Genome sequence analysis of *Bacillus subtilis* PTA-271 isolated from a *Vitis vinifera* (cv. Chardonnay) rhizospheric soil: an highlight on some of its biocontrol traits

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ABSTRACT

Background: *Bacillus subtilis* strains have been widely studied for their innumerous benefits in agriculture, including viticulture. Providing numerous assets, *B. subtilis* spp. are widely described as promising grapevine-protectors against a broad spectrum of pathogens, ranging from biotroph to necrotroph. *B. subtilis* spp. may both elicit host defenses and promote host vigor, but may also directly antagonize pathogens and detoxify their aggressive molecules. This study reports the draft genome sequence of the *Bacillus subtilis* PTA-271, isolated from the rhizospheric soil of healthy *Vitis vinifera* cv. Chardonnay at Champagne Region in France, attempting to draw outlines of its full biocontrol capacity.

Results: The PTA-271 genome has a size of 4,001,755 bp, with 43.78% of G + C content and 3,945 protein coding genes. The draft genome of PTA-271 highlights (1) a functional swarming motility system hypothesizing a colonizing capacity and a strong interacting capacity, (2) strong survival capacities and (3) a set of genes encoding for bioactive substances. Bioactive compounds are known both (i) to stimulate plant growth or defenses such as hormones and elicitors, and (ii) to counteract pathogen aggressiveness such as effectors and many kinds of detoxifying enzymes.

Conclusions: The plurality of the encoded biomolecules by *Bacillus subtilis* PTA-271 genome appears as strengths for PTA-271 biocontrol potential towards plants, offering a big potential against a broad spectrum of pathogens, especially those responsible for the complex grapevine trunk diseases.

Key-words: genome draft*, Bacillus subtilis* PTA-271, biocontrol value, grapevine trunk diseases, *Botrytis cinerea*, wide protective spectrum.

BACKGROUND

Bacillus subtilis is a gram-positive endospore-forming bacterium from *Bacillus* genera considered as a promising plant beneficial organism that can survive in the soil for a long time-period under harsh environmental conditions [1-3]. Benefits of species from the *Bacillus* group are well described in many sectors of industry, agriculture and viticulture [4-10]. Focusing on the *B. subtilis* species, it has been described to provide plants with a broad range of benefits that include their induced systemic resistance (ISR) upon pathogen attacks, their growth promotion, or the direct control of plant pathogens [9-12].

Primed defenses during ISR are regulated either by jasmonic acid (JA) and ethylene (ET) signaling or by salicylic acid (SA) signaling [13-15, 21, 23, 27, 32, 111]. Beneficial microorganisms may thus modulate the plant hormonal balance or directly elicit the plant defenses [12, 16]. Contributing to such plastic events are the *Bacillus spp.* described to secrete ACC deaminase (EC 4.1.99.4) that breaks down the ET precursor ACC into ammonia and ketobutyrate in plant cells, altering thus ET synthesis and the ET-dependent defenses in crop plants [17-20]. In contrast, bacterial food source compounds may induce the synthesis of hormones in bacterial populations [18]. Beneficial microorganisms might thus produce some hormones (common to plants) or their precursors (i.e. SA, auxins, gibberellins, cytokinins, polyamines…) and possibly interfere with the plant hormonal balance [18, 21-26]. Numerous bacterial elicitors of ISR are also reported in several plant species, such as exopolysaccharides (EPS), lipopolysaccharides (LPS), siderophores such as the iron-regulated pyoverdin, iron, flagella, biosurfactants, N-acyl-L-homoserine lactone, N-alkylated benzylamine and volatile compounds (i.e. phthalic acid methyl ester, phenylacetic acid, nitric oxide, acetoin and 2-butanone) [27-34]. Some of them have already been identified as elicitors in some species of *B. subtilis* or *Bacillus* genera [28, 34-36].

Changes in the phytohormonal-balance may also impact plant growth and development, since the reduction of ET may promote plant growth [18-20]. The nutrient-acquisition by plants through both the microbiota mineralizing capacity and chelating properties also conditions plant growth and development [3, 7, 18, 24-26, 37] as well as volatile compounds derived from beneficial microorganisms [38, 39]. Efficient beneficial effects of *Bacillus spp*. also assume bacterium and microbiota preservation, upon both abiotic and biotic stressful conditions. Interestingly, when protecting itself by extrusion transporters, detoxifying enzymes, quenching enzymes and pathogen homologous enzymes, bio-control agent (BCA) additionally contributes directly to plant protection.

Finally, *B. subtilis* may produce an extensive range of antimicrobial molecules, chelators and lytic enzymes to alter pathogen fitness and aggressiveness [40-44]. According to literature, these beneficial molecules include ribosomally synthesized antimicrobial peptides (RP, including the post-translationally modified peptides RiPP), non-ribosomally synthesized peptides (NRP), polyketides (PK), as well as other uncommon antimicrobial volatile compounds (the inorganic and organic ViCs and VOCs, respectively) and other terpenoid secondary metabolites as listed in Table 1 [13, 40-48].

To date, *B. subtilis* species were reported both to elicit plant defenses by mean of elicitors or by interfering with phytohormone signaling and to antagonize plant pathogens and were also described as protective against a broad spectrum of pathogens ranging from biotrophs to necrotrophs [9-14, 16, 21-23, 27, 30-34, 40-49, 52]. Focusing on *B. subtilis* PTA-271, its protective effect was already published in grapevine against *Neofusicoccum parvum* and *Botrytis cinerea* [9-11], the causal agents of Botryosphaeria dieback and grey mold respectively. These beneficial characteristics of *B. subtilis* species, combined with the fact it was a non-pathogenic species able to sporulate in order to resist to climate changes and common disinfectants [53, 54, 77, 121], make this microorganism suitable to control a wide spectrum of pathogens among which the most widely dangerous grapevine trunk disease (GTD) pathogens with no currently efficient control strategies [9, 55]. In this study, we report the draft genome sequence of the *B. subtilis* strain PTA-271 and analyze and compare with other known *Bacillus* strains sequences, to expand our knowledge on the *B. subtilis* PTA-271 valuable properties in order to design most efficient sustainable biocontrol strategies for viticulture.

METHODS

1.- *B. subtilis* PTA-271 GENERAL INFORMATION AND FEATURES

B. subtilis PTA-271 was isolated in 2001 (Table 2) from the rhizospheric soil of healthy Chardonnay grapevines (*V. vinifera* L., cv Chardonnay) from a vineyard located in Champagne (Marne, France). Rhizospheric samples were directly suspended in a sterile 0.85% NaCl solution (1g of soil: 10 ml of NaCl) and bacterial isolates were obtained by serial dilutions of the soil samples (10^7, 10^3, 10^2 cfu/g soil) in triplicate onto LB-agar (Luria–Bertani-agar), King's B-agar and glycerol–arginine-agar plates by incubating at 30°C for 24-72 h. All different colonies were then re-isolated on LB-agar, cultured in LB at 30 °C for 24 h and screened for their protective role against *Botrytis cinerea* by using grapevine plantlet leaf assays pretreated with bacterium [10]. Selected biocontrol microorganisms were then identified, calculated to establish the density formula and stored in a sterile 25% glycerol solution at -

80°C for complementary purposes. The classification and general features of *B. subtilis* PTA-271 are in Table 2. The taxonomic information for this strain was already described by Trotel-Aziz *et al.* (2008) [10] and remains unaltered to this date.

2.- *B. subtilis* PTA-271 GENOMIC SEQUENCING INFORMATION

2.1.- Genome project history

B. subtilis PTA-271 was designated for sequencing because of its efficient capacity to protect grapevine against several pathogens such as *Botrytis cinerea* or *Neofusicoccum parvum*, the causal agents of grey mold or Botryosphaeria dieback respectively [9-11, 58]. As previously shown [9-11], this beneficial microorganism can modulate grapevine defenses, but may also directly antagonize the growth of pathogens and detoxify aggressive molecules. Such multi-target beneficial levers are adding guaranties for a wide spectrum of protection, in addition to physical and chemical tolerant characteristics (endospore-forming bacterium, large range of pH and salinity, Table 2). Altogether, there are advantages to sequence the *B. subtilis* PTA-271 genome to better understand its key beneficial levers and further develop the best as possible sustainable biocontrol strategies whatever the field conditions or parameters (pH, salinity, etc …).

The whole genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JACERQ010000000. The version described in this paper is version JACERQ010000000 and all related information is represented in Table 3.

2.2.- Genomic DNA preparation

Genomic DNA of *B. subtilis* PTA-271 was extracted using the Wizard® Genomic DNA Purification kit (Promega), from the pellet of a 1 mL-overnight culture incubated at 28 °C in LB medium. DNA integrity was confirmed on a 0.65% agarose gel electrophoresis in TAE buffer. DNA concentration and quality were read from 1 µL of DNA with the NanoDrop-ONE spectrophotometer (Ozyme).

2.3.- Library preparation and genome sequencing

DNA library for bacterial genome sequencing was prepared from 0.5 nanograms of high-quality genomic DNA using the Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, USA) and sequenced using paired-end (PE) 2x300 bp on the MiSeq® Illumina® platform at Genoinseq

(Cantanhede, Portugal). All the procedures were performed according to standard manufacturer protocols.

2.4.- Genome assembly and annotation

Sequenced reads were demultiplexed automatically by the Illumina® Miseq® sequencer using the CASAVA package (Illumina, San Diego, USA) and quality-filtered with Trimmomatic version 0.30 [59]. High-quality adapter-free reads were assembled with SPAdes version 3.9.0 [60] and contigs with size <500 bp or coverage lower 10x were removed from the assembly. Assembly metrics were calculated with Quast version 4.6.1 [61]. Contigs were checked for contamination and completeness using CheckM 1.0.9 [62]. Coding gene predictions were made with Prodigal version 2.6 [63], rRNA and tRNA genes were detected using Barrnap version 0.8 and CRISPR regions were detected by Minced version 0.2.0. Coding gene annotation was performed with Prokka version 1.12 [64] using the following repositories: SwissProt (The UniProt Consortium, 2017), HAMAP [65], TIGRFAMs [66] and Pfam [67]. Coding genes were also annotated for Pathway using KEGG [68], for peptidases using MEROPS [69] and for carbohydrate-active enzymes with dbCaN [70].

RESULTS AND DISCUSSION

1.- *B. subtilis* PTA-271 GENOME PROPERTIES AND COMPARISON WITH OTHER BACILLUS STRAINS

1.1.- General features of the genome

The general features of *B. subtilis* PTA-271 are in Table 4 and Figure 1, performed using Artemis version 16.0.0. The draft genome sequence of *B. subtilis* PTA-271 presented an estimated genome size of 4,001,755 bp divided in 20 contigs. The G + C content of this sequence was 1,751,999 bp, representing about 43.78% of the whole genome. Genome analysis showed that *B. subtilis* PTA-271 contained 4,038 genes, among which 3,945 (97.69%) were protein coding genes. This genome draft predicts 92 RNA genes among which 11 rRNA genes were identified and no CRISPR repeats. From 4,001,755 bp of the genome size, 3,550,299 bp correspond to coding genes representing 88.73% of the whole genome. From this, 3,440 genes had function prediction, 3,183 were assigned to COG categories described in Table 5, and 3,517 genes had Pfam domain descriptions.

1.2.- Insights

According to Table 5, the majority of the proteins in *B. subtilis* PTA-271 genome are *Proteins not assigned in COG's* that represented 19.31% (762) of the whole genome, *Amino acid transport & metabolism* that represented 8.31% (328), *Transcription* (313) and *Carbohydrate transport & metabolism* (313) that represented 7.93% of the genome. Two biocontrol-useful-categories in *B. subtilis* PTA-271 genome are (1) *Secondary metabolites biosynthesis, transport and catabolism*, representing 2.30% (91) of the genome, and (2) *Other defense mechanisms* encoding proteins relevant for plant-bacteria interactions, representing 1.49% (59) of the whole genome sequence.

2.- *B. subtilis* PTA-271 MULTI-STRENGTHS FOR PLANT SUSTAINABLE BIOCONTROL

Bacillus species offer a broad range of benefits to plants, covering: (1) plant growth promotion, (2) induced systemic plant defenses and protection against pathogens, and (3) prevention of pathogen fitness or aggressiveness, by producing many compounds able to interact with the host plants, the pathogens or their tripartite intricate communication. As previously cited, these compounds include hormone and many elicitors, as well as many antimicrobial molecules, but also a range of many other substances and mechanisms contributing to increase both the plant capacity to recruit beneficial microorganisms and the tripartite communication within plant microbiota including also pathogens (i.e. surfactants, biofilm key forming-elements, quorum -sensing or -quenching molecules, among others). Considering this, the genome analysis of *B. subtilis* PTA-271 tried to highlight some useful characteristics directly or indirectly beneficial for a sustainable plant protection against a broad spectrum of pathogens.

2.1.- Motility, adhesion and plant root colonizing capacity

Motility of a bacterium is due to the flagellum, enabling it to move towards a vital nutrient source (chemotaxis). In this sense, *B. subtilis* PTA-271 contains genes (Supplementary Table S1) encoding for (i) flagella maintenance, such as *flhF*, *flhA*, *flhB*, *flgC*, *flgB*, *fliE*, *fliF* and *fliG*, and (ii) chemotaxis, such as *cheY*, *cheD*, *cheW, cheA* and *cheB*.

Once reaching a comfortable area, adhesion is due to bacterium pili, allowing the initiation of biofilm formation where both chemotaxis and gene exchanges among microorganisms of microbiota can be amplified [72]. To this end, *B. subtilis* PTA-271 has genes from the comG operon

(Supplementary Table S1), essential for DNA binding to competent cells upon transformation of *B. subtilis* [73].

B. subtilis spp. are also described for their strong swarming motility [74]. The gene *swrC* encoding for swarming motility protein was identified in the genome of *B. subtilis* PTA-271 (Supplementary Table S1). Swarming motility requires the production of both functional flagella, pili and surfactant to reduce surface tension [75].

Motility and adhesion are both considered advantageous characters for a successful host colonization and *B. subtilis* spp. are already described to grow in biofilm mode involved in root colonization [76]. To this end, *B. subtilis* PTA-271 encodes the transcription factor *Spo0A* (S19-40_02177, Supplementary Tables S1 and S2), described to be required for the surface-adhered cells transition to a three-dimensional biofilm structure [77] and to repress *AbrB* (S19-40_03988), described as a negative regulator of biofilm formation [77].

The genes identified above in *B. subtilis* PTA-271 support additional investigations towards (1) a tripartite communication within plant microbiota and (2) grapevine root colonization from the rhizospheric soil where it was already identified [10]. Some authors consider that (1) all of the microbial genera described as common inhabitants of the rhizosphere are also endophytics [78] and that (2) whatever their localization, beneficial microorganisms that successfully colonize the plant, particularly by the root system [79], would be advantageous both for plant growth promotion and for plant biocontrol. Indeed, the *B. subtilis* spp. flagellum contains flagellin proteins that are recognized as elicitors of plant defenses [80] as indicated below. Surfactin is another elicitor as indicated below too, and also a biosurfactant involved in the formation of stable biofilm essential for the successful colonization of host-plants [81].

2.2.- Plant growth promotion through trophic- and morphogenic- effects

Plant nutrition depends on the soil retention capacity of minerals and on nutrient availabilities, thus both on chelating process, on mineralization by decomposers and on the bioavailability of minerals towards the plant consumer. Upon nitrogen starvation, some bacteria are described to upregulate the *ure* gene cluster, since urea is an easy nitrogen source. Such *ure* genes are also predicted in *B. subtilis* PTA-271 genome containing *ureA* (S19-40_00755), *ureB* (S19-40_00756) and *ureC* (S19-40_00757). This cluster of genes is known to be controlled by the global nitrogen-regulatory protein *TnrA* (Supplementary Table S2), also predicted in *B. subtilis* PTA-271 genome and consolidating this bacterium as a good plant partner as non-competitive for nitrogen. Regarding other nutrient access

that also depends on soil solubilizing activity and nutrient bioavailability, it is well known that phosphate-solubilizing bacteria (PSB) may take advantage of low molecular weight molecules [51, 82]. Similarly, genes of *B. subtilis* PTA-271 are predicted to encode for proteins involved in the production of gluconic acid and precursor of citric acid (S19-40_03830, S19-40_03828). These organic acids may lower the soil pH to solubilize phosphate and thus increase its availability to the plant [83]. Bacterial secondary metabolites (i.e. PyrroloQuinoline Quinone, PQQ) are also known to control gluconic acid production [84], and *B. subtilis* PTA-271 has three genes related to PQQ production *pqqL, pqqF and pqqC* (S19-40_00233, S19-40_00234, S19-40_00247) [85]. Additionally, as in the other *Bacillus spp*., *B. subtilis* PTA-271 contains the phytase gene *phy* (S19-40_03630) encoding for phosphatases able to hydrolyze the organic complex in order to liberate phosphate and make it available for plants [86, 87]. Iron is another very important nutrient for plant growth and development. *B. subtilis* PTA-271 possesses the *fur* gene (Supplementary Table S2) that encodes for a ferric uptake regulatory protein coordinating the homeostasis of iron uptake depending on its availability in soil [88]. *B. subtilis* PTA-271 appears thus as a good plant auxiliar as non-competitive for iron. However, the soil contains an abundant ferric form (Fe^{3+}) that is weakly available for plants [89]. Fortunately, some bacteria producing siderophores with high specificity and affinity for iron, can bind, extract and transport iron near the plant roots [90]. *B. subtilis* PTA-271 genome also predicted the production of such siderophores, namely the catecholic siderophore 2,3-dihydroxybenzoate-glycine-threonine trimeric ester bacillibactin encoded by 5 genes (*dhbA, dhbB, dhbC, dhbE, dhbF*: S19-40_01242, S19-40_01245, S19-40_01243, S19-40_01244, S19-40_01246, respectively). Altogether, *B. subtilis* PTA-271 appears as a good candidate to improve plant iron uptake. Surfactants produced by beneficial bacteria may also contribute to increase the availability of hydrophobic nutrients. In this sense, *B. subtilis* PTA-271 is suspected to produce surfactin from its identified genes *srfAD, srfAC, srfAB and srfAA* (S19-40_02068, S19-40_02069, S19-40_02070, S19-40_02071, respectively). Surfactin is a powerful biosurfactant due to its amphiphilic nature that strongly anchor with lipid layers, thus interfering with the structure of biological membranes [91].

Plant root morphology is also described to impact nutrient uptake and thus plant growth thanks to the stimulation of lateral root formation and root air formation, while primary root elongation was inhibited [92, 93]. Plant hormone productions (i.e. auxins, cytokinins, gibberellins) are key elements for root morphology changes. Some beneficial bacteria seem able to produce some of them, including *B. subtilis* PTA-271. This latter has genes encoding for tryptophan, the main precursor of the auxin IAA (indole-3-acetic acid), namely from the *trp* group, *trpA* (S19-40_02736), *trpB* (S19-40_02737), *trpC* (S19-40_02739), *trpD* (S19-40_02740), *trpE* (S19-40_02741), *trpF* (S19-40_02738), trpP (S19-40_02553), *trpR* (S19-40_03152) and *trpS* (S19-40_02410). Once synthesized,

bacterial IAA has two main functions: (i) increase the plant root surface and length for a deeper soil prospecting capacity and nutrient acquiring capacity [51, 94] and (ii) release the cell walls of rootlets to facilitate molecule exudations and benefit to rhizospheric bacteria [51]. *B. subtilis* PTA-271 has also genes that encode for cytokinin synthesis such as *yvdD* (Supplementary Table S2), known as a plant growth regulator (i.e. cell division, organogenesis) in combination with IAA. Gibberellins (GA) produced by some bacteria may also affect the plant growth and survival by interfering with the plant signaling pathways through secondary metabolites changes [93]. GA pathways is not fully encoded by *B. subtilis* PTA-271 which only contains *ispD* linked to 2-C-methyl-D-erythritol 4-phosphate (MEP) and *GerC3_HepT* linked to geranylgeranyl diphosphate (GGPP) production (as indicated below), two successive precursors of GA and ABA synthesis in plants. But from GGPP, no genes were detected for the kaurene pathway required to complement GA synthesis in *B. subtilis* PTA-271 genome.

Genes encoding for some plant growth regulators were presents in *B. subtilis* PTA-271 genome, such as *speA* (S19-40_00456) encoding for arginine decarboxylase (ADC), *speB* (S19-40_00673) encoding for agmatinase (leading to putrescine, Put), *speG* (S19-40_00166) encoding for spermidine synthase (Spd) and *speE* (S19-40_00672) encoding for spermine synthase (Spm). Additionally, genes encoding for S-adenosyl-methionine (SAM) decarboxylase (*speH*, S19-40_01619) and putative SAM-methyltransferase (S19-40_00450) exist in *B. subtilis* PTA-271 genome and are needed to complete Spd and Spm synthesis from Put. These polyamines (PAs) are known to promote flowering and to play important roles in inducing cell division, promoting regeneration of plant tissues and cell cultures [95], as delaying senescence [96]. Volatile compounds (VOCs) produced by some beneficial rhizospheric bacteria have also been identified as elicitors promoting plant growth. Those suspected to be produced by *B. subtilis* PTA-271 looking at the genes identified in its genome are (1) acetoin which producing pathway is known to be encoded by *acuA* (S19-40_01690) and *acuC* (S19-40_01692) among others genes encoding for acetoin utilization proteins, and (2) 2,3-butanediol known to be produced by *butA* and *butC* (encoding for S19-40_03395 and S19-40_00056, respectively) [28, 97]. VOCs are especially reported to interact with some of the previously cited plant hormones (i.e. auxins, ethylene, among others) [98-100].

2.3.- Host protection due to host induced immunity and to Microbiota preservation

HOST INDUCED IMMUNITY to prevent biotic stress

Primed defenses during ISR are regulated by phytohormones, depending on either JA and ET signaling or SA signaling [13-15, 21, 23, 27, 32, 111]. Beneficial microorganisms may thus modulate the plant hormonal balance or directly elicit the plant defenses [12, 16, 23, 32]. Literature reports

that *Bacillus spp.* could inhibit ET synthesis and related defense responses by breaking the ET precursor ACC, using an ACC deaminase [17, 19, 20]. But, no gene encoding for ACC deaminase was detected in *B. subtilis* PTA-271 genome. In contrast, the *metK* gene encoding for S-adenosylmethionine (SAM) synthase (S19-40_01774) leading to SAM, the ACC precursor, was identified in *B. subtilis* PTA-271 genome. By synthesizing the ET precursor SAM, *B. subtilis* PTA-271 would appear ISR-useful to plants that possess the complementary metabolic machinery for ET synthesis. Genes encoding for PAs are previously cited from *B. subtilis* PTA-271 genome (*speA*, *speB, speE*, *speG, speH*), and PAs and ET biosynthetic pathways are interrelated from decarboxylated SAM [101]. Although their physiological functions are distinct and at times antagonistic, the balance between the two would enable to manipulate the plant senescence process [102]. SA is another phytohormone for which several genes encoding its metabolic pathways (from synthesis to hydrolysis) are identified in *B. subtilis* PTA-271 genome, among which *pchA* encoding for the salicylate biosynthesis isochorismate synthase (S19-40_01801).

Many other elicitors also induced host immunity, coming from beneficial microorganisms (MAMPs, microbial associated molecular patterns) but also from the plant host (DAMPs, damaged associated molecular patterns). MAMPs can act from the external surface of a beneficial microorganism (i.e. flagellin) or result from a secretion outside or inside the host (i.e. surfactin, fengycin, NO, acetoin, 2-butanone, phthalic acid methyl ester) [43,66-72]. In *B. subtilis* PTA-271, *hag* gene encodes for flagellin protein from bacterial flagellum (Supplementary Table S1) often recognized by plant pattern recognition receptors (PRRs) normally cell surface localized receptor kinases or LRR-RLP proteins [103], such as FLS2 and EF-Tu [104, 105] described to activate host defenses through mitogen-activated protein kinase cascades (MAPK) [106]. Lipopeptides are other elicitors encoded by genes identified in the genome of *B. subtilis* PTA-271, such as the previously cited surfactin and fengycin. Alkalanization of host extracellular medium by surfactin provokes ions -influx and -efflux activating in turn systemic host defenses through intracellular changes of signaling compounds [107], then the production of antimicrobicidal phenolic compounds [108]. Fengycin is another elicitor of plant defenses that also enhance the production of plant phenolics compounds [108, 109]. Genes that encode for fengycin in *B. subtilis* PTA-271 are *fenA, fenB, fenC*, and *fend* (S19-40_00076, S19-40_00077, S19-40_00073, S19-40_00074). VOCs produced by rhizospheric bacteria, such as the previously reported 3-hydroxy 2-butanone and acetoin, are also well known to induce ISR through SA-independent pathway, but merely through the ET one that remains to be deeply investigated [99]. No other genes encoding for other VOC elicitors such as the phthalic acid methyl ester were identified in *B. subtilis* PTA-271, in contrast with *B. subtilis* IAGS174 described by Akram et al. (2015). Among inorganic volatile compound (VIC), the ubiquitous nitric oxide (NO) is a signal

molecule scavenging reactive oxygen species (ROS) and regulating the level of PAs and hormonal balance (i.e. ABA versus SA) to reprogram or switch plant development upon stress [110]. Different genes related to NO metabolic pathways are found in *B. subtilis* PTA-271 genome, among which the gene *nos* encoding for a NO synthase oxygenase (S19-40_03258). Many other elicitors are additionally encoded by the genome of *B. subtilis* PTA-271 such as those cited above (i.e. siderophores, iron, flagella) and those cited below (i.e. N-acyl-L-homoserine lactone). Maybe their beneficial effect on plant vigor and their detrimental effect on pathogen fitness are the contributors to host protection. Exopolysaccharides (EPS) and lipopolysaccharides (LPS) are also reported as elicitors in several *Bacillus* genera [28, 34-36]. Among the EPS encoding genes identified in *B. subtilis* PTA-271 are S19-40_00800, S19-40_00870, S19-40_00999, S19-40_01009, S19-40_01427. Among the LPS encoding genes identified in *B. subtilis* PTA-271 are *lptB, lapA, lapB* (S19-40_01170, S19-40_01479, S19-40_03936) [27, 30-32, 34, 111].

DAMP elicitors are products of lytic enzymes (i.e. chitosan, glucans, ….) from microorganisms (either beneficial or pathogenic) that may elicit plant defenses [27, 34, 111, 112]. Genes encoding for lytic enzymes are identified in *B. subtilis* PTA-271 genome, such as those encoding for chitosanase and ß-glucanase (Supplementary Table S3). Many other genes also encode for lytic enzymes in the spore cortex (Supplementary Table S4) for which the roles remain unclear. No other genes encoding for ISR elicitors such as N-alkylated benzylamine were identified in *B. subtilis* PTA-271 although described in literature [27, 30-32, 34, 111].

MICROBIOTA QUALITY AND STRENGTHS PRESERVATION

Biologists showed that plant root exudates (i.e. sugars, organic acids, amino acids, lipophilic compounds, etc…), as energy and carbon sources, would enable a plant to selectively recruit some beneficial bacterial subspecies (i.e. biosurfactant producers) and then to modulate its own rhizospheric microbiota composition and its agronomic fitness in turn [113]. Biosurfactant producers such as suspected for *B. subtilis* PTA-271, as mentioned above, can additionally facilitate biofilm formation and the bioavailability of root exudates, which are both essential for a successful colonization of host-plants [81]. SA was also shown to mediate changes in the composition of root exudates, and in turn in the type of microorganisms recruited by the plant [114] and as indicated above *B. subtilis* PTA-271 has the genes to produce SA. Altogether, *B. subtilis* PTA-271 looks to benefit of key levers to influence actively the qualitative plant microbiota.

Bacterial auto-inducers (AI), low-molecular weight signal molecules, also activate the interactive competences of a bacterium in a quorum-sensing (QS) dependent manner. Indeed, efflux pump systems mediate QS-signals at a target concentration of AI, activating the transcription of

target genes [115]. The furanosyl-borate-diester (AI-2) is described as universal for interspecies communication both in gram-positive and gram-negative bacteria [116]. Genome analysis of *B. subtilis* PTA-271 shows that this bacterium contains the *luxS* gene (S19-40_01786) responsible for AI-2 production. Another class of AI also produced by Gram-positive bacteria for their intercellular communication is that of oligopeptides or auto-inducing peptide (AIP), consisting of 5-34 amino acids residues such as CSP, EntF, AM373, AD1, F10, PD1, OB1 and EDF [117, 118]. Genome analysis of *B. subtilis* PTA-271 shows that this bacterium may encode for the AIP precursors *EntF* (S19-40_01246) and *AM373* (S19-40_03157).

When interacting with a plant, *Bacillus* species are also exposed to its host defenses that also include reactive oxygen species (ROS) [119]. Genes encoding for resistance to hydroperoxide such as *ohrA, ohrB* and *ohrR* (S19-40_00615, S19-40_00613, S19-40_00614) are identified in the genome of *B. subtilis* PTA-271, supporting a complex system of sensing, protection and regulation of ROS to ensure survival.

Additionally, *B. subtilis* PTA-271 has the genes to withstand to extreme environment conditions such as nutrient limitation by sporulation (turning on endospore form) [120]. Indeed, endospore is an environmentally resistant cell, metabolic dormant, able to resist extreme temperatures, desiccation and ionizing radiation for thousands of years [121]. Several genes are involved in the sporulation process of *B. subtilis* PTA-271 (Supplementary Table S4), among which: (1) the *spo* genes responsible for the control of the sporulation [122], (2) the *ger* genes responsible for the control of the germination depending on the alleviation of stressful environmental conditions [123], (3) the *cot* genes involved in the formation of the spore over coating envelope (endospore external layer) [124], and (4) the *cw* genes encoding for the spore cortex lytic enzymes. The sporulation capacity of *B. subtilis* PTA-271 represents a great asset for its survival upon extreme environmental conditions over long lasting periods, preserving then the beneficial strengths of this microorganisms for plant profits [3].

HOST INDUCED IMMUNITY to prevent abiotic stress

To exert beneficial effects, a microorganism had to stay metabolically active upon abiotic stress. Beneficial bacteria need thus to survive abiotic stress such as dehydration, wounding, cold, heat or salinity that in turn lead to a water status regulation. For this end, bacterial species are described to control their intracellular solute pools [125, 126]. In this sense, *B. subtilis* PTA-271 has genes encoding for two potassium uptake proteins *KtrA* and *KtrB* (S19-40_01338, S19-40_01337) enabling survival in high salinity environments [125, 126].

As previously described upon biotic stress conditions, some phytohormones are also useful for plant defense against abiotic stress, such as abscisic acid (ABA), gibberellins (GA) and ethylene (ET) [127] which precursors are encoded by genes also identified in the genome of *B. subtilis* PTA-271. Indeed, the identified *GerC3_HepT* encodes for GGPP synthase (S19-40_02907) and *pcrB* encodes for geranylgeranylglyceryl phosphate synthase (S19-40_03154), GGPP being a common precursor of GA- and ABA- synthesis [128]. Upstream of GGPP, MEP is another common precursor of GA- and ABA-synthesis [129] and two *ispD* genes were found to encode for cytoplasmic MEP cytidylyltransferases (S19-40_00851 and S19-40_03933) in *B. subtilis* PTA-271 genome. Additionally, *ispF* encodes for a 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (S19-40_03932) and *ispE* for a 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (S19-40_03980). From GGPP, the kaurene pathway may lead to GA, while the phytoene path may lead to ABA and in the genome of *B. subtilis* PTA-271, *yisP* (a *crtb* KEGG gene) encodes for a 15-cis-phytoene/all-trans-phytoene synthase (S19-40_02475). Similarly, and as already mentioned above, ET pathway seems not to be entirely encoded by *B. subtilis* PTA-271 genome from which was only identified the *metK* gene enabling to produce SAM, a precursor of ACC required for ET synthesis in plants. Altogether these data indicate that *B. subtilis* PTA-271 genome may encode for key precursors of phytohormones that may influence actively ABA and ET contents in plants. In plants, ABA, GA and ET signaling pathways may interfere altogether through different transcription factors (TF) or small proteins (i.e. GiD, DELLA, EIN, ERF, ABI, XERICO, …) that may also physically interact [130, 131]. In the genome of *B. subtilis* PTA-271, many sigma factors and many TF exist, among which those encoded by *ykuD*, *yciB*, *slrA*, *yocK, carD, infA*, *infB*, *infC, IF5B*, *tsf*, *efp*, *tuf* and *fusA* genes (Supplementary Table S2). It is noteworthy to understand that useful TF upon abiotic stress could also be useful upon biotic stress. The set of genes under common regulatory controls (i.e. operons) are also listed in the same Supplementary Table S2.

As mentioned above, *B. subtilis* PTA-271 has the genes to produce PAs, known to protect plant cells upon water deficit [132], temperature changes [133] and salinity [134]. Polyamines are known to increase the activity of various antioxidant enzymes in plants and may contribute to produce H_2O_2 as a signaling molecule that can activate plant antioxidant defense responses [135].

Interestingly, the genome of *B. subtilis* PTA-271 also encodes for genes to detoxify compounds accumulating in the environment, such as the arsenite detoxifying system with *arsR* (Supplementary Table S2) [136]. *B. subtilis* PTA-271 genome has also genes that are involved in the degradation of organic pesticides or nitroaromatic compounds by encoding for resistance genes against quaternary ammonium compounds *sugE, qacC* (S19-40_00985, S19-40_01079) or else against catechol (*mhqR, mhqA*) (S19-40_00558, S19-40_00645) [137], among others (Supplementary Table S5).

2.4.- Other biocontrol activity by direct confrontation with pathogens or aggressive molecules

Upon direct confrontation, *Bacillus* species need first to protect themselves against antimicrobial attacks from the other aggressive species that may also compete for resources [138]. As already mentioned, *B. subtilis* PTA-271 has antimicrobial resistance genes encoding for efflux pump systems to detoxify several types of drugs such as pathogen's antibiotic and ROS (i.e. hydroperoxide), as for the previously cited compounds accumulating in the environment (i.e. quaternary ammonium compounds, catechol and arsenate). Efflux pump systems also allow bacteria to adjust their internal environment by using transporters mediating drug extrusion from the cell [139, 140], whether specific to a substance or a group of substances. Some specific transporters are encoded by the genome of *B. subtilis* PTA-271, as for the resistance proteins against: (1) tetracyclin (S19-40_01293, S19-40_01359, S19-40_01919) encoded by *tetA*, *tetR*, *tetD* [141, 142]; (2) fosfomycin (S19-40_00125) encoded by *fosB* [143]; (3) erythromycin (S19-40_03633 and S19-40_03632) encoded by *msrA* and *msrB*, respectively [144]; (4) bacillibactin (S19-40_00235) encoded by *ymfD* [145]; (5) bacitracin (S19-40_01756, S19-40_01755 and S19-40_00770) encoded by *BceA*, *BceB* and *BcrC* [146]; (6) bleomycin (S19-40_01406) encoded by *ble*; (7) riboflavin (S19-40_03749, S19-40_01917) encoded by *ribZ* and *rfnT*, among many others. Non-specific transporters are also designated as multidrug transporters [139, 140], such as those encoded in *B. subtilis* PTA-271 genome by *mepA* (S19-40_00070, S19-40_03635) [147], *ebrA* (S19-40_00188) and *ebrB* (S19-40_00189) [148], *ykkD* (S19-40_00619) and *ykkC* (S19-40_00620) [149], *bmrA* (S19-40_00951) and *bmr3* (S19-40_01151) [150], *emrY* (S19-40_02033) [150], among others.

In addition to the extruding transporters, *Bacillus* species may also detoxify the pathogen aggressive molecules (i.e. toxins) by the mean of antitoxins or detoxifying enzymes coming from multigenic families of proteins such as the transferases and CYP450. In *B. subtilis* PTA-271, the main transferase encoding genes are for glutathione-S-transferases GST, malonyl-transferases MT, glucosyl-transferases GT and many others as indicated in the Supplementary Table S5. Among *B. subtilis* PTA-271 CYP450 encoding genes are those for mono-oxygenases and dioxygenases as indicated in the Supplementary Table S5. By mean of such detoxifying systems, *B. subtilis* PTA-271 might thus contribute to decrease pathogen aggressiveness**.**

Beneficial bacteria may also directly target pathogen aggressiveness by using quenching enzymes against the pathogen QS-dependent production of aggressive molecules [142-143]. For that, *B. subtilis* PTA-271 like other *Bacillus* species share *aiiA* encoding for *N*-acetyl homoserine lactonase

hydrolyzing the lactone ring of AHLs (Acyl-homoserine lactones) that would have been useful for the QS production of pathogen virulent factors [46, 151]. Looking at *B. subtilis* PTA-271 genome, genes encoding for quenching enzymes (Supplementary Table S6) may thus produce lactonases, but also β-lactamases, deaminases, deacetylases and other (de)acylases. By mean of such quenching enzymes, *B. subtilis* PTA-271 might contribute to decrease pathogen aggressiveness.

Polyketide synthases (PKS) and other acetyltransferases are also described to produce polyketides (PK) as beneficial molecules. Polyketides are a large group of natural products built from acyl-coenzyme A, essential for bacterial antagonism. Many PK produced by *Bacillus* are bactericidal agents that play a vital role in controlling plant pathogens [152, 153]. Regarding *B. subtilis* PTA-271 genome (Supplementary Table S7), 15 genes encode for PKS and many others for acetyltransferases or share similar part of the PKS functions. By mean of PKS, *B. subtilis* PTA-271 might contribute to antagonize pathogens. According to antiSMASH 5.1.0 [154], *B. subtilis* PTA-271 genome contains 11 secondary metabolites gene clusters, among which: 1 polyketide synthase cluster (PKS) and 1 hybrid PKS-NRPS cluster (Supplementary Table S8).

Additional genes encoding for an extensive range of beneficial molecules produced by *Bacillus* spp. are also identified in *B. subtilis* PTA-271 (Supplementary Table S3), such as those encoding for antimicrobial molecules or effectors (i.e. antibiotics, surfactants, hydrogen cyanide …), chelators (i.e. siderophores) and lytic enzymes (i.e. chitosanases, glucanases, cellulases, proteases, chitinases) able to directly alter pathogen fitness and aggressiveness [40, 47, 155].

Among the genes identified in *B. subtilis* PTA-271 to encode for RP (ribosomally synthesized antimicrobial peptides) and NRP (non-ribosomally synthesized peptides) antimicrobial molecules (Supplementary Table S3) are those known to produce: Baillaene (*pksD)*, subtilosin *(sboA, albG, albE, albD, albB, albA)* and bacilysin (*bacE, bacF, bacG*). According to COG categories, 2.30% of *B. subtilis* PTA-271 genome is devoted to the production of such secondary metabolites, considered as one of the most important features in biocontrol activities. Genes encoding for lipopeptides, as other NRP antimicrobial molecules, are also identified in *B. subtilis* PTA-271 [41, 156, 158, 160, 163]. Among their products, the previously cited elicitors of plant defenses: (1) fengycin is also a powerful antifungal substance described as particularly active against filamentous fungi [157]. It interferes with the integrity of biological membranes until their complete disruption at high concentrations [158]. Fengycin causes structural deformations of the pathogen hyphae, suppressing their proliferation in plant and thus prevent phytotoxins production [159]. (2) Surfactin is another powerful antimicrobial molecule [160] whose encoding gene is identified in *B. subtilis* PTA-271.

Aside from these secondary metabolites, *B. subtilis* PTA-271 has also genes encoding for uncommon antimicrobials volatile compounds either inorganic (VIC) or organic (VOC), such as: (1) 1 VIC: hydrogen cyanide (HCN) encoded by *hcnC* (S19-40_01178) to antagonize a pathogen. As a potent inhibitor of cytochrome C oxidase and several other metalloenzymes, HCN is extremely toxic to aerobic microorganisms at very low concentrations [161, 162]. (2) The 2 previously reported VOC elicitors acetoin and 2,3-butanediol are also well known to work as weapons against some pathogens [28, 97]. According to antiSMASH 5.1.0 [154], *B. subtilis* PTA-271 genome contains 11 secondary metabolites gene clusters, among which: 3 NRPS clusters and 2 RiPPs clusters (Supplementary Table S8).

As described above, *B. subtilis* PTA-271 has also genes encoding for siderophores such as Bacillibactin (Supplementary Table S3), known to deprive pathogen growth of iron while providing it for plant growth [163].

Lytic enzymes (CWDE) such as cellulases, proteases, chitinases, glucanases, are other important feature of *Bacillus* spp. that may both alter pathogen fitness and produce DAMPs as previously mentioned. Concerning the CWDE encoding genes in *B. subtilis* PTA-271 genome, are found: 1 chitosanase encoded by *csn*, 1 β-glucanase encoded by *bglS*, 1 β-glucanase / cellulase (S19-40_00094) encoded by *eglS*, and about 80 proteases (Supplementary Table S3). These enzymes are considered as powerful fungicides since they are responsible for the degradation of key structural components of fungal cell walls [164-166].

3- *B. subtilis* PTA-271 GENOME COMPARISON WITH OTHER GENOMES

To understand the magnitude of the differences between *B. subtilis* PTA-271 and other *Bacillus* strains, the PTA-271 genome has been compared to the complete genomes of 5 type-strains and 32 non-type strains, represented in Table 6. Type-strains are living culture organisms descending from strains designated as “nomenclatural types”, according to the International Code of Nomenclature of Prokaryotes [167]. Among them are the type strains *B. subtilis* NCIB 3610, *B. subtilis* 168, *B. subtilis* 9407, *B. amyloliquefaciens subsp. plantarum* strain FZB42, and *B. velezensis* KTCT 13012 [168-171]. Among non-type strains showing ≥99% of thr *16S* ribosomal gene similarity with PTA-271 are 31 distinct strains of *B. subtilis* and 1 *Bacillus velezensis*. For this genomic comparison, we used the GGDC 2.1 web server [172], the DSMZ phylogenomics pipeline to estimate DNA-DNA hybridization (DDH) [172], and the JSpecies WS web server to estimate the Average Nucleotide Identity (ANI) through pairwise comparisons [173]. The DDH value was estimated using the recommended formula (formula two) for draft genomes, at the GGDC website [174]. The ANI values were calculated using

Ezbiocloud [175]. The whole data analysis enabled to obtain the intergenomic distances between genomes and their probability of belonging to the same species or subspecies. The general comparison of genomes is reported in Table 6, while the intergenomic distances (DDH estimate and ANI) are shown in Table 7.

Among the type strain genomes, the closer strain to *B. subtilis* PTA-271 was *B. subtilis* 9407, with a 0.0104 distance, a DDH estimate of 91.60%, and an ANIm of 99.02%. As expected, the most distant strain was *B. velezensis* KTCT 13012, with a 0.2268 distance, a DDH estimate of 19.40% and a 0% probability of being the same species, corroborated with an ANIm percentage of 77.02%. Concerning the non-type strain genomes, the closer strains to PTA-271 were *B. subtilis* QB5413, *B. subtilis* SRCM 104005, and *B. subtilis* QB61 with distances of 0.0112, 0.0119 and 0.0119 respectively, and DDH estimates of 90.90%, 90.20% and 90.20% respectively. The most distant strains was *B. velezensis* strain ATR2, with a distance of 0.2144 and a DDH estimate of 20.50% corroborated with an ANIm percentage of 77.1%. The most distant *B. subtilis* strain to PTA-271 was *B. subtilis* subsp. *subtilis* RO-NN-1 with a distance of 0.203 and a DDH of 82.60%.

CONCLUSION

With a genome size of 4,001,755 bp containing 97.69% of protein encoding genes, the draft genome of *B. subtilis* PTA-271 highlights all the qualities of a promising plant beneficial microorganism. The most relevant genes encode for: (1) a functional swarming motility system highlighting advantageous colonizing capacity of host and a strong interacting capacity within plant microbiota; (2) a strong survival capacity, due to sporulation but also to complex detoxifying systems, auto-inducing metabolic paths and recruiting capacities for adding microbiota values; and (3) the delivery of many bioactive substances (i.e. hormones, elicitors, effectors and quenchers, siderophores and lytic enzymes, etc …), facilitating either the stimulation of plant growth or defenses, or else disturbing pathogen fitness or aggressiveness. Interestingly, the *B. subtilis* PTA-271 genome capacity to produce a wide range of phytohormone analogous (i.e. SA, ET precursor, ABA, PAs, etc …) as well as diverse direct effectors and lytic enzymes against plant pathogens, highlight a big potential valuable for biocontrol strategies. Altogether, plurality of the biomolecules encoded by the genome of *B. subtilis* PTA-271 appears as strength to combat a broad spectrum of plant pathogens (ranging from biotrophs to necrotrophs), and looks especially as highly useful against hemibiotrophs such as those responsible of the complex grapevine trunk diseases, as reported by previous works [9].

Abbreviations: ABA: absissic acid; BCA: bio-control agent; DAMPs: damaged associated molecular patterns; EPS: exopolysaccharides; ET: ethylene; GTD: grapevine trunk diseases; ISR: induced systemic resistance; JA: jasmonic acid; LPS: lipopolysaccharides; MAMPs: microbial associated molecular patterns; NO: nitric oxide; NRP: non-ribosomally synthesized peptides; PK: polyketides; RP: ribosomally synthesized antimicrobial peptides; RiPP: post-translationally modified RP; ROS: reactive oxygen species; SA: salicylic acid; ViC: inorganic volatile compound; VOC: organic volatile compound.

DECLARATIONS

Ethics approval and consent to participate: Not applicable.

Consent for publication: All authors approved the final version and consent for publication.

Availability of data and material: The whole genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JACERQ010000000. The version described in this paper is version JACERQ010000000 and all related information is represented in Table 3.

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Figure legend

Figure 1. Circular map of the *Bacillus subtilis* PTA-271 genome. Map generated with CGView server [35].

Table legends

Table 1. *Bacillus subtilis* known antimicrobial molecules, chelators and lytic enzymes.

Table 2. Classification and features of *Bacillus subtilis* PTA-271 according to MIGS recommendations [56].

Table 3. *Bacillus subtilis* PTA-271 genomic sequencing information.

Table 4. Genome statistics.

Table 5. Number of genes associated with general COG functional categories.

Table 6. Comparative NCBI genome analysis of *Bacillus subtilis* PTA-271 with strains showing ≥99% of *16s* similarity.

Table 7. Comparative genome distances analysis with other strains, using DNA-DNA hybridization and average nucleotide identities.

Supplementary material

Table S1. *Bacillus subtilis* PTA-271 encoding genes for motility, adhesion and plant root colonizing capacity.

Table S2. *Bacillus subtilis* PTA-271 encoding genes for some Transcriptional regulators and Operons.

Table S3. *Bacillus subtilis* PTA-271 encoding genes for antimicrobial molecules, other effectors and lytic enzymes.

Table S4. *Bacillus subtilis* PTA-271 encoding genes for sporulation.

Table S5. *Bacillus subtilis* PTA-271 encoding genes for some CYP450 and for Transferases.

Table S6. *Bacillus subtilis* PTA-271 encoding genes for lactonases, β-lactamases, deaminases, deacetylases.

Table S7. *Bacillus subtilis* PTA-271 encoding genes for PKS and other acetyltransferases.

Table S8. Anti-SMASH 5.1.0 prediction of gene clusters responsible for secondary metabolite production in *Bacillus subtilis* PTA-271.