Plant growth-promoting activities and genomic analysis of the stress-resistant *Bacillus megaterium* STB1, a bacterium of agricultural and biotechnological interest

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ABSTRACT

In this work, the stress-resistant *Bacillus megaterium* STB1 is characterized and its ability to promote plant growth under normal and stress conditions is demonstrated. The genomic sequence of this bacterium, and a detailed analysis of the genes involved in facilitating its stress resistance and plant growth-promoting activities is also reported.

The *B. megaterium* STB1 genome is rich in genetic elements involved in multiple stress resistance, xenobiotic degradation, pathogen antagonistic activities, and other traits related to soil and rhizosphere colonization. Moreover, genes participating in the biosynthesis of auxins and cytokinins, the modulation of polyamines, GABA, brassinosteroids and ethylene levels were also found.

Ultimately, this study brings new insights into the role of *B. megaterium* as a plant growth-promoting bacterium and opens new opportunities for the development of novel strategies for agriculture and biotechnology.

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1. Introduction

The increasing demand for food production and environmental clean-up solutions brings new challenges in agricultural and environmental biotechnology, amongst them, the recovery of degraded soils and their conversion to arable lands (bioremediation), as well as the development of sustainable agricultural products to replace the use of chemical fertilizers and pesticides that lead to unacceptable pollution levels worldwide. Beneficial soil and plant-associated bacteria can promote plant growth and stress resistance, hence, constituting a sustainable alternative to the excessive use of chemicals in agriculture [1]. These plant-growth-promoting bacteria (PGPB) can bind to or live inside of plant tissues and facilitate plant growth and protection through a variety of mechanisms, including nitrogen fixation, phosphate solubilization, modulation of plant hormone levels, production of antimicrobial compounds, among others [2].

Bacteria such as *Bacillus* spp., including *Bacillus megaterium*, are commonly found in soils and are members of the microbiome of several plant hosts worldwide [3], acting mostly as PGPB [4,5]. These strains effectively colonize soils and plant tissues, and, produce a wide range of bioactive compounds that are involved in plant-growth promotion [6,7] and antiphytopathogen activities [8]. *Bacillus* species are also a source for a wide range of metabolites and enzymes of biotechnological and industrial interest [9]. Nevertheless, one of the most important characteristics of *Bacillus* strains is the ability to form spores, thereby, increasing their capability to resist to a wide range of stress conditions [10] and enable their application as PGPB inoculants for agriculture and bioremediation processes [11]. Thus, obtaining spore-forming and stress-resistant *Bacillus* strains with a high level of plant-growth-promoting abilities is important for the development of novel products to be applied in agricultural/bioremediation setups, especially those subjected to stressful conditions (e.g. high salinity).

Previous studies conducted by our group have led to the isolation of several spore-forming *Bacillus* spp. strains from stressful environments, amongst them, *Bacillus megaterium* strain STB1 that was isolated from a rhizospheric soil obtained from a contaminated estuarine environment in Portugal. This strain resisted increased salinity and heavy metal concentrations and had the ability to promote the germination and early growth of several plants. Here, we report a detailed biochemical and genomic

characterization of strain *B. megaterium* STB1, as well as it plant growth-promoting activities. The obtained results bring new insights into *B. megaterium* genomics and the factors involved in its ability to interact with a host plant and promote its growth.

2. Materials and methods

2.1. Bacillus megaterium *STB1 characterization*

B. megaterium STB1 was isolated from an estuarine soil collected from the Mitrena area (Setúbal, Portugal) (38°30′24″N 8°48′47″W) which contains industries producing paper and chemical fertilizers, among other pollutants, which over time has resulted in the contamination of the surface waters and soils of this area. The bacterium was maintained in 20 % glycerol stocks at -80 °C and cultured in Tryptic Soy Agar (TSA) and Broth (TSB), at 28 °C with 150 rpm shaking, whenever necessary.

The strain was tested for the ability to produce indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, protease, lipase, esterase, amylase, cellulase, catalase, siderophores, ammonia, acetoin, H_2S, nitrate/nitrite reduction, motility and to solubilize phosphate as previously described [12]. Phytohormone degradation abilities were tested according to the methodology described by Nascimento et al. [13].

2.2. Tomato plant-growth promoting assay under normal and salt stress conditions

Commercial tomato (*Solanum lycopersicum* cv. super marmande) seeds were disinfected using 70 % ethanol (1 min), 1 % sodium hypochlorite solution (3 min) and five washes with sterile distilled water to remove any traces of the former solutions. Seeds were pre-germinated on 1 % agar plates at 25 °C. After germination, one seedling was placed in each pot (volume of 300 mL) containing a non-sterilized red clay soil (pH in H_2O – 5.3; organic matter 15.62 %; P and K – 0.49 mg.kg and 0.13 cmolc.kg respectively; Ca, Mg, Al and H^+Al,– 0.74, 0.43, 1.00, and 1.36 cmolc.kg, respectively), obtained from Florianópolis, Brazil. The seedlings were inoculated with 2.5 ml of a STB1 bacterial inoculum (OD_{600} = 0.5) obtained following the growth of strain STB1 in TSB medium at 28°ªC for 72 h with shaking at 150 rpm. The non-inoculated controls received 2.5 ml of sterile TSB medium. The experiment consisted on four treatments: normal conditions, non-inoculated control; normal conditions, inoculation with STB1; salt stress conditions, non-inoculated control; salt stress conditions inoculation with STB1. The study followed a completely randomized design with 10 replications. All pots received Hoagland and Arnon [14] nutrient solution (containing 200 mM NaCl, in the case of stress treatments) every three days. The experiment was conducted under greenhouse conditions (average temperature of 23.3 °C) in the Universidade Federal de Santa Catarina, Florianópolis, Brazil. The plants were harvested 35 days after inoculation, roots and shoots were excised from one another and dried at 60 °C for 3 days, before dry weights were measured on an analytical scale.

Statistical analysis was conducted with SPSS Statistics v.22 software (SPSS Inc., IBM Company, USA), using ANOVA and the means were compared using Tukey's test.

2.3. Genome sequencing and analysis

Strain STB1 genome sequencing was conducted following the methodology described by Urón et al. [15]. Briefly, the genomic
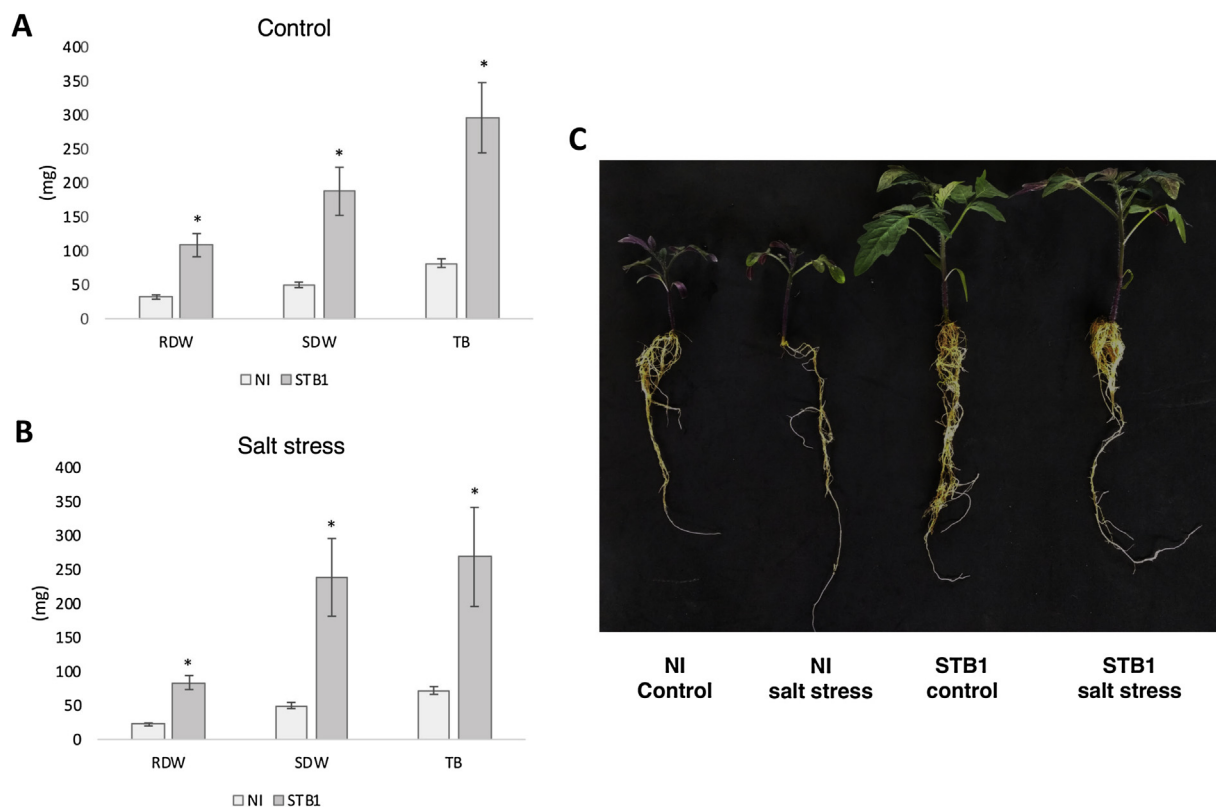

Fig. 1. Results obtained from the tomato plant growth promotion assay under normal and salt stress conditions (200 mM NaCl), 35 days after inoculation. A) control conditions; B) salt stress conditions; C) representative plants from each treatment.
NI- non-inoculated; STB1- inoculation with *Bacillus megaterium* STB1; RDW- Root Dry Weight; SDW- Shoot Dry Weight; TB- Total Biomass.
*represents significant statistical differences (p<0.05).

DNA library was constructed using Illumina TruSeq DNA Nano kit (automated), and sequenced in the Illumina MiSeq platform using the MiSeq V3 reagent kit (2 × 300 bp). A total of 2,272,938 reads were obtained and assembled using the SOAPdenovo v2.04 software [16]. The final genome sequence of *B. megaterium* STB1 was constructed based on a guided assembly against the complete genome sequence of *Bacillus megaterium* ATCC 14581 (CP009920.1) using MAUVE 2.4.0 progressive alignments [17]. A chromosome and one plasmid were assembled. The chromosome of strain STB1 (5,002,401 bp) is a scaffold of 13 contigs (N50 = 1,566,135 bp), which were joined by introducing 100 Ns in the assembly gap regions as indicated in the NCBI submission guidelines. Plasmid pSTB1A results from one contig (118,698 bp) containing plasmid replication genes and a lower GC%. The genome annotation was performed using the NCBI Prokaryotic annotation pipeline [18]. The final near-complete genome sequence of *B. megaterium* STB1 is available in the NCBI database under the accession number CP025700-1.

Functional genome annotation was performed using Blast-KOALA [19]. Genomic islands were predicted in IslandViewer [20]. CAZyme analysis was executed in the dbCAN website using HMMER3 (hmmscan) [21]; antibiotic and secondary metabolite analysis were performed in antiSMASH [22].

Sequences described in the manuscript and supporting information were verified by BLASTp searches (default parameters) against the UniProt/SwissProt database using the Geneious software v9.1 (https://www.geneious.com). Genomic average nucleotide identity (ANI) was calculated using OrthoANIu (https://www.ezbiocloud.net/tools/ani) [23]. Genome circular views were created using CGview [24].

3. Results and discussion

3.1. General characterization of Bacillus megaterium STB1

Bacillus megaterium STB1 is a Gram-positive, pale yellow, sporulating bacterium, able to grow from 7° to 45 °C (maximum tested) and in the presence of up to 5 % NaCl (maximum tested). It is a catalase positive and motile bacterium, able to use glucose and lactose as carbon sources, but non-fermentative. It does not produce acetoin nor H_2S, and a nitrate/nitrite reduction test was negative. This bacterium produced several extracellular enzymes including protease, cellulase and amylase, but not lipase or esterase under the tested conditions. Strain STB1 produced ammonia and siderophores, however, it was not able to solubilize phosphate or zinc. IAA or IAA-like compounds [25] were found in the supernatant of strain STB1 (17.01 ± 0.1 μg/ml) when the bacterium was grown in TSB medium supplemented with 500 μg/ml tryptophan. This strain catabolized 4-aminobutyrate (GABA) but was not able to degrade the plant hormones ACC, IAA, SA, N6-isopentenyladenine, kinetin, jasmonic acid and abscisic acid.

3.2. Bacillus megaterium STB1 promotes tomato growth under normal and salt-stress conditions

B. megaterium STB1 significantly promoted tomato plant growth under both normal and salt stress conditions, leading to increased root, shoot and leaf development (Fig. 1A–C). The inoculation of tomato seedlings with *B. megaterium* STB1 resulted in a significantly ~3.5-fold increase in the root (32 vs 108 mg), and shoot dry weight (49.9 vs 187.4 mg), which resulted in an expressive increase in tomato total dry biomass (81.9 vs. 295.8 mg) when compared to the non-inoculated control, under normal conditions (Fig. 1A and C). Similar results were observed under stress conditions, where tomato seedlings inoculated with *B. megaterium* STB1 presented a significantly increased root

(22.2 vs 83.8 mg) and shoot dry weight (50 vs 238.5 mg), and consequently, total dry biomass (72.2 vs. 268.6 mg) when compared to non-inoculated plants (Fig. 1B and C).

This data indicates a potentially significant agricultural impact of *B. megaterium* STB1, which is consistent with previous reports demonstrating the increased plant growth promoting abilities of several other *B. megaterium* strains, including induced plant protection against salt stress [26–28].

3.3. Bacillus megaterium STB1 genome general characteristics

The genome of *Bacillus megaterium* STB1 includes a single circular chromosome of approximately 5 Mbp with an average GC content of 38.2 %, and plasmid pSTB1A of approximately 118 Kb with an average GC content of 33.7 % (Fig. 2).

DNA sequencing predicted a total of 5271 open reading frames (ORFs) in the genome, in which 5195 correspond to putative protein coding sequences (CDS) and 88 to ribosomal RNA and tRNA genes.

BlastKoala analysis resulted in the functional annotation of 2513 from a total of 5195 CDS (48.4 %). Environmental (609) and genetic (590) information processing functions were assigned for most of the annotated CDS, followed by carbohydrate (299) and amino acid (295) metabolism.

DbCAN analysis indicated a total of 37 proteins predicted to belong to the Glycoside Hydrolase (GH) family, 36 to Glycosyl Transferases (GT), 29 to Carbohydrate Esterases (CE), 10 to Carbohydrate Binding Modules (CBM) and 7 to Auxiliary Activities (AA).

The complete elements for sec (secretion system), tat (twin arginine) and a type VII secretion system were identified and represent the major secretion systems found in STB1 (**Table S1**).

3.4. Phylogenomics

Phylogenetic analysis based on the partial 16S rRNA sequences of all described *Bacillus* species showed that strain STB1 clusters closely to the *B. megaterium* ATCC 14581 type strain (data not shown). To confirm this result, genomic average nucleotide identity (ANI) analysis were conducted. The analysis showed that strain STB1 genome presents a high ANI (>95 %, which is the cut-off value for defining bacteria belonging to the same species) to the genomes of other *B. megaterium* strains: QM B1551 (97.23 %); DSM 329 (97.20 %); ATCC 14581 (97.12 %), hence, indicating that strain STB1 belongs to the *B. megaterium* species.

3.5. Genetic elements involved in B. megaterium STB1 stress resistance, soil and plant colonization abilities

3.5.1. Osmotic stress tolerance

B. megaterium STB1 genome analysis revealed the presence of several genes that are involved in osmotic stress tolerance, including: sodium and chloride transporters, the osmoprotectant transport system, osmolyte biosynthesis and transport (e.g. glycine-betaine, proline, glutamate, glutamine), and, membrane integrity and protection mechanisms (e.g. cardiolipin, lipoteichoic acid production, fatty acid desaturases) (**Table S2**). The abundance of genetic elements involved in osmotic stress tolerance in the strain STB1 genome is in agreement with the functional data showing that strain STB1 tolerates high salinity concentrations, and previous reports on the increased salinity tolerance of other *B. megaterium* strains, many of which are used as inoculants for increasing salt tolerance in various crops [27,28].

3.5.2. Temperature stress resistance and spore formation

The genome of *B. megaterium* STB1 contains several cold shock (csp) and heat shock (hsp) proteins, as well as other chaperone
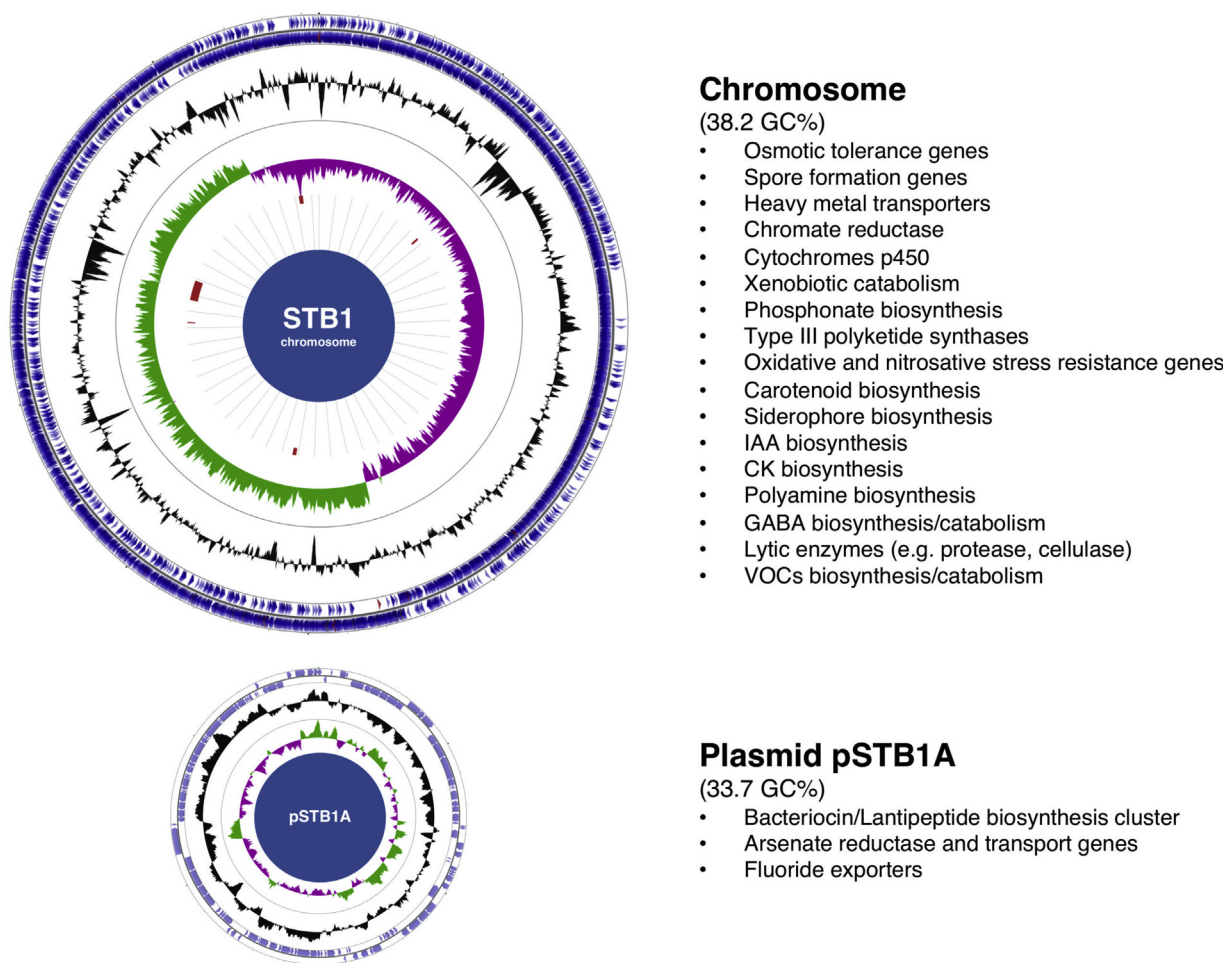
Chromosome
(38.2 GC%)
- Osmotic tolerance genes
- Spore formation genes
- Heavy metal transporters
- Chromate reductase
- Cytochromes p450
- Xenobiotic catabolism
- Phosphonate biosynthesis
- Type III polyketide synthases
- Oxidative and nitrosative stress resistance genes
- Carotenoid biosynthesis
- Siderophore biosynthesis
- IAA biosynthesis
- CK biosynthesis
- Polyamine biosynthesis
- GABA biosynthesis/catabolism
- Lytic enzymes (e.g. protease, cellulase)
- VOCs biosynthesis/catabolism

Plasmid pSTB1A
(33.7 GC%)
- Bacteriocin/Lantipeptide biosynthesis cluster
- Arsenate reductase and transport genes
- Fluoride exporters

Fig. 2. Schematic representation of *Bacillus megaterium* STB1 genome.

proteins involved in the response to temperature stress (**Table S3**). The STB1 genome harbors six *cspA*, three *cspB*, four *cspC* genes and a *cspE* gene, which are all involved in the stabilization of DNA and RNA, and, consequently, impact the transcription and translation efficiency under cold stress [29], as well as other stress conditions [30]. In addition, the genome contains several chaperones involved in heat stress response including *dnaJ*, *dnaK*, *groES*, *groEL*, as well as other several small *hsp* genes [31].

A total of 172 genes predicted to be involved in the several phases of spore formation and spore germination processes were detected in the STB1 genome (**Table S4**). This number is similar to those observed in other *B. megaterium* strains (165 spore-related proteins in *B. megaterium* QM B1551; 157 spore-related proteins in *B. megaterium* DSM 319; 174 spore-related proteins in *B. megaterium* JX285).

3.5.3. Heavy metals resistance

Heavy metal transport/resistance genes are abundant in the *B. megaterium* STB1 genome (**Table S5**). These include several transport genes (for zinc, cobalt, copper, cadmium, manganese, nickel, arsenate, chromate, and fluoride) as well as genes encoding arsenate and chromate reductases (three *arsC* genes, and one *chrR* gene). One of the arsenate reductase genes is present in a cluster (*arsADCBRB*, C0569_25700-25725) that also contains several arsenate transport/resistance genes and is found on the plasmid pSTB1A (**Table S5**). BLASTn analysis revealed that this cluster is rare, and close homologs were only found in the *B. weihaiensis* Alg07 chromosome and the *B. oceanisediminis* 2691 plasmid pBO1.

Interestingly, *B. megaterium* STB1, *B. weihaiensis* Alg07 and *B. oceanisediminis* 2691 were all isolated from similar marine/ estuarine environments.

The large number of heavy metal resistance genes in the *B. megaterium* STB1 genome suggests that this strain is able to deal with high levels of heavy metals, which is consistent with previous reports demonstrating the uptake and heavy metal resistance of several *B. megaterium* strains [32–34].

3.5.4. Oxidative and nitrosative stress resistance

The *B. megaterium* STB1 genome contains multiple genes involved in the oxidative stress response, including two Cu-Zn superoxide dismutase genes, two Fe-Mn superoxide dismutase genes, three catalase genes, two organic hydroperoxide resistance genes and single copies of genes encoding thiol peroxidase, heme-dependent peroxidase, glutathione peroxidase, peroxiredoxin and a non-heme chloroperoxidase (**Table S6**). This collection of genes indicates a strong adaptation and resistance to reactive oxygen species (ROS), which are commonly produced and accumulated by bacteria under stressful conditions and are also known to be involved in the plant defense response.

Furthermore, the genome contains a farnesyl diphosphate synthase (*ispA*) gene and three gene clusters involved in carotenoid synthesis (**Table S6**). The carotenoid cluster 1 (C0569_00970-00985) encodes hydroxyneurosporene desaturase (C0569_00970) and 4,4′-diaponeurosporenoate glycosyltransferase (C0569_00975) homologs, as well as an acyltransferase (C0569_00980) and bisanhydrobacterioruberin hydratase CruF-like (C0569_00985)

domain-containing proteins. Cluster 2 (C0569_14990-15000) encodes a diapophytoene synthase, a diapophytoene desaturase and a diapophytofluene desaturase homolog, all presenting high identity (97.5 %, 99 %, 98.8 %) to the *B. megaterium* DSM319 enzymes involved in the synthesis of the C30 carotenoids, 4,4′-diapophytoene and 4,4′-diaponeurosporene [35]. Cluster 3 (C0569_21280-90) encodes a 4,4′-diapolycopene oxygenase (C0569_21280), 4,4′-diaponeurosporenoate glycosyltransferase (C0569_21285) and glycosyl-4,4′-diaponeurosporenoate acyltransferase (C0569_21290) homologs. Accordingly, the sequence data suggests that *B. megaterium* STB1 is able to produce 4,4′-diapophytoene and 4,4′-diaponeurosporene, as well as modified versions of these compounds, all of which play a protective role in the response against oxidative stress.

Two nitric oxide dioxygenase genes were also identified in the genome (**Table S6**) and may account for strain STB1 resistance to nitrosative stress.

3.5.5. Metabolism of xenobiotics

B. megaterium strains are known for their ability to resist and degrade several xenobiotic compounds including herbicides, pesticides, nitro compounds, azo dyes, barbiturates, epoxides, steroids, and other toxic compounds [36–41]. Not surprisingly, genomic analysis revealed several genes encoding enzymes involved in xenobiotic degradation in *B. megaterium* STB1 (**Table S7**), including two nitroreductases (C0569_01050; C0569_01380) presenting high identity (99.6 %) to *B. megaterium* Mes11 NfrA1 and NfrA2 nitroreductases, respectively, involved in the degradation of the herbicide mesotrione and other nitro compounds [36]; an epoxide hydrolase (C0569_00825) sharing 98.3 % identity to the *B. megaterium* ECU1001 epoxide hydrolase involved in the degradation of α-naphthyl glycidyl ether and the production of optically active epoxides [39]; an aryldialkylphosphatase (C0569_03085) with 51 % identity to *Sulfolobus solfataricus* MT4 thermostable phosphotriesterase involved in the degradation of organo-phosphorus insecticides [42]; an alkyl/aryl-sulfatase (C0569_23470) participating in the degradation of sodium dodecyl sulfate and 4-nitrocatechol; a nitronate monooxygenase involved in the degradation of toxic nitronates; five azoreductases involved in the degradation of aromatic azo compounds; and a catechol-2,3-dioxygenase (C0569_05375) involved in the degradation of catechol, a common metabolite in several aromatic compound degradation pathways.

In addition, the genome of strain STB1 contains several cytochrome p450 encoding genes (**Table S7**), including a cytochrome P450/NADPH–P450 reductase homolog (C0569_02245) (98.1 % identity to *B. megaterium* ATCC 14581 CYP102A1) involved in fatty acid hydroxylation [43]; a CYP109E1 homolog (C0569_04895) (98.5 % identity to *B. megaterium* DSM 319 CYP109E1) involved in the hydroxylation of steroids, vitamin D3 (cholecalciferol), cholesterol, statins and terpenes [44–46,47]; a CYP106A1 homolog (C0569_20645) (95.4 % identity to *B. megaterium* DSM 319 CYP106A1) involved in the hydroxylation of the pentacyclic triterpene 11-keto-β-boswellic acid and several steroids [48,49]; a CYP109A2 homolog (C0569_21565) (97.3 % identity to *B. megaterium* DSM 319 CYP109A2) involved in vitamin D3 hydroxylation [50]; and a unknown cytochrome P450 (C0569_21365) with 96 % identity to a *B. megaterium* DSM 319 WP_080514752.1 cytochrome P450.

3.5.6. Nitrogen, sulfur and phosphorus acquisition

B. megaterium STB1 possesses nitrate and nitrite reductase genes, as well as several genes involved in nitrate/nitrite transport (**Table S8**). Additionally, urea degradation (urease) and transport genes, and three ammonium transporter genes (*amtB*) were found in the genome (**Table S8**).

Genes involved in the assimilatory sulfate reduction pathway, and sulfate transport (*cysPUWA*, *ylnA*, *sulP*) were detected (**Table S8**), as well as sulfonate transport and degradation genes (*ssuABCD*). Three *ssuD* genes, encoding alkanesulfonate monooxygenase, were identified, suggesting that strain STB1 actively obtains sulfur sources (sulfite) by degrading sulfonates. The STB1 genome also contains two dimethyl-sulfide (DMS) monooxygenase (*dmoA*) gene homologs involved in the degradation of DMS; and a dimethylsulfone monooxygenase (*sfnG*) gene homolog involved in the DMS degradation pathway. Curiously, DMS is volatile compound typically found in marine and estuarine environments and is also produced by plants.

Phosphate transport system genes, as well as the alkaline phosphatase genes *phoA* and *phoD* are present in the genome of strain STB1 (**Table S8**), and account for its main organic phosphorus acquisition abilities.

3.5.7. Iron acquisition

The *B. megaterium* STB1 genome contains several genes involved in iron transport (**Table S9**), as well as in the biosynthesis of a siderophore (C0569_05785-5815), with the putative siderophore biosynthesis genes being somewhat homologous to the rhizobactin biosynthesis genes (*rhaABCDEF*) involved in the production of the hydroxamate-type siderophore rhizobactin from *Sinohizobium meliloti* 1021. Moreover, BLASTn analysis revealed that the siderophore cluster (C0569_05785-5815) is highly conserved amongst *B. megaterium* strains, including *B. megaterium* ATCC 19213 (98 % identity), that is known to produce the hydroxamate-type siderophores schizokinen and N-deoxyschizokinen [51]. This analysis also indicated that the *B. megaterium* ATCC 19213 genome contains only one siderophore biosynthesis gene cluster. Hence, the data suggests that the *B. megaterium* STB1 siderophore gene cluster (C0569_05785-5815), also found in most *B. megaterium* strains, is likely involved in the production of schizokinen and N-deoxyschizokinen. Additionally, a gene cluster encoding a siderophore transport system was found in *B. megaterium* STB1 and contains the *yusV*, *yfhA*, *yfiZ* and *yfiY* gene homologs, which have been shown to play a role in *B. subtilis* schizokenin transport [52].

3.5.8. Competition- antagonistic activities

Genomic analysis revealed the presence of several gene clusters involved in the production of antimicrobial compounds, including a gene cluster involved in phosphonate biosynthesis, a cluster containing a type III polyketide synthase gene, and a bacteriocin/lantipeptide gene cluster (**Table S10**).

The phosphonate biosynthesis cluster found in the *B. megaterium* STB1 chromosome contains at least six genes, including phosphonopyruvate mutase (*pepM*), phosphonopyruvate decarboxylase and 2-aminoethylphosphonate-pyruvate transaminase gene homologs. Curiously, this gene cluster also contains two genes encoding proteins related to endospore coat formation as well as peptidoglycan biosynthesis (Mur-like, catalytic domain superfamily), suggesting that it may be involved in the production of a phosphonoglycan. BLAST analysis revealed that this cluster is only found in *B. megaterium* strains, thus, suggesting that this is a species-specific trait.

Bacterial type III polyketide synthases have been linked to the production of phenolic lipids such as alkylresorcinols and alkylpyrones, possessing antimicrobial activity [53]. A gene encoding a type III polyketide synthase from the chalcone/stilbene synthase family (C0569_19065), and an adjacent isoprenylcysteine carboxyl methyltransferase gene (C0569_19070) were found in the genome of strain STB1. The encoded proteins are somewhat homologous to *B. subtilis* 168 BpsA and BpsB proteins (52.4 % and 45.3 % identity, respectively) which are involved in the production of several alkylresorcinols and alkylpyrones [54]. BLASTp analysis

revealed that C0569_19065-70 homologs are commonly found in other *B. megaterium* strains indicating that this is a common trait in this species.

A bacteriocin/lantipeptide gene cluster was found in strain STB1 in plasmid pSTB1A. The cluster consists of an operon containing at least ten genes, three of which are involved in lantipeptide biosynthesis while the others are involved in lantipeptide/bacteriocin modification and transport (**Table S10**). The C0569_25480 gene encodes a type 2 lantipeptide synthetase LanM, and genes C0569_25490-95 encode Type 2 lantibiotic, SP_1948 family proteins. BLAST analysis indicates that the LanM homolog C0569_25480 is rare and close homologs are only found in five other sequenced *B. megaterium* strains, namely, strains AFS083949, AFS069568, AFS058476 (obtained from corn), AFS013177 (grass) and CHCC20162 (soil), hence, suggesting that this is a new class of lantipeptide. Interestingly, most of these strains were found associated with plants, suggesting a role for this product in the associative lifestyle with a plant host.

Altogether, these results are in agreement with reports indicating the antagonistic activities and production of antifungal and antibacterial compounds by *B. megaterium* strains [5,55–57].

3.5.9. Motility, chemotaxis and attachment to plant surfaces

The flagella biosynthesis and regulation operons were detected in the genome (**Table S11**), as well as, several genes involved in gas vesicle formation (*gvp*). Gas vesicles are intracellular hollow organelles commonly found in aquatic bacteria, which allow buoyancy and enable cells to move upwards in liquid to access oxygen [58]. Strain STB1 was isolated from an estuarine environment, so it is possible that it produces gas vesicles in order to survive a periodic aquatic environment. Nevertheless, the *gvp* genes are found in most *B. megaterium* strains [9,58], indicating that this is a species-specific trait.

Several chemotaxis genes, such as *cheABYWR* and *motAB*, five methyl-accepting chemotaxis proteins and a heme-based aerotactic transducer HemAT homolog were detected (**Table S11**). Moreover, The *B. megaterium* STB1 genome contains multiple genes involved in exopolysaccharide production, including capsule biosynthesis genes, which may facilitate bacterial attachment to plant roots. In fact, two clusters containing polysaccharide biosynthesis genes were found (**Table S11**). In addition, several genes involved in the production of teichoic acids, which play a role in biofilm formation and root colonization [59] were also identified (**Table S3**).

3.5.10. Utilization of root exudates

PGPB typically thrive in the rhizosphere, the portion of the soil near to plant roots that is directly influenced by plant exuded compounds. Root exudates differ amongst plants but are mainly composed by sugars, organic acids, amino acids, peptides, proteins, phenolics and flavonoids [60]. The genome of *B. megaterium* STB1 is rich in genetic elements involved in the catabolism/conversion of carbohydrates and responsible for the main carbon cycling pathways (**Table S12**). Genes encoding proteins involved in the degradation of fructose, ribose, arabinose, lactose, galactose, mannose, GDP-mannose, sucrose, trehalose, raffinose, stachyose, cellobiose and other hexoses, sugar acids (gluconate and several of its keto forms, galactonate, lactate, galacturonate, tagaturonate and altronate), sugar alcohols (myo-inositol, mannitol) and complex sugars (cellulose, starch, glycogen) were found in the genome (**Table S12**). Additionally, several glucosidase encoding genes are present in the genome and may account for the catabolism of several alpha and beta-glucosides (**Table S12**). Genes encoding proteins related to sugar transport systems, which are vital in carbohydrate acquisition, were also abundantly found in the STB1 genome (**Table S13**).

The genetic elements encoding the tricarboxylic acid (TCA) and glyoxylate shunt pathways, including succinate, fumarate, malate and citrate degradation/conversion genes are present in the genome (**Table S14**), as well as genes encoding enzymes involved in the degradation of acetate, formate, malonate and tartrate, thus, suggesting the ability of this bacterium to degrade and use a wide range of organic acids as carbon sources. Moreover, three phenolic acid decarboxylase and a polyphenol oxidase encoding gene were also detected (**Table S14**) and may account for this bacterium ability to catabolize several phenolic compounds. The phenolic acid decarboxylase encoded by C0569_13590 presents high identity (88.2 %) to *B. subtilis* 168 enzyme, PadC, involved in the decarboxylation of ferulic, p-coumaric and caffeic acids [61]. The genes C0569_24570 and C0569_24565 encode enzymes with a high homology to *B. subtilis* 168 BsdCD enzymes (63 % and 81.4 % identity, respectively) involved in the decarboxylation and detoxification of phenolic derivatives such as 4-hydroxybenzoate and vanillate [62].

Amino acid transport and degradation genes are abundant in the genome (**Table S15**) and several peptide transport and degradation genes were also identified (**Table S16**), including those encoding aminopeptidases, dipeptidases, oligoendopeptidases and proteases, such as bacillolysin (C0569_22935).

Genes encoding cytochrome p450 enzymes, including a CYP102A1 homolog (C0569_02245) that is known to hydroxylate resveratrol [63], are found in the genome, suggesting that strain STB1 also possesses the ability to modify/catabolize plant flavonoids.

3.6. Genetic elements involved in B. megaterium *STB1 plant growth promotion activities*

3.6.1. IAA biosynthesis

Several *Bacillus* strains, including *B. megaterium* STB1, are known to produce auxins, such as IAA, that play an important role in plant growth promotion and plant-microbe interactions [64]. Recently, Shao et al. [6] proposed that IAA production in *B. amyloliquefaciens* SQR9a occurs via a tryptophan-dependent indole-3-pyruvate (IPyA) pathway. Similarly, the genome sequence of *B. megaterium* STB1 harbors genetic elements consistent with the production of IAA via the IPyA pathway (**Table S17**). These include several aminotransferase genes, including *patB*, which may be involved in the conversion of tryptophan to IPyA; pyruvate oxidase, pyruvate decarboxylase and several phenolic acid decarboxylase genes, including a *yclB* homolog (C0569_24575), which encodes an enzyme involved in the conversion of IPyA to indole-3-acetaldehyde (IAAld); and aldehyde dehydrogenase genes, such as *dhaS*, encoding an enzyme responsible for the conversion of IAAld to IAA [6].

Curiously, the STB1 genome harbors a gene encoding an amidase and with 44.5 % identity to *Arabidopsis thaliana* amidase 1 enzyme, involved in the conversion of indole-3-acetamide (IAM) to IAA [65]. This suggests that *B. megaterium* STB1 is also able to produce IAA via the IAM pathway. In this regard, it is not unusual for bacteria that synthesize IAA to do so by more than a single pathway [64].

3.6.2. Cytokinin biosynthesis

Elements involved in the production of cytokinins, such as, the *miaA* (C0569_06005) and *miaB* (C0569_06035) genes encoding the tRNA dimethylallyltransferase and tRNA-2-methylthio-N6-dimethylallyladenosine synthase enzymes involved in the production of N6-(dimethylallyl)adenosine (iPR) and 2-methylthio-N6-(dimethylallyl)adenosine (2MeSiPR), respectively; the *yvdD* gene (C0569_21570) encoding a Lonely Guy (LOG) family protein (cytokinin riboside 5′-monophosphate phosphoribohydrolase)

involved in the hydrolytic removal of ribose 5′-monophosphate from nitrogen N6-modified adenosines; and a bifunctional cytochrome P450/NADPH–cytochrome P450 reductase gene (C0569_02245) with homology to *A. thaliana* cytokinin hydroxylase involved in the biosynthesis of trans-zeatin, were found in the STB1 genome (**Table S17**). These results suggest that *B. megaterium* STB1 is able to synthesize several cytokinins, which is consistent with previous reports demonstrating cytokinin production by many *Bacillus* species and its important role in *Bacillus* (including *B. megaterium*) plant growth promotion abilities [66–68].

3.6.3. Polyamine production and modulation of ethylene levels

Polyamines such as putrescine, spermine and spermidine have an important role in the plant-growth promoting abilities of *Bacillus* strains, including *B. megaterium* [69,70]. Furthermore, spermidine production by *B. subtilis* has been shown to decrease the activity of the tobacco ACC oxidase gene (ACO1) responsible for ethylene production, thereby lowering plant ethylene levels that affect a range of plant-microbe interactions [69,71]. Several polyamine metabolism and transport genes are found in the genome of *B. megaterium* STB1, including, a *speB* gene encoding agmatinase (involved in putrescine production), four *speE* genes encoding spermidine synthase and several polyamine transport genes (*potABCD*, *pupP*) (**Table S17**), consistent with an important role for polyamine metabolism in *B. megaterium* STB1 plant growth promotion abilities.

3.6.4. GABA metabolism

Under stress conditions plants produce and accumulate GABA [72]. However, high concentrations of GABA can have deleterious effects on cell elongation and overall plant stress resistance [73,74]. GABA metabolism genes are abundantly found in *B. megaterium* STB1 (**Table S17**). In this sense, strain STB1 possesses the *gadB* gene encoding glutamate decarboxylase involved in GABA production, as well as, four *gabT* and three *gabD* genes, encoding GABA aminotransferase and succinate-semialdehyde dehydrogenase involved in GABA degradation. Moreover, strain STB1 possess two *gabP* genes, encoding GABA permease, involved in GABA uptake. These observations are consistent with the possibility that *B. megaterium* STB1 acquires and uses GABA as a nutrient source in the rhizosphere and, consequently, modulates plant GABA levels. Interestingly, plants defective in the ability to degrade GABA are often hypersensitive to salt stress [74], thus, *B. megaterium* STB1 GABA modulation abilities may be linked to its ability to induce salt stress tolerance in tomato.

3.6.5. Modulation of plant brassinosteroids levels by cytochromes p450

A recent study by Asari and colleagues [67] showed that *B. amyloliquefaciens* UCMB5113 inoculation of the roots of *A. thaliana* led to the modification of plant brassinosteroids levels. In this sense, brassinolide, homocastasterone, castasterone, and teasterone levels were all increased in UCMB5113-inoculated plants. Interestingly, *B. megaterium* and other *Bacillus* species metabolize/modify steroids via cytochrome P450 enzymes. These results are consistent with the possibility that *B. megaterium* STB1, possessing several cytochrome p450 (**Table S7**) involved in steroid metabolism, may be able to metabolize/modify brassinosteroids that are involved in plant growth regulation.

3.6.6. Plant growth promotion activities by modulation of VOCs

Bacillus strains are known to produce several VOCs that positively impact plant growth and defense responses [7]. The main production of bacterial VOCs results from nitrogen, sulfur, carbohydrate, amino acid, fatty acid, ketone and alcohol metabolism [75] and genes/enzymes involved in these pathways are commonly found in the *B. megaterium* STB1 genome (**Table S8, S12, S14, S15, S18**), thus, suggesting a high level of VOCs production ability by this strain.

In addition, genomic analysis revealed the presence of several genes/pathways involved in the synthesis and degradation of VOCs such as acetoin, butanediol, DMS, 1-3-propanediol and nitric oxide (**Table S19**), that are known to directly impact plant development [7,76].

4. Conclusions

B. megaterium STB1 is a versatile and stress-resistant PGPB with great potential for agriculture and biotechnology. Its genome sequence revealed the presence of multiple genes involved in important functions regarding soil and plant colonization, plant growth promotion and other relevant features of biotechnological interest (e.g. osmotic, temperature, oxidative, nitrosative and heavy metal stress resistance; carbohydrate, organic acid, amino acid, phenolic acids and xenobiotic, including steroids, terpenoids, herbicides, insecticides, nitronates, metabolism; lytic activities and antagonistic activities mediated by lytic enzymes, bacteriocins, phosphonates; VOCs modulation and multiple plant hormone biosynthesis/modulation abilities). Ultimately, the data obtained in this study brings new insights into the genomic properties governing the successful beneficial interactions between important soil microorganisms like *B. megaterium* and plants, and their potential use in the development of novel sustainable agricultural and biotechnological applications.

Author contributions

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2019.e00406.

References

[1] A. Sessitsch, G. Brader, N. Pfaffenbichler, D. Gusenbauer, B. Mitter, The contribution of plant microbiota to economy growth, Microb. Biotechnol. (2018), doi:http://dx.doi.org/10.1111/1751-7915.13290.

[2] B.R. Glick, Plant growth-promoting Bacteria: mechanisms and applications, Scientifica (Cairo) 2012 (2012) 1–15, doi:http://dx.doi.org/10.6064/2012/963401.

[3] R. Mendes, P. Garbeva, J.M. Raaijmakers, The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms, FEMS Microbiol. Rev. 37 (2013) 634–663, doi:http://dx.doi.org/10.1111/1574-6976.12028.

[4] A. Esringü, M. Turan, A. Güneş, M.R. Karaman, Roles of *Bacillus megaterium* in remediation of Boron, lead, and cadmium from contaminated soil, Commun. Soil Sci. Plant Anal. 45 (2014) 1741–1759, doi:http://dx.doi.org/10.1080/00103624.2013.875194.

[5] P. Trivedi, A. Pandey, Plant growth promotion abilities and formulation of *Bacillus megaterium* strain B 388 (MTCC6521) isolated from a temperate Himalayan location, Indian J. Microbiol. 48 (2008) 342–347, doi:http://dx.doi.org/10.1007/s12088-008-0042-1.

[6] J. Shao, S. Li, N. Zhang, X. Cui, X. Zhou, G. Zhang, Q. Shen, R. Zhang, Analysis and cloning of the synthetic pathway of the phytohormone indole-3-acetic acid in the plant-beneficial *Bacillus amyloliquefaciens* SQR9, Microb. Cell Fact. 14 (2015) 130, doi:http://dx.doi.org/10.1186/s12934-015-0323-4.

[7] R. Sharifi, C.-M. Ryu, Revisiting bacterial volatile-mediated plant growth promotion: lessons from the past and objectives for the future, Ann. Bot. 122 (2018) 349–358, doi:http://dx.doi.org/10.1093/aob/mcy108.

[8] G. Yu, J. Sinclair, G. Hartman, B. Bertagnolli, Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*, Soil Biol. Biochem. 34 (2002) 955–963, doi:http://dx.doi.org/10.1016/S0038-0717(02)00027-5.

[9] M. Eppinger, B. Bunk, M.A. Johns, J.N. Edirisinghe, K.K. Kutumbaka, S.S.K. Koenig, H.H. Creasy, M.J. Rosovitz, D.R. Riley, S. Daugherty, M. Martin, L.D.H. Elbourne, I. Paulsen, R. Biedendieck, C. Braun, S. Grayburn, S. Dhingra, V. Lukyanchuk, B. Ball, R. Ul-Qamar, J. Seibe, E. Bremer, D. Jahn, J. Ravel, P.S. Vary, Genome sequences of the biotechnologically important *Bacillus megaterium* strains QM B1551 and DSM319, J. Bacteriol. 193 (2011) 4199–4213, doi:http://dx.doi.org/10.1128/JB.00449-11.

[10] W.L. Nicholson, P. Fajardo-Cavazos, R. Rebeil, T.A. Slieman, P.J. Riesenman, J.F. Law, Y. Xue, Bacterial endospores and their significance in stress resistance, Antonie Van Leeuwenhoek 81 (2002) 27–32, doi:http://dx.doi.org/10.1023/A:1020561122764.

[11] Y. Bashan, L.E. De-Bashan, S.R. Prabhu, J.-P. Hernandez, Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013), Plant Soil 378 (2014) 1–33, doi:http://dx.doi.org/10.1007/s11104-013-1956-x.

[12] C.S.L. Vicente, F. Nascimento, M. Espada, P. Barbosa, M. Mota, B.R. Glick, S. Oliveira, Characterization of bacteria associated with pinewood nematode *Bursaphelenchus xylophilus*, PLoS One 7 (2012)e46661, doi:http://dx.doi.org/10.1371/journal.pone.0046661.

[13] F.X. Nascimento, B.R. Glick, M.J. Rossi, Isolation and characterization of novel soil- and plant-associated bacteria with multiple phytohormone-degrading activities using a targeted methodology, Access Microbiol. (2019), doi:http://dx.doi.org/10.1099/acmi.0.000053.

[14] D.R. Hoagland, D.I. Arnon, The water-culture method for growing plants without soil, Circ. Calif. Agric. Exp. Stn. (1950) 347.

[15] P. Urón, A.J. Giachini, B.R. Glick, M.J. Rossi, F.X. Nascimento, Near-complete genome sequence of *Pseudomonas palleroniana* MAB3, a beneficial 1-aminocyclopropane-1-Carboxylate deaminase-producing bacterium able to promote the growth of mushrooms and plants, Genome Announc. 6 (2018) e00242–18, doi:http://dx.doi.org/10.1128/genomeA.00242-18.

[16] R. Luo, B. Liu, Y. Xie, Z. Li, W. Huang, J. Yuan, G. He, Y. Chen, Q. Pan, Yunjie Liu, J. Tang, G. Wu, H. Zhang, Y. Shi, Yong Liu, C. Yu, B. Wang, Y. Lu, C. Han, D.W. Cheung, S.-M. Yiu, S. Peng, Z. Xiaoqian, G. Liu, X. Liao, Y. Li, H. Yang, Jian Wang, T.-W. Lam, Jun Wang, SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler, Gigascience 1 (2012) 18, doi:http://dx.doi.org/10.1186/2047-217X-1-18.

[17] A.C.E. Darling, B. Mau, F.R. Blattner, N.T. Perna, Mauve: multiple alignment of conserved genomic sequence with rearrangements, Genome Res. 14 (2004) 1394–1403, doi:http://dx.doi.org/10.1101/gr.2289704.

[18] S.V. Angiuoli, A. Gussman, W. Klimke, G. Cochrane, D. Field, G.M. Garrity, C.D. Kodira, N. Kyrpides, R. Madupu, V. Markowitz, T. Tatusova, N. Thomson, O. White, Toward an online repository of standard operating procedures (SOPs) for (Meta)genomic annotation, Omi. A J. Integr. Biol. 12 (2008) 137–141, doi:http://dx.doi.org/10.1089/omi.2008.0017.

[19] M. Kanehisa, Y. Sato, K. Morishima, BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences, J. Mol. Biol. 428 (2016) 726–731, doi:http://dx.doi.org/10.1016/j.jmb.2015.11.006.

[20] B.K. Dhillon, M.R. Laird, J.A. Shay, G.L. Winsor, R. Lo, F. Nizam, S.K. Pereira, N. Waglechner, A.G. McArthur, M.G.I. Langille, F.S.L. Brinkman, IslandViewer 3: more flexible, interactive genomic island discovery, visualization and analysis: figure 1, Nucleic Acids Res. 43 (2015) W104–W108, doi:http://dx.doi.org/10.1093/nar/gkv401.

[21] Y. Yin, X. Mao, J. Yang, X. Chen, F. Mao, Y. Xu, dbCAN: a web resource for automated carbohydrate-active enzyme annotation, Nucleic Acids Res. 40 (2012) W445–W451, doi:http://dx.doi.org/10.1093/nar/gks479.

[22] K. Blin, T. Wolf, M.G. Chevrette, X. Lu, C.J. Schwalen, S.A. Kautsar, H.G. Suarez Duran, E.L.C. de Los Santos, H.U. Kim, M. Nave, J.S. Dickschat, D.A. Mitchell, E. Shelest, R. Breitling, E. Takano, S.Y. Lee, T. Weber, M.H. Medema, antiSMASH 4.0-improvements in chemistry prediction and gene cluster boundary identification, Nucleic Acids Res. 45 (2017) W36–W41, doi:http://dx.doi.org/10.1093/nar/gkx319.

[23] S.H. Yoon, Smin Ha, J. Lim, S. Kwon, J. Chun, A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie van

[24] Leeuwenhoek, Int. J. Gen. Mol. Microbiol 110 (2017) 1281–1286, doi:http://dx.doi.org/10.1007/s10482-017-0844-4.

[24] J.R. Grant, P. Stothard, The CGView Server: a comparative genomics tool for circular genomes, Nucleic Acids Res. 36 (2008) W181–4, doi:http://dx.doi.org/10.1093/nar/gkn179.

[25] E. Glickmann, Y. Dessaux, A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria, Appl. Environ. Microbiol. 61 (1995) 793–796.

[26] A. Chinnaswamy, T. Coba de la Peña, A. Stoll, D. de la Peña Rojo, J. Bravo, A. Rincón, M.M. Lucas, J.J. Pueyo, A nodule endophytic *Bacillus megaterium* strain isolated from *Medicago polymorpha* enhances growth, promotes nodulation by *Ensifer medicae* and alleviates salt stress in alfalfa plants, Ann. Appl. Biol. 172 (2018) 295–308, doi:http://dx.doi.org/10.1111/aab.12420.

[27] A. Sharma, P. Singh, S. Kumar, P.L. Kashyap, A.K. Srivastava, H. Chakdar, R.N. Singh, R. Kaushik, A.K. Saxena, A.K. Sharma, Deciphering diversity of salt-tolerant Bacilli from saline soils of eastern indo-gangetic plains of India, Geomicrobiol. J. 32 (2015) 170–180, doi:http://dx.doi.org/10.1080/01490451.2014.938205.

[28] J.I. Vílchez, Q. Tang, R. Kaushal, W. Wang, S. Lv, D. He, Z. Chu, Heng Zhang, R. Liu, Huiming Zhang, Genome sequence of *Bacillus megaterium* strain YC4-R4, a plant growth-promoting rhizobacterium isolated from a high-salinity environment, Genome Announc. 6 (2018), doi:http://dx.doi.org/10.1128/genomea.00527-18.

[29] S. Phadtare, Recent developments in bacterial cold-shock response, Curr. Issues Mol. Biol. 6 (2004) 125–136.

[30] R. Keto-Timonen, N. Hietala, E. Palonen, A. Hakakorpi, M. Lindström, H. Korkeala, Cold shock proteins: a minireview with special emphasis on csp-family of Enteropathogenic *Yersinia*, Front. Microbiol. 7 (2016) 1151, doi:http://dx.doi.org/10.3389/fmicb.2016.01151.

[31] J. Guzzo, Biotechnical applications of small heat shock proteins from bacteria, Int. J. Biochem. Cell Biol. 44 (2012) 1698–1705, doi:http://dx.doi.org/10.1016/J.BIOCEL.2012.06.007.

[32] K.H. Cheung, J.-D. Gu, Chromate reduction by Bacillus megaterium TKW3 isolated from marine sediments, World J. Microbiol. Biotechnol. 21 (2005) 213–219, doi:http://dx.doi.org/10.1007/s11274-004-3619-9.

[33] G. Fierros Romero, A. Rivas Castillo, M. Gómez Ramírez, R. Pless, N. Rojas Avelizapa, Expression analysis of Ni- and V-Associated resistance genes in a *Bacillus megaterium* strain isolated from a mining site, Curr. Microbiol. 73 (2016) 165–171, doi:http://dx.doi.org/10.1007/s00284-016-1044-6.

[34] S. Selenska-Pobell, P. Panak, V. Miteva, I. Boudakov, G. Bernhard, H. Nitsche, Selective accumulation of heavy metals by three indigenous *Bacillus* strains, *B. cereus*, *B. Megaterium* and *B. sphaericus*, from drain waters of a uranium waste pile, FEMS Microbiol. Ecol. 29 (1999) 59–67, doi:http://dx.doi.org/10.1111/j.1574-6941.1999.tb00598.x.

[35] P. Hartz, M. Milhim, S. Trenkamp, R. Bernhardt, F. Hannemann, Characterization and engineering of a carotenoid biosynthesis operon from *Bacillus megaterium*, Metab. Eng. 49 (2018) 47–58, doi:http://dx.doi.org/10.1016/j.ymben.2018.07.017.

[36] L. Carles, P. Besse-Hoggan, M. Joly, A. Vigouroux, S. Morera, I. Batisson, Functional and structural characterization of two *Bacillus megaterium* nitroreductases biotransforming the herbicide mesotrione, Biochem. J. 473 (2016) 1443–1453, doi:http://dx.doi.org/10.1042/BJ20151366.

[37] A. Hanano, M. Shaban, D. Almutlk, I. Almousally, The cytochrome P450BM-1 of *Bacillus megaterium* A14K is induced by 2,3,7,8-Tetrachlorinated dibenzo-p-dioxin: biophysical, molecular and biochemical determinants, Chemosphere 216 (2019) 258–270, doi:http://dx.doi.org/10.1016/j.chemosphere.2018.10.103.

[38] B.H. Kim, A.J. Fulco, Induction by barbiturates of a cytochrome P-450-dependent fatty acid monooxygenase in *Bacillus megaterium*: relationship between barbiturate structure and inducer activity, Biochem. Biophys. Res. Commun. 116 (1983) 843–850, doi:http://dx.doi.org/10.1016/S0006-291X(83)80219-8.

[39] X.-D. Kong, S. Yuan, L. Li, S. Chen, J.-H. Xu, J. Zhou, Engineering of an epoxide hydrolase for efficient bioresolution of bulky pharmaco substrates, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 15717–15722, doi:http://dx.doi.org/10.1073/pnas.1404915111.

[40] M.P. Shah, Bioremedial application of *Bacillus megaterium* PMS82 in microbial degradation of acid orange dye, Int. J. Environ. Bioremed. Biodegrad. 2 (2014) 93–99, doi:http://dx.doi.org/10.12691/IJEBB-2-3-1.

[41] M. Smitha, R. Singh, Novel *Bacillus* consortium for degradation of 2,4-dinitrotoluene: a xenobiotic compound, Br. Microbiol. Res. J. 15 (2016) 1–10, doi:http://dx.doi.org/10.9734/BMRJ/2016/25837.

[42] L. Merone, L. Mandrich, M. Rossi, G. Manco, A thermostable phosphotriesterase from the archaeon *Sulfolobus solfataricus*: cloning, overexpression and properties, Extremophiles 9 (2005) 297–305, doi:http://dx.doi.org/10.1007/s00792-005-0445-4.

[43] S.S. Boddupalli, R.W. Estabrook, J.A. Peterson, Fatty acid monooxygenation by cytochrome P-450BM-3, J. Biol. Chem. 265 (1990) 4233–4239.

[44] A. Abdulmughni, I.K. Jóźwik, N. Putkaradze, E. Brill, J. Zapp, A.-M.W.H. Thunnissen, F. Hannemann, R. Bernhardt, Characterization of cytochrome P450 CYP109E1 from *Bacillus megaterium* as a novel vitamin D3 hydroxylase, J. Biotechnol. 243 (2017) 38–47, doi:http://dx.doi.org/10.1016/j.jbiotec.2016.12.023.

[45] I.K. Jóźwik, F.M. Kiss, Ł. Gricman, A. Abdulmughni, E. Brill, J. Zapp, J. Pleiss, R. Bernhardt, A.-M.W.H. Thunnissen, Structural basis of steroid binding and oxidation by the cytochrome P450 CYP109E1 from *Bacillus megaterium*, FEBS J. 283 (2016) 4128–4148, doi:http://dx.doi.org/10.1111/febs.13911.

[46] N. Putkaradze, M. Litzenburger, M.C. Hutter, R. Bernhardt, CYP109E1 from *Bacillus megaterium* acts as a 24- and 25-hydroxylase for cholesterol, ChemBioChem 20 (2019) 655–658, doi:http://dx.doi.org/10.1002/cbic.201800595.

[47] N. Putkaradze, M. Litzenburger, A. Abdulmughni, M. Milhim, E. Brill, F. Hannemann, R. Bernhardt, CYP109E1 is a novel versatile statin and terpene oxidase from *Bacillus megaterium*, Appl. Microbiol. Biotechnol. 101 (2017) 8379–8393, doi:http://dx.doi.org/10.1007/s00253-017-8552-6.

[48] E. Brill, F. Hannemann, J. Zapp, G. Brüning, J. Jauch, R. Bernhardt, A new cytochrome P450 system from *Bacillus megaterium* DSM319 for the hydroxylation of 11-keto-β-boswellic acid (KBA), Appl. Microbiol. Biotechnol. 98 (2014) 1703–1717, doi:http://dx.doi.org/10.1007/s00253-013-5029-0.

[49] F.M. Kiss, D. Schmitz, J. Zapp, T.K.F. Dier, D.A. Volmer, R. Bernhardt, Comparison of CYP106A1 and CYP106A2 from *Bacillus megaterium* – identification of a novel 11-oxidase activity, Appl. Microbiol. Biotechnol. 99 (2015) 8495–8514, doi:http://dx.doi.org/10.1007/s00253-015-6563-8.

[50] A. Abdulmughni, I.K. Jóźwik, E. Brill, F. Hannemann, A.-M.W.H. Thunnissen, R. Bernhardt, Biochemical and structural characterization of CYP109A2, a vitamin D 3 25-hydroxylase from *Bacillus megaterium*, FEBS J. 284 (2017) 3881–3894, doi:http://dx.doi.org/10.1111/febs.14276.

[51] K.B. Mullis, J.R. Pollack, J.B. Neilands, Structure of schizokinen, an iron-transport compound from *Bacillus megaterium*, Biochemistry 10 (1971) 4894–4898.

[52] J. Ollinger, K.-B. Song, H. Antelmann, M. Hecker, J.D. Helmann, Role of the Fur regulon in Iron transport in *Bacillus subtilis*, J. Bacteriol. 188 (2006) 3664–3673, doi:http://dx.doi.org/10.1128/JB.188.10.3664-3673.2006.

[53] M. Stasiuk, A. Kozubek, Biological activity of phenolic lipids, Cell. Mol. Life Sci. 67 (2010) 841–860, doi:http://dx.doi.org/10.1007/s00018-009-0193-1.

[54] C. Nakano, H. Ozawa, G. Akanuma, N. Funa, S. Horinouchi, Biosynthesis of aliphatic polyketides by type III Polyketide synthase and methyltransferase in *Bacillus subtilis*, J. Bacteriol. 191 (2009) 4916–4923, doi:http://dx.doi.org/10.1128/JB.00407-09.

[55] S. Jock, B. Völksch, L. Mansvelt, K. Geider, Characterization of *Bacillus* strains from apple and pear trees in South Africa antagonistic to *Erwinia amylovora*, FEMS Microbiol. Lett. 211 (2002) 247–252, doi:http://dx.doi.org/10.1016/S0378-1097(02)00701-2.

[56] H.K. Jung, S.D. Kim, An antifungal antibiotic purified from *Bacillus megaterium* KL39, a biocontrol agent of red-pepper *Phytophthora*-blight disease, J. Microbiol. Biotechnol. 15 (2005) 1001–1010.

[57] I.A. Malanicheva, D.G. Kozlov, I.G. Sumarukova, O.V. Efremenkova, V.A. Zenkova, G.S. Katrukha, M.I. Reznikova, O.D. Tarasova, S.P. Sineokii, G.I. El'-Registan, Antimicrobial activity of *Bacillus megaterium* strains, Microbiology 81 (2012) 178–185, doi:http://dx.doi.org/10.1134/s0026261712020063.

[58] N. Li, M.C. Cannon, Gas vesicle genes identified in *Bacillus megaterium* and functional expression in *Escherichia coli*, J. Bacteriol. 180 (1998) 2450.

[59] Z. Xu, H. Zhang, X. Sun, Y. Liu, W. Yan, W. Xun, Q. Shen, R. Zhang, *Bacillus velezensis* wall teichoic acids are required for biofilm formation and root colonization, Appl. Environ. Microbiol. 85 (2018) e02116–18, doi:http://dx.doi.org/10.1128/aem.02116-18.

[60] T.S. Walker, H.P. Bais, E. Grotewold, J.M. Vivanco, Update on root exudation and rhizosphere biology root exudation and rhizosphere biology 1, Plant Physiol. 132 (2014) 44–51, doi:http://dx.doi.org/10.1104/pp.102.019661.

[61] J.F. Cavin, V. Dartois, C. Diviès, Gene cloning, transcriptional analysis, purification, and characterization of phenolic acid decarboxylase from *Bacillus subtilis*, Appl. Environ. Microbiol. 64 (1998) 1466–1471.

[62] B. Lupa, D. Lyon, L.N. Shaw, M. Sieprawska-Lupa, J. Wiegel, Properties of the reversible nonoxidative vanillate / 4-hydroxybenzoate decarboxylase from *Bacillus subtilis*, Can. J. Microbiol. 54 (2008) 75–81, doi:http://dx.doi.org/10.1139/w07-113.

[63] D.H. Kim, T. Ahn, H.C. Jung, J.G. Pan, C.H. Yun, Generation of the human metabolite piceatannol from the anticancer-Preventive agent resveratrol by bacterial cytochrome P450 BM3, Drug Metab. Dispos. 37 (2009) 932–936, doi:http://dx.doi.org/10.1124/dmd.108.026484.

[64] D. Duca, J. Lorv, C.L. Patten, D. Rose, B.R. Glick, Indole-3-acetic acid in plant-microbe interactions, Antonie Van Leeuwenhoek 106 (2014) 85–125, doi:http://dx.doi.org/10.1007/s10482-013-0095-y.

[65] S. Pollmann, D. Neu, E.W. Weiler, Molecular cloning and characterization of an amidase from *Arabidopsis thaliana* capable of converting indole-3-acetamide into the plant growth hormone, indole-3-acetic acid, Phytochemistry 62 (2003) 293–300.

[66] T.N. Arkhipova, S.U. Veselov, A.I. Melentiev, E.V. Martynenko, G.R. Kudoyarova, Ability of bacterium *Bacillus subtilis* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants, Plant Soil 272 (2005) 201–209, doi:http://dx.doi.org/10.1007/s11104-004-5047-x.

[67] S. Asari, D. Tarkowská, J. Rolčík, O. Novák, D.V. Palmero, S. Bejai, J. Meijer, Analysis of plant growth-promoting properties of *Bacillus amyloliquefaciens* UCMB5113 using *Arabidopsis thaliana* as host plant, Planta 245 (2017) 15–30, doi:http://dx.doi.org/10.1007/s00425-016-2580-9.

[68] Y.-G. Park, B.-G. Mun, S.-M. Kang, A. Hussain, R. Shahzad, C.-W. Seo, A.-Y. Kim, S.-U. Lee, K.Y. Oh, D.Y. Lee, I.-J. Lee, B.-W. Yun, *Bacillus aryabhattai* SRB02 tolerates oxidative and nitrosative stress and promotes the growth of soybean by modulating the production of phytohormones, PLoS One 12 (2017) e0173203, doi:http://dx.doi.org/10.1371/journal.pone.0173203.

[69] S. Xie, H.-J. Wu, H. Zang, L. Wu, Q. Zhu, X. Gao, Plant growth promotion by spermidine-producing *Bacillus subtilis* OKB105, Mol. Plant Microbe Interact. 27 (2014) 655–663, doi:http://dx.doi.org/10.1094/MPMI-01-14-0010-R.

[70] C. Zhou, Z. Ma, L. Zhu, X. Xiao, Y. Xie, J. Zhu, J. Wang, Rhizobacterial strain *Bacillus megaterium* BOFC15 induces cellular polyamine changes that improve plant growth and drought resistance, Int. J. Mol. Sci. (2016) 17, doi:http://dx.doi.org/10.3390/ijms17060976.

[71] F.X. Nascimento, M.J. Rossi, B.R. Glick, Ethylene and 1-aminocyclopropane-1-carboxylate (ACC) in plant–bacterial interactions, Front. Plant Sci. 9 (2018) 1–17, doi:http://dx.doi.org/10.3389/fpls.2018.00114.

[72] A.M. Kinnersley, F.J. Turano, Gamma aminobutyric acid (GABA) and plant responses to stress, Crit. Rev. Plant Sci. 19 (2000) 479–509, doi:http://dx.doi.org/10.1080/07352680091139277.

[73] H. Renault, A. El Amrani, A. Berger, G. Mouille, L. Soubigou-Taconnat, A. Bouchereau, C. Deleu, γ-Aminobutyric acid transaminase deficiency impairs central carbon metabolism and leads to cell wall defects during salt stress in *Arabidopsis* roots, Plant Cell Environ. 36 (2013) 1009–1018, doi:http://dx.doi.org/10.1111/pce.12033.

[74] H. Renault, A. El Amrani, R. Palanivelu, E.P. Updegraff, A. Yu, J.P. Renou, D. Preuss, A. Bouchereau, C. Deleu, GABA accumulation causes cell elongation defects and a decrease in expression of genes encoding secreted and cell wall-related proteins in *Arabidopsis thaliana*, Plant Cell Physiol. 52 (2011) 894–908, doi:http://dx.doi.org/10.1093/pcp/pcr041.

[75] S. Schulz, J.S. Dickschat, Bacterial volatiles: the smell of small organisms, Nat. Prod. Rep. 24 (2007) 814–842, doi:http://dx.doi.org/10.1039/b507392h.

[76] C. Molina-Favero, C.M. Creus, M. Simontacchi, S. Puntarulo, L. Lamattina, Aerobic nitric oxide production by *Azospirillum brasilense* Sp245 and its influence on root architecture in tomato, Mol. Plant Microbe Interact. 21 (2008) 1001–1009, doi:http://dx.doi.org/10.1094/mpmi-21-7-1001.