, J., De Bruin, I., Nybroe, O., & Ongena, M. (2010). Natural functions of cyclic lipopeptides from *Bacillus* and *Pseudomonas*: More than surfactants and antibiotics. *FEMS Microbiology Reviews, 34*, 1037–1062.

Ramírez, C. A., & Kloepper, J. W. (2010). Plant growth promotion by Bacillus amyloliquefaciens FZB45 depends on inoculum rate and P-related soil properties. *Biology and Fertility of Soils, 46*, 835–844. doi:10.1186/1475-2859-8-63.

Renna, M. C., Najimudin, N., Winik, L. R., & Zahler, S. A. (1993). Regulation of the *Bacillus subtilis alsS*, *alsD*, and *alsR* genes involved in post-exponential phase production of acetoin. *Journal of Bacteriology, 175*, 3863–3875.

Rudrappa, T., Biedrzycki, M. L., Kunjeti, S. G., Donofrio, N. M., Czymmek, K. J., Paré, P. W., & Bais, H. P. (2010). The rhizobacterial elicitor acetoin induces systemic resistance in *Arabidopsis thaliana. Communicative & Integrative Biology, 3*, 130–138.

Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M., Wei, H. X., Pare, P. W., & Kloepper, J. (2003). Bacterial volatiles promote growth in *Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America, 100*, 4927–4932.

Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Kloepper, J. W., & Paré, P. W. (2004). Bacterial volatiles induce systemic resistance in Arabidopsis. *Plant Physiology, 134*, 1017–1026.

Sasser, J. N., & Freckman, D. W. (1987). *A world perspective on nematology: The role of the society*. Hyattsville: Society of Nematologists.

Schneider, K., Chen, X. H., Vater, J., Franke, P., Nicholson, G., Borriss, R., & Suessmuth, R. D. (2007). Macrolactin is the polyketide biosynthesis product of the *pks2* cluster of *Bacillus amyloliquefaciens* FZB42. *Journal of Natural Products, 70*, 1417–1423.

Scholz, R., Molohon, K. J., Nachtigall, J., Vater, J., Markley, A. L., Süssmuth, R. D., Mitchell, D. A., & Borriss, R. (2011). Plantazolicin, a novel microcin B17/streptolysin S-like natural product from *Bacillus amyloliquefaciens* FZB42. *Journal of Bacteriology, 193*, 215–224.

Scholz, R., Vater, J., Budiharjo, A., Wang, Z., He, Y., Dietel, K., Schwecke, T., Herfort, S., Lasch, P., & Borriss, R. (2014). Amylocyclicin, a novel circular bacteriocin produced by *Bacillus amyloliquefaciens* FZB42. *Journal of Bacteriology, 196*, 1842–1852.

Singh, B., & Satyanarayana, T. (2011). Microbial phytases in phosphorous acquisition and plant growth promotion. *Physiology Molecular Biology Plants, 17*, 93–103.

Van Loon, L. C. (1997). Induced resistance in plants and the role of pathogenesis-related proteins. *European Journal of Plant Pathology, 103*, 753–765.

Wang, S., Wu, H., Qiao, J., Ma, L., Liu, J., Xia, Y., & Gao, X. (2009). Molecular mechanism of plant growth promotion and induced systemic resistance to tobacco mosaic virus by Bacillus spp. *Journal of Microbiology and Biotechnology, 19*(10), 1250–1258.

Wu, L., Wu, H., Chen, L., Yu, X. F., Borriss, R., & Gao, X. (2015a). Difficidin and bacilysin *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against *Xanthomonas oryzae* from rice pathogens. *Scientific Reports, 5*, 12975. doi:10.1038/srep12975.

Wu, L., Wu, H. J., Qiao, J., Gao, X., & Borriss, R. (2015b). Novel routes for improving biocontrol activity of Bacillus based bioinoculants. *Frontiers in Microbiology, 6*, 1395. doi:10.3389/fmicb.2015.01395.

Xia, Y., Xie, S., Ma, X., Wu, H., Wang, X., & Gao, X. (2011). The *purL* gene of *Bacillus subtilis* is associated with nematicidal activity. *FEMS Microbiology Letters, 322*, 99–107.

Yao, A. V., Bochow, H., Karimov, S., Boturov, U., Sanginboy, S., et al. (2006). Effect of FZB24 *Bacillus subtilis* as a biofertilizer on cotton yields in field tests. *Archives Phytopathology Plant Protection, 39*, 1–6.