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Research article

Characterization and genome mining of *Bacillus subtilis* BDSA1 isolated from river water in Bangladesh: A promising bacterium with diverse biotechnological applications

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ABSTRACT

The metabolic versatility of *Bacillus subtilis* makes it useful for a wide range of applications in biotechnology, from bioremediation to industrially important metabolite production. Understanding the molecular attributes of the biocontrol characteristics of *B. subtilis* is necessary for its tailored use in the environment and industry. Therefore, the present study aimed to conduct phenotypic characterization and whole genome analysis of the *B. subtilis* BDSA1 isolated from polluted river water from Dhaka, Bangladesh to explore its biotechnological potential. The chromium reduction capacity at 100 ppm Cr (VI) showed that *B. subtilis* BDSA1 reduced 40 % of Cr (VI) within 24hrs at 37 °C. Exposure of this bacterium to 200 ppm cadmium resulted in 43 % adsorption following one week of incubation at 37 °C. Molecular detection of *chrA* and *czcC* gene confirmed chromium and cadmium resistance characteristics of BDSA1. The size of the genome of the *B. subtilis* BDSA1 was 4.2 Mb with 43.4 % GC content. Genome annotation detected the presence of numerous genes involved in the degradation of xenobiotics, resistance to abiotic stress, production of lytic enzymes, siderophore formation, and plant growth promotion. The assembled genome also carried chromium, cadmium, copper, and arsenic resistance-related genes, notably *cadA, czcD, czrA, arsB* etc. Genome mining revealed six biosynthetic gene clusters for bacillaene, bacillibacin, bacilysin, subtilosin, fengycin and surfactin. Importantly, BDSA1 was predicted to be non-pathogenic to humans and had only two acquired antimicrobial resistance genes. The pan-genome analysis showed the openness of the *B. subtilis* pan-genome. Our findings suggested that *B. subtilis* BDSA1 might be a promising candidate for diverse biotechnological uses.

1. Introduction

The widespread presence of heavy metals such as cadmium, chromium, nickel, arsenic, lead, and others is causing significant environmental pollution, especially with the advancement of industrialization [1]. Bangladesh is in the most precarious position due to the abundance of tanneries, pharmaceutical, textile, fertilizer and other industries around Dhaka City, that release untreated wastewater into the Buriganga, Turag, Shitalakhya, and Balu rivers, causing heavy metal pollution of the aquatic environment [2]. In

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addition to being harmful for humans and animals, such heavy metal contamination promotes the emergence of antibiotic and metal resistance through co-resistance and cross-resistance [3]. Therefore, effective strategies are obvious to control heavy metal pollution and curb the occurrence of antimicrobial resistant bacteria in the environment. Traditional methods for the detoxification and degradation of metals such as adsorption, chemical oxidation or reduction reactions, chemical precipitation, and electrochemical process are often costly and inefficient, especially for low metal concentrations [4]. Moreover, these methods lack specificity, require substantial space, and often demand high energy consumption [5]. Thus, there is a need for bioremediation using microorganisms with potential for remediation of polluted environments and production of eco-friendly secondary metabolites. Microorganisms have developed various mechanisms, including biosorption, biotransformation, bioaccumulation, and biomineralization to detoxify heavy metals [6]. Bioremediation of heavy metals offers an alternative and effective means of decontaminating metal-polluted environments [7].

The use of bacteria, belonging to the *Bacillus* genus, might be one way of bioremediating pollutants [8]. *B. subtilis* is one of the well-described species in this genus that has enormous potential in biotechnology because of its direct industrial application and satisfactory bioremediation potential [9,10]. Its ability to survive in harsh environmental conditions for extended times and rapid multiplication rate have made it a popular biocontrol agent [11]. A growing body of literature reported the potential use of *B. subtilis* in the bioremediation of chromium, cadmium, nickel, mercury, and copper [12–14]. Apart from its bioremediation potential, *B. subtilis* is often regarded as a cell factory for microbial production of industrially important chemicals, enzymes and antimicrobial compounds because of its sophisticated protein secretion system and remarkable adaptability [15]. These beneficial compounds include volatile organic or inorganic compounds, antimicrobial peptides, non-ribosomally synthesized peptides (NRP), polyketides, and others [11]. Usually, the secondary metabolite-encoding genes are typically found in clusters and encode multifunctional enzyme complexes [16]. Some notable secondary metabolites produced by *B. subtilis* include bacilysin, surfactin, fengycin, and others [17]. Furthermore, it also facilitates pathogen inhibition and plant growth promotion (PGP) by producing phytohormones, siderophores, lipopeptides, and phytases [18]. Mechanism of bioremediation, metabolite production, and PGP activity vary between strains; thus, individual strains specificities may influence species ratios and metabolic conditions, which in turn may affect hosts and microbial communities interactions [11].

Bioinformatics-assisted recognition of specific functional genes or gene clusters in genome sequences, popularly known as genome mining, is faster and more effective in the detection of bacterial metabolites than conventional screening strategies [17]. It also provides a more comprehensive evaluation of the safety of using *B. subtilis*, expanding from traditional phenotypic examination to molecular mechanism and genetic features [19]. Several previous studies conducted whole genome analysis of *B. subtilis* strain EB1, XF-1, MBI-600, Bbv57 to evaluate their ability to serve numerous biotechnological purposes [20–23].

In pursuing sustainable solutions to heavy metal pollution and harnessing the untapped potential of microbial metabolites, we explained the bioremediation and secondary metabolite production potential of indigenous *B. subtilis* BDSA1. The main objective of the study was to assess the biotechnological potential of BDSA1 through the characterization of resistance to heavy metals, and whole-genome analysis.

2. Materials and methods

2.1. Isolation and presumptive identification of Bacillus spp.

B. subtilis BDSA1 was isolated from polluted water samples collected from the Sitalakshya river, Dhaka, Bangladesh (23°43′18.48"N; 90°30′1.8"E) [24]. Briefly, collected samples were diluted (10^{-01} to 10^{-08}) using normal saline. Each dilution was spread on Luria-Bertani agar (LB) supplemented with 50 ppm chromium ($K_2Cr_2O_7$) and incubated at 37 °C for 24hrs. The glycerol stock (25 %) of the pure culture of this bacterium was stored at −80 °C for long-term maintenance. The isolate was retrieved from stock by inoculating a loopful of inoculum on LB agar followed by incubation at 37 °C for 24–48hrs. Then, several biochemical tests, including catalase, oxidase, IMViC, carbohydrate fermentation, gelatin, starch hydrolysis and hemolysis tests were done for presumptive identification with the help of Bergey's Manual of Systematic Bacteriology [25].

2.2. Tolerance to heavy metal

The extent of metal tolerance of BDSA1 was determined according to the procedure described earlier [26]. A single colony of freshly grown bacteria from LB agar (without metal supplementation) was inoculated in 10 ml LB broth supplemented with varying concentrations of chromium or cadmium (100–700 ppm) and incubated overnight at 37 °C. LB broth with no heavy metal was used as a control. Bacterial growth was determined by measuring OD at 600 nm. The minimum concentration of the heavy metals at which no turbidity was observed was considered as the Minimum Inhibitory Concentration (MIC).

2.3. Reduction of hexavalent chromium

The bacterial ability to reduce hexavalent chromium was estimated using 1,5-diphenylcarbazide (DPC) method [27]. The isolate was grown in 50 ml LB broth amended with 100 ppm Cr for 24hrs and cultures were harvested by centrifugation at 6000×*g* for 10 min. After adding DPC reagent to the collected supernatant from control and test cultures, the absorbance of the color produced by Cr^{6+}-DPC complex was measured spectrophotometrically at 540 nm. A standard curve was prepared to estimate the concentration of Cr (VI) in the samples.

2.4. Biosorption of cadmium

Cadmium biosorption potential of BDSA1 was determined by following the protocol as described by Syed et al., 2015 [28]. The tested isolate was inoculated on LB broth containing 200 ppm cadmium and kept at 37 °C for 7 days. Then, the culture was centrifuged at 8000×*g* for 10 min and the concentration of cadmium in the supernatant was measured by Atomic absorption spectrometry (AAS). Finally, the biosorption efficiency of the bacteria was calculated using the following formula.

$$\left(C_i - C_f\right) / C_i \times 100\ (\%)$$

where C_i is the initial concentration, and C_f is the final concentration of cadmium.

2.5. Detection of Cr and Cd resistant genes

Genomic DNA from *B. subtilis* BDSA1 was extracted using the Monarch Genomic DNA purification kit (New England Biolabs) according to the manufacturer's instructions. The concentration and purity of genomic DNA were measured using Nanodrop 2000 spectrophotometer (Thermo scientific, USA). The extracted DNA was then subjected to polymerase chain reaction using gene (*chrA* and *czcC*) specific primer. The details of primer sequences, PCR conditions, and amplicon size are available in Supplementary Table 1. The total volume of each PCR reaction was 25 μL, consisting of 12.5 μL 2X mastermix, 1 μL forward primer, 1 μL reverse primer, 8.0 μL nuclease-free water and 2.5 μL template DNA. The amplified products were separated and visualized on 1.5 % agarose gel electrophoresis.

2.6. Antibiotic susceptibility pattern

The susceptibility of *B. subtilis* BDSA1 to a wide array of antibiotics was assessed by following the Kirby-Bauer disc diffusion method [29]. The used discs were gentamycin (CN10), streptomycin (S10), imipenem (IMI10), meropenem (MRP10), vancomycin (VA30), ampicillin (AMP10), chloramphenicol (C10), doripenem (DOR10), tetracycline (TE30), colistin (CT10), cefotaxime (CTX30), and ceftriaxone (CRO30). Freshly grown bacteria standardized to 0.5 McFarland was evenly spread on Mueller-Hinton agar using sterile cotton swab. Antibiotic discs were placed on the surface of the bacterial lawn and incubated at 37 °C for 16–18hr. Then, the diameter of the zone of inhibition was measured and classified as sensitive (S), intermediate (I), and resistant (R) in accordance with the guidelines of Clinical and Laboratory Standards Institute (CLSI) [30].

2.7. Bacterial genome sequencing and assembly

Bacterial DNA was sent to Macrogen Genome Center, South Korea for paired-end whole genome sequencing using Illumina NextSeq 500 platform. The quality parameters of raw sequences were checked using FastQC. Following adapter trimming by Trimmomatic v0.36 [31], quality-filtered trimmed reads were assembled using SPAdes genome assembler v3.15.2. using –isolate parameters for high coverage isolate and multi-cell Illumina data. Finally, the quality of the assembled genome was checked with QUAST v5.2.0 [32]. Contigs size <200bp were removed and the draft genome was submitted to NCBI GenBank.

2.8. Phylogenetic analysis and functional annotation

The assembled genome was uploaded to the Type (Strain) Genome Server (TYGS) [33] and The Genome Taxonomy Database (GTDB)-Tk v2.3.2 [34] for whole genome-based taxonomic identification and classification of bacteria. TYGS also constructed a whole-genome phylogenetic tree based on the Genome Blast Distance Phylogeny (GBDP) approach. Further phylogenetic analysis was conducted using The Bacterial Genome Tree Service integrated in BV-BRC, using codon tree method [35] and visualized using iTOL [36]. PubMLST (Public databases for molecular typing and microbial genome diversity) [37] and K-merFinder v3.2 [38] tools were employed to identify and determine the sequence type (ST) of the isolate. Genome annotation and identification of target genes were accomplished using RAST (Rapid Annotation using Subsystem Technology) webserver [39] and PROKKA (Galaxy Version 1.14.6) [40] integrated in Galaxy with default parameters. EggNOG-mapper v2 [41] was utilized for genome-wide functional annotation and determining the composition of the cluster of orthologous genes (COG). Moreover, we also used KEGG annotations in the KEGG Automatic Annotation Server (KAAS) v2.1 [42] with the Bidirectional Best Hits (BBH-method) to study the metabolic pathways in the assembled genome.

2.9. Detection of antimicrobial- and metal resistance associated genes

Contigs in the assembled genome were screened for determining antimicrobial resistance genes using ABRicate with different databases including CARD [43] and ResFinder [44] (Minimum DNA identity and coverage = 80 %). Genes associated with metal resistance were screened by performing blastP using the BacMet (version 2.0) database [45].

2.10. Characterization of virulence and other properties

The Virulence Factor Database (VFDB) [46], PathogenFinder v1.1 [47], PHASTER [48], CRISPRFinder [49] and PlasmidFinder [50] were utilized to detect bacterial virulence factors, pathogenic capability, prophage, CRISPR and plasmid sequences, respectively in the draft genome. The genome mining for the identification of secondary metabolites biosynthetic gene cluster and bacteriocins were performed with the help of AntiSMASH (bacterial version) [51] and BAGEL4 [52].

2.11. Pan-genome analysis

The genome sequences of 36 different strains of *B. subtilis* were downloaded from NCBI Genbank database (Supplementary Table 2). The sequences were subjected to pan-genome analysis using IPGA v1.09 [53] and PanExplorer [54]. Roary [55] was selected for genome clustering and pan-genome analysis procedures.

3. Results and discussion

Heavy metal pollution substantially threatens human health, food production, and the natural environment globally. The search for novel microbial strains by exploring indigenous sources is essential for enabling innovative measures to counter environmental pollution. Using beneficial microorganisms for bioremediation, enhancement of plant growth, and production of valuable metabolites can facilitate the development of sustainable strategies for the environment, agriculture, and industry. Therefore, a heavy metal-resistant *B. subtilis* was characterized through laboratory experiments and *in silico* genome exploration that provide adequate genomic insights into different biological characteristics of the strain BDSA1. Fig. 1 illustrates the workflow of this study.

3.1. Phenotypic characterization of metal- and antibiotic resistance of BDSA1

The *B. subtilis* BDSA1 could tolerate up to 600 ppm of cadmium and chromium, though the growth tend to decrease with the increasing metal concentration (Supplementary Fig. 1). The finding was in agreement with previous studies that reported the tolerance range of chromium reducing microorganisms between 100 ppm and 4000 ppm [56,57]. Such broad variations in the tolerance level might be due to chemical composition of the media and its consistency (solid or liquid), type of microorganism, microbial adaptation and resistance mechanisms, environmental factors and others [58]. For example, the toxicity of metals is greater in liquid than solid media due to more free availability of metals in liquid cultures [59].

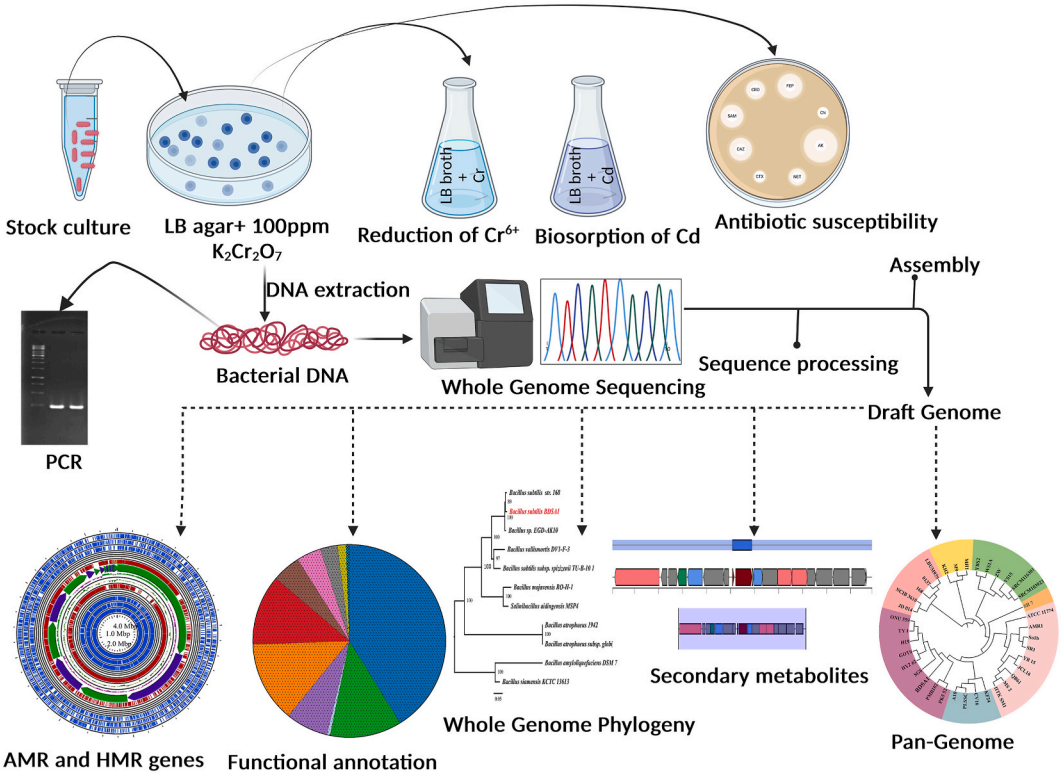

Figure 1. Graphical representation of the entire work

The isolate reduced 40 % of 100 ppm hexavalent Chromium to non-toxic trivalent form within 24hrs at 37 °C at pH = 7.0. The chromium reduction ability of *B. subtilis* is well-documented. Earlier studies reported the influence of numerous factors such as temperature, pH of the media, initial chromium concentration, presence of other heavy metals, exposure time, and others on the extent of chromium reduction by different strains of *B. subtilis*. For example, *B. subtilis* MNU16 reduced 75 % of 50 ppm of Cr(VI) at 30 °C within 72hrs [60]. *B. subtilis* CRB-1 achieved a 100 % reduction of 50 ppm Cr (VI) at pH range 7–9.0, temperature 42 °C with aerobic condition [61]. Alkaliphilic *B. subtilis* also caused complete chromium reduction at pH = 9.0 [57]. On the other hand, *B. subtilis* QH-1 reduced only 23.22 % of 100 ppm initial concentration of Cr (VI) at pH = 7.0 within 24hrs [62]. Overall, a comparison of this evidence indicated that optimization of media pH, incubation time and temperature, and initial Cr (VI) concentration might result in greater chromium reduction by BDSA1.

The *B. subtilis* BDSA1 also caused adsorption of 43 % of 200 ppm cadmium after 7 days of incubation at 37 °C, similar to *B. subtilis* strain NSPA13 and KC6, though the percentage of adsorption varied depending on experimental conditions and initial cadmium concentration [13,63].

We confirmed the presence of *chrA* and *czcC* gene in bacterial genomic DNA, as evident from distinct band at 214 and 232 bp, respectively in agarose gel (Supplementary Fig. 2). The occurrence of *chrA* gene reaffirmed the chromate resistance characteristic of BDSA1. Zhu et al., 2019 demonstrated the involvement of *chrA* (chromate efflux pump) in bacterial chromate resistance by heterologous expression [61].

The bacterium exhibited resistance to the action of streptomycin, ceftriaxone, cefotaxime and sensitivity to gentamicin, ampicilin, imipenem, meropenem and trimethoprim. Intermediate resistance was observed to tetracycline, chloramphenicol and vancomycin (Supplementary Table 3). Table 1 provides a comparison of the antibiotic resistance profiles and hemolytic characteristics on blood agar of different reported strains of *B. subtilis*.

3.2. Genomic identification and characterization of BDSA1

Whole genome sequencing (WGS) has emerged as a powerful tool for accurate characterization and functional analysis of bacterial strains at the genomic level [70]. The total size of the assembled genome of BDSA1 was 4.2 Mb, distributed into 47 contigs with 43.4 % GC content (Table 2). Whole genome taxonomic identification using TYGS and GTDB revealed the isolate as *Bacillus subtilis*. Codon tree-based phylogenetic analysis suggested that *B. subtilis* BDSA1 was similar to *B. subtilis* strain 168 (Fig. 2A), whereas GBDP based whole genome phylogenetic tree identified *B. subtilis* NCIB 3610 as the closest neighbor of BDSA1 (Fig. 2B). Analysis of sequence data using pubMLST (Locus*: ilvD, tpiA, pycA, rpoD, glpF, pta, purH*) and K-merFinder also confirmed the identification of *Bacillus subtilis.* As MLST-2.0 predicted the housekeeping genes *pycA and purH* as novel allele, the isolate might be designated as a novel sequence type (ST). Nowadays, ANI and dDDH are used to identify bacterial species in which an ANI value of ≥96 %, and a *in silico* ddH value of ≥70 % are considered cut-off for species delineation. *B. subtilis* BDSA1 had dDDH value = 88.9 % and ANI value = 98.47 % with *B. subtilis* NCIB 3610, validating the whole genome identification (Fig. 3A) [71].

According to PathogenFinder, the probability of the *B. subtilis* BDSA1 being a human pathogen is 0.296, in a scale of 1 as the highest value. The PHASTER server predicted genome regions that contained one intact, four incomplete, and one questionable prophage sequences (Supplementary Table 4). PlasmidFinder identified two plasmid sequences in BDSA1 genome that did not have any heavy metal or antibiotic resistant genes. As evident from the MGEFinder, two copies of insertion sequences, ISBpu1 were found in node_3 and node_6. Both sequences were transposases that originated from *B. pumilus* and carried the *aadK* gene and *mphK* gene. Presence of two more insertion sequences ISSep2, from *Staphylococcus epidermidis* and *Proteus vulguris* and ISSau6 from *Staphylococcus aureus* were also detected in node 13. The genome also had three confirmed CRISPR regions. VFDB analysis resulted in detection of only one virulence factor, *bslA* (hydrophobin). The genes encoding virulence factors such as hemolysin, enterotoxin, cytotoxin and other toxins were not found in the genome.

3.3. Genome annotation of BDSA1

Genome annotation using RAST predicted 4438 protein coding sequences and 37 tRNA in the draft assembly, whereas PROKKA-based annotation detected 4227 CDS, 6 rRNA and 43 tRNA in the draft genome (Fig. 3B). The discrepancy in the number of predicted coding sequences might be due to differences in their underlying algorithms, reference databases, and criteria for gene calling [39,40]. RAST-annotation revealed a total of 330 subsystems including carbohydrates, protein metabolism, amino acids and derivatives, co-factors, vitamins and others (Fig. 4A).

Table 1
Comparison of antibiotic resistance and hemolytic activity in various B. subtilis strains

B. subtilis strains	Antibiotic resistance	Hemolytic activity	Reference
BDSA1	Streptomycin, Ceftriaxone, Cefotaxime	Non-hemolytic	This study
RZS-01	Nor-floxacin and Amoxicillin	na	[64]
G8	Tiamulin, Oxacillin, Lincomycin	na	[65]
MBTDCMFRI Ba37	Bacitracin, Ampicillin and Colistin	Non-hemolytic	[66]
CU1	No resistance	Non-hemolytic	[67]
VKPM B2335 (BS3)	Chloramphenicol, Oxacillin, Cefotaxime, Ceftriaxone	Non-hemolytic	[68]
IDCC1101	Streptomycin	Non-hemolytic	[69]

Table 2
Different characteristics of *B. subtilis* BDSA1 assembled genome

Characteristics		Terms
Taxonomy		Firmicutes>Bacilli>Bacillales>Bacillaceae>*Bacillus*>*Bacillus subtilis*
Genome statistics	Number of contigs	47
	Size	4,221,385 bp
	GC content (%)	43.4
	Contig N50 value	502218
	Contig L50 value	3
Genomic features	CDS	4438
	tRNA	37
	rRNA	9
Genome quality	Completeness	100%
	Contamination	0.4%
	Overall remark	Good
Annotation features	Transporter (TCDB)	326
	Drug target (Drug Bank)	71
	Antibiotic resistance (PATRICK)	44
Protein features	Proteins with functional assignments	3658
	Proteins with EC	1077
	Proteins with GO assignments	902
	Proteins with Pathway assignments	794
	Hypothetical proteins	780
Genome availability	Bio Project	PRJNA898679
	Bio Sample	SAMN31620504
	GenBank accession	JAPFBY000000000.1

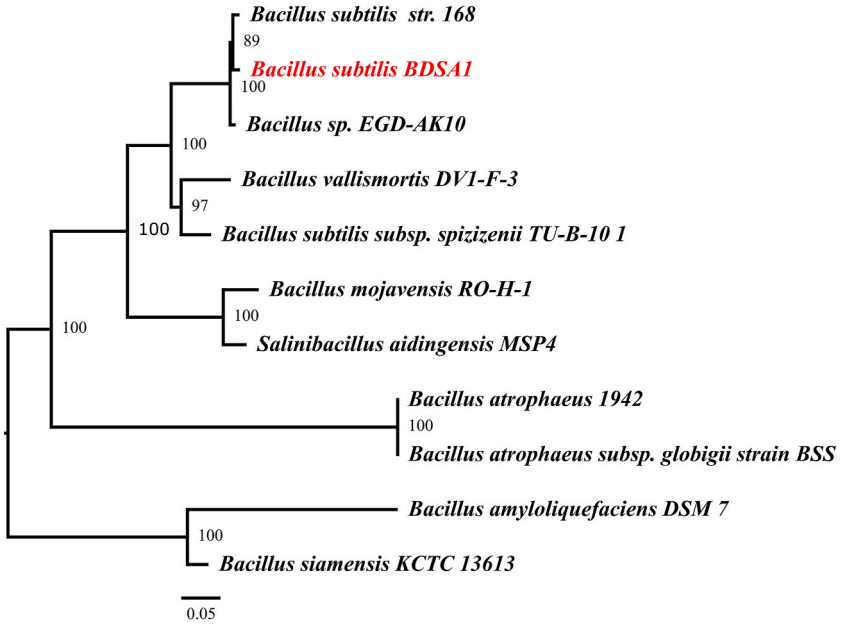

Figure 2. aPhylogenetic analysis of *B. subtilis* BDSA1 based on codon tree in PATRIC

Based on EggNOG mapping, 16.21 % of proteins were involved in information storage and processing activities including replication, transcription, translation, ribosomal biogenesis, recombination and repair. 14.85 % of proteins were related to cellular processes and signaling, including activities such as cell wall biogenesis, signal transduction, cell cycle regulation, intracellular trafficking, and post-translational modification. Additionally, about 29.82 % of proteins were associated with metabolic functions (amino acid transport, carbohydrate metabolism, energy production, conversion, and inorganic ion transport) (Fig. 4B). 26.01 % of proteins were poorly characterized. KEGG pathway enrichment analysis suggested that most of the pathways were associated with metabolism of carbohydrate, amino acid, lipid, energy and biosynthesis of other compounds (Fig. 4C). In addition, BDSA1 harbored 39 genes that were linked to xenobiotics degradation and metabolism pathways including, benzoate, aminobenzoate, xylene, naphthalene, styrene, atrazine, and DDT degradation. Similar to our findings, Li et al., 2019 and Kumar et al., 2023 identified 37 and 34 genes in *B. subtilis* DM2 and *B. subtilis* EB-1 respectively that might be responsible for the degradation of petroleum hydrocarbon and xenobiotics [20,72].

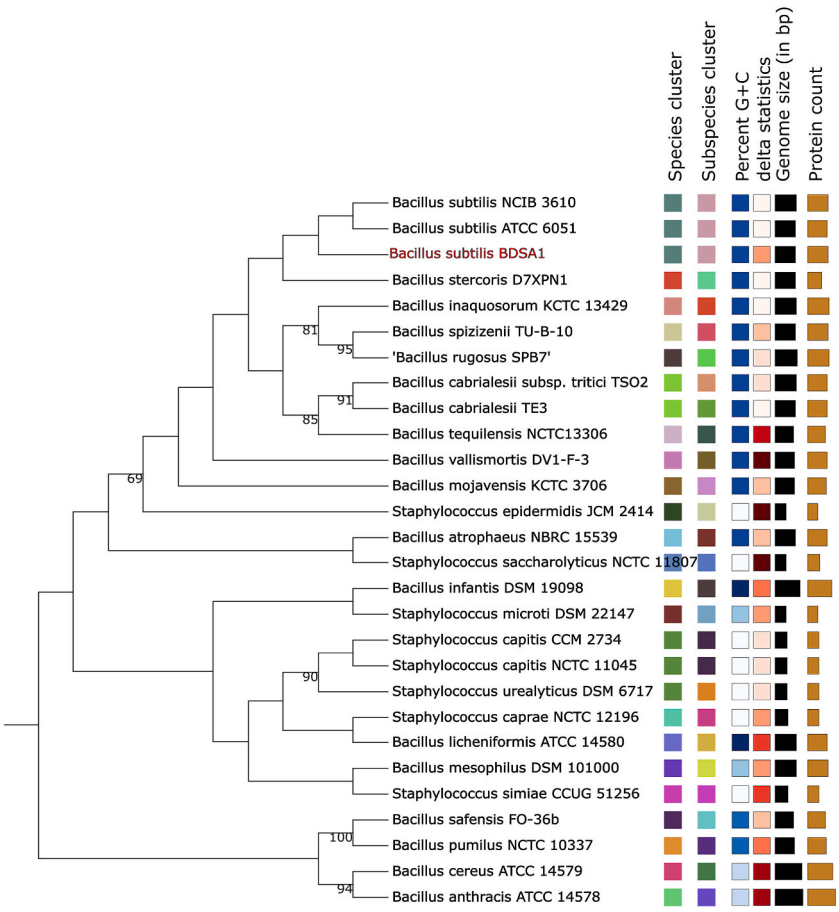

Figure 2. bPhylogenetic analysis of *B. subtilis* BDSA1 based on Genome Blast Distance Phylogeny (GBDP) approach in TYGS

Genome annotation of BDSA1 also showed the presence of different genes associated with resistance to oxidative and osmotic stress, metabolic regulation, biofilm formation, and production of degradative enzymes (Table 3). For example, BDSA1 had *opuA* operon (multicomponent glycine betaine transport system), consisting of three structural genes that allow bacteria to uptake osmoprotectant [73]. Biofilms of *B. subtilis* have been associated with probiotic and biocontrol activities, as well as potential industrial applications [74]. *tasA* and *bslA*, encoding amyloid fiber protein and self-assembling bacteria hydrophobin, are required for biofilm formation in *B. subtilis* and their presence in BDSA1 indicated its biofilm-forming ability [75]. The strain also carried genes for production of degradative enzymes such as endoglucanase, phytase, amylase, xylanase that have potential applications in lignocellulosic biomass degradation, poultry production and others [76].

B. subtilis BDSA1 harbored numerous genes involved in plant growth promoting mechanisms including regulation of auxin production, GABA (Gamma-aminobutyric acid) formation, spermidine acetyltransferase, siderophore production. Prior studies utilized genome analysis for determining the biocontrol potential of different strains of *B. subtilis*, notably Bbv57, MBI600, CTXW 7-6-2 against phytopathogens [22,23,77]. Phosphate solubilization-associated genes *pstA*, *pstB*, *pstS* were identified in the BDSA1 genome, which together account for the phosphate transport system. Based on the occurrence of these genes, other studies also reported *B. subtilis* RS10, *B. subtilis* EA-CB0575, and *B. subtilis* QH-21 as phosphate solubilizers [18,62,78]. Iqbal et al., 2021 identified the phosphate transport system in *B. subtilis* RS10 genome and their role in plant growth was experimentally validated through *in vitro* experiments [78]. Siderophore-producing bacteria increase the availability of iron for plant development [79]. Siderophore-formation genes in the BDSA1 genome suggested its ability to sequester rhizospheric iron for plant metabolism. Annotated genome also found to carry genes for tryptophan synthase, anthranilate phosphoribosyltransferase, and phosphoribosylanthranilate isomerase that function in tryptophan synthesis and regulation of auxin biosynthesis. Taghavi et al., 2010 demonstrated the association between tryptophan-related genes and auxin formation [80]. In addition, spermidine production by *B. subtilis* inhibited ethylene production, affecting plants-microbe interactions [81]. Overall, the genomic characteristics of BDSA1 suggested its potential in plant growth promotion which needs further experimentation to elaborate the extent of PGPR activities.

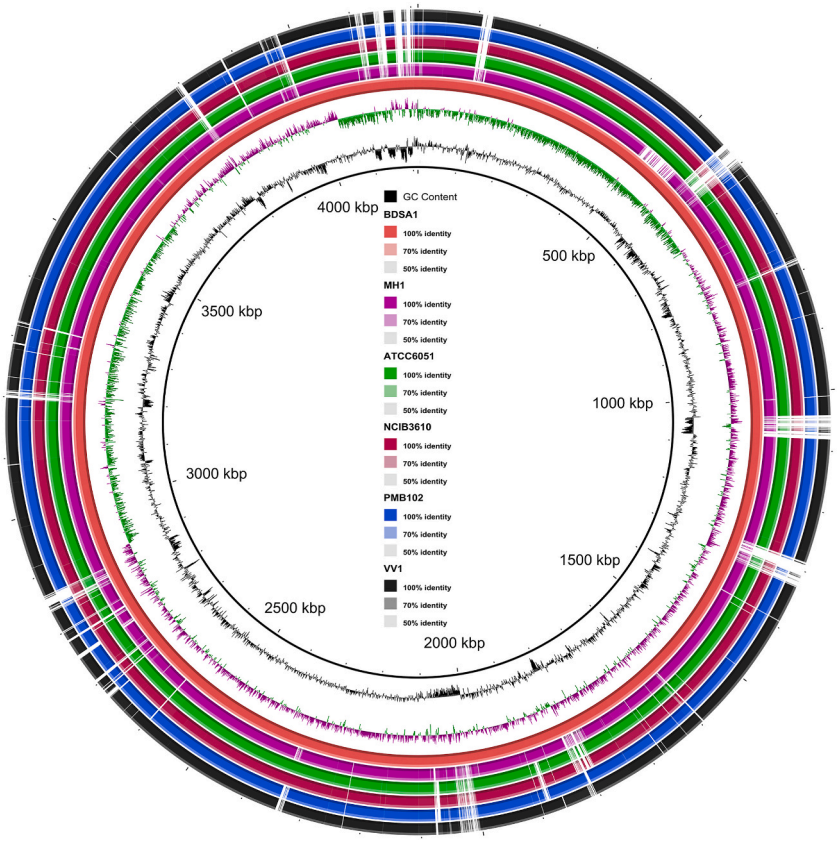

Figure 3. aWhole genome comparison of BDSA1 with closely related strains

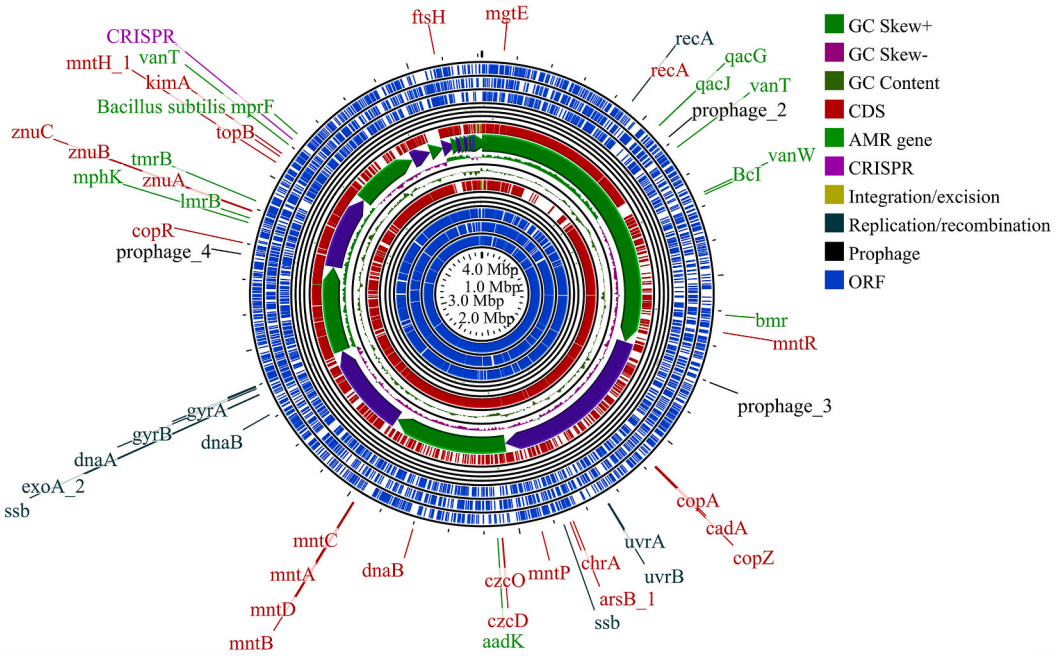

Figure 3. bCircular genome map of *B. subtilis* BDSA1 with different annotation characteristics of the isolate

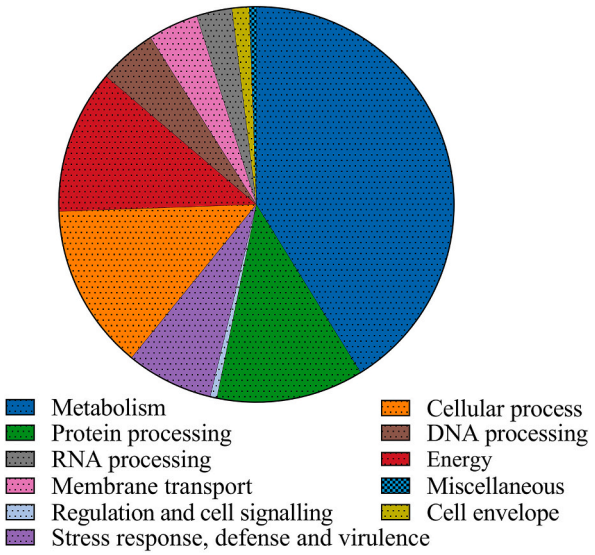

Figure 4. aFunctional annotation of *B. subtilis* BDSA1: Distribution of subsystems by RAST

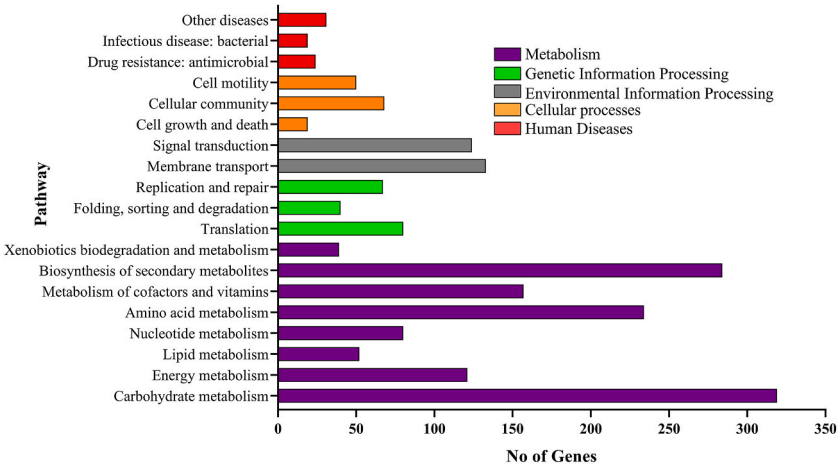

Figure 4. bFunctional annotation of *B. subtilis* BDSA1: Pathway enrichment analysis

3.4. Genomic analysis of AMR and HMR properties

The search for AMR genes in BDSA1 genome against CARD database yielded no perfect hit and 16 strict hits for AMR genes, whereas, according to ResFinder analysis, only 2 acquired resistance genes, namely *aadK* (aminoglycoside 6-adenylyltransferase) and *mphK* (macrolide phosphotransferase) were detected. The variations in the number of AMR gene predictions could occur because CARD encompasses both intrinsic and acquired resistance genes, leading to a higher count, whereas ResFinder targets mainly acquired resistance genes, contributing to the observed discrepancies [82]. However, phenotypic resistance to ceftriaxone, cefotaxime and aztreonam aligned with the CARD-based AMR analysis. The acquired resistance gene *aadK* encodes a streptomycin-modifying enzyme and thus confers streptomycin resistance [83]. Overall, the results of antibiotic susceptibility testing were consistent with *in silico* AMR analysis.

Adaptation and resistance to heavy metals is well-recognized in *B. subtilis*. A number of metal resistance associated genes, such as *chrA*, *czcD*, *arsB*, *cadA* were found that were responsible for bacterial resistance to chromium (Cr), cadmium (Cd), arsenic (As), Copper (Cu), manganese (Mn), Nickel (Ni) etc (Table 4). Among these genes, *cadA* encodes cadmium-translocating ATPase, which is thought to be a multifunctional metal-exporting pump for the extrusion of Zn^{2+}, Cd^{2+}, Ag^{2+}, and Pb^{2+} [84]. Copper-translocating P-type ATPase (*copA*), Cobalt-zinc-cadmium resistance protein (*czcD*), Arsenical pump membrane protein (*arsB*), were also present in tested genome. The three copper resistance-associated genes in BDSA1 were arranged (*csoR-copZ-copA*) in the same way as the copper resistance system seen in *B. subtilis* and *B. megaterium* [85]. Besides, *czrA* regulon responds to different mono and divalent cations that consist of *cadA* and *czcD*, a determinant of resistance to Zn(II), Cu, Ni(II), and Co(II) [86]. We also observed manganese transport systems

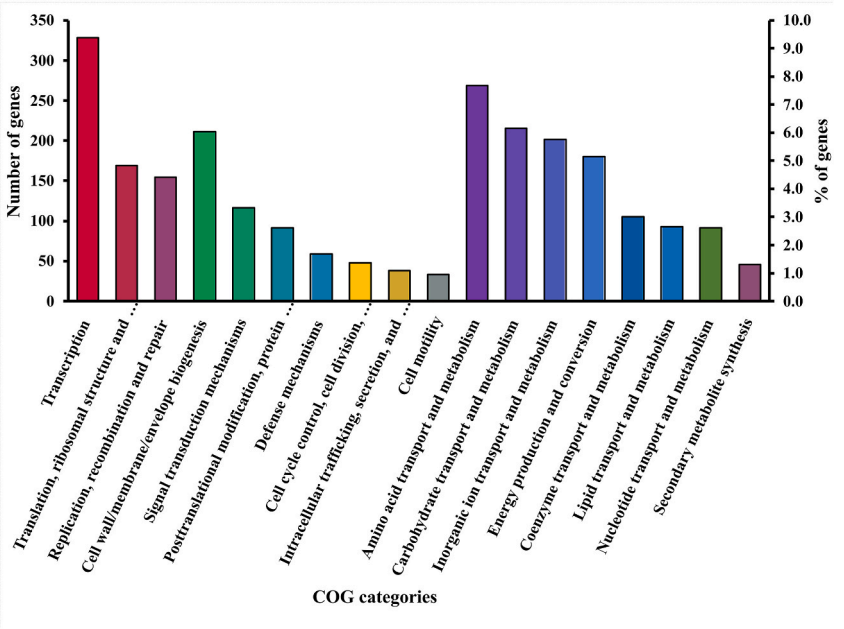

Figure 4. cFunctional annotation of *B. subtilis* BDSA1: COG annotations

Table 3
Representative genes related to biocontrol properties of *B. subtilis* BDSA1

Gene	Product/Pathway involvement	Description
Opu operon (opuAA, opuAB, opuAC)	High affinity osmopro- tectant glycine betaine uptake system	**Resistance to osmotic, oxidative and others abiotic stress**
yfkM, yugL	General stress protein	
nsrR,	Nitrosative resistance gene	
perR	Peroxide operon regulator	
nasABCDE	Nitrate assimilation operon	**Nitrogen metabolism**
pstA, pstB, pstS	Phosphate transport	**Phosphate solubilization**
dhbABCD	Bacillibactin biosynthesis	**Siderophore formation**
yclNOPQ	Ferric-Petrobactin uptake operon	
eglS	Endoglucanase	**Lytic/Degradative enzyme**
xynABC, xynD	Xylanase	
csn	Chitosanase	
phy	3-phytase	
tasA,	Biofilm matrix component	**Biofilm formation**
bslA, bslB	Biofilm surface layer protein	
alsS. alsD	Acetoin biosynthesis	**Plant growth promotion and regulation**
treA, treP, treR	Trehalose biosynthesis	
trpABCD, trpF	Tryptophan biosynthesis and regulation of auxin biosynthesis	
gabD, gabP	Biosynthesis of gamma-aminobutyric acid (GABA)	
pelC	Pectate lyase	
paiA, bltD	Spermine/spermidine acetyltransferase	

encoded by *mntABCD* and *mntH* along with their regulator *mntR* and manganese efflux *mneS*, *mneP* in different contigs of the genome.

3.5. Secondary metabolites analysis

B. subtilis produces a wide range of chemically diverse secondary metabolites exerting diverse biological activities [87]. The BDSA1 genome was found to carry six biosynthetic gene clusters encoding bacillaene, bacilysin, fengycin, bacilibactin, subtilosin and surfactin. Occurrence of these gene clusters is often reported in different strains of *B. subtilis* and in others species of *Bacillus* genus, though little information is available regarding their detection, quantification and antimicrobial use [23,77]. These gene clusters were composed of core and additional biosynthetic genes, transport-related genes, regulatory genes and others (Fig. 5). Fengycin and surfactin are lipopeptides produced by *B. subtilis* strains that are known to have a broad range of antifungal and antibacterial activities, respectively [88,89]. Expression of the genes for biosynthesis of a catechol siderophore, named bacillibactin, allows *B. subtilis* to

Table 4
Some notable antimicrobial and metal-resistance genes and their mechanism of actions

Gene		Location				Function	Resistance	Mechanism of resistance/Pathway involvement
		Start (bp)	End (bp)	Strand	Node			
Antimicrobial resistance genes	*ykkC*	41106	41444	+	1	Small multidrug resistance efflux protein	Aminoglycoside Phenicol Tetracycline	Antibiotic efflux
	ykkD	41444	41761	+				
	rphB	773517	776113	-		Rifampin phosphotransferase protein	Rifamycin	Antibiotic inactivation
	bmr	1110143	1111312	+		MFS efflux pump	Fluoroquinolone phenicol	Antibiotic efflux
	blt	39890	41092	+			Fluoroquinolone	
	aadK	59216	60070	-	3	Aminoglycoside nucleotidyltransferase	Aminoglycoside	Antibiotic inactivation
	mphK	77240	78160	+		Macrolide phosphotransferase	Macrolide	
	lmrB	90052	91485	-	6	chromosomally-encoded efflux pump	Lincosamide	Antibiotic efflux
	tmrB	140609	141202	-		ATP-binding tunicamycin resistance protein	Nucleoside	Reduced permeability
	mprF	27270	29840	-		Integral membrane protein that modifies phosphatidylglycerol	Peptide antibiotic	Antibiotic target alteration
Metal resistance associated genes	*chrA*	589,219	589,755	+	2	Chromate transport protein	Chromium	Responsible for induction of resistance
	copA	307,753	310,167	-	2	Copper-translocating P-type ATPase	Copper	Activation of copZA operon by CueR and repressed by YfmP
	czcD	45,711	46,748	-	3	Cobalt-zinc-cadmium resistance protein CzcD	Cadmium, Zinc, Cobalt	Efflux of metal ion
	mntR	1,158,929	1,159,357	-	1	Transcriptional regulator	Manganese, Magnesium	Represses the expression of Mn(II) uptake
	czrA	794,932	795,255	-	1	A repressor for the czr operon	Zinc, Cadmium	Part of the czrSRCBA resistance operon
	arsB	580,023	581,237	+	2	Arsenical pump membrane protein	Arsenic	Arsenite extrusion pump
	cadA	305,484	307,583	-	2	Cadmium efflux ATPase	Cadmium, Zinc	Chromosomal determinant to cadmium resistance
	csoR	310,531	310,836	-		Copper-sensing transcriptional repressor	Copper	Negatively regulates expression of the copZA operon
	corA	1,175,964	1,176,917	-		Cobalt/magnesium transport protein	Magnesium, Cobalt, Nickel, Manganese	Mediates magnesium ions influx and efflux as well as cobalt and nickel uptake

compete with other microbes for iron acquisition in iron-deficient conditions [90]. Bacillaene is a polyketide that inhibits protein synthesis and, thus, exerts antibacterial activity, whereas bacilysin show antimicrobial effect by disrupting the cell membrane integrity [91,92]. In addition, BAGEL4 predicted 5 gene clusters of interest linked to the production of secondary metabolites such as UviB, comX4, subtilosin, Lanthipeptide_class_IV and sactipeptides. Subtilosin A, a ribosomally synthesized and post-translationally modified peptide, proved to interfere with the phospholipid bilayer and thus showed a broad spectrum of antibacterial activity [93].

3.6. Pan-genome analysis

Roary-based pan-genome analysis showed 7348 clusters, including 1574 strain-specific genes (21.4 %), 2225 core genes (30.3 %) and 3549 dispensable genes (48.3 %) (Fig. 6A). In the core-pan rarefaction curve, it was evident that the number of the pan genome genes increased gradually with the addition of new strains, whereas the core genome exhibited the opposite trend (Fig. 6B). No distinct plateau in core/pan-genome ratio indicated the open state of the *B. subtilis* pan-genome. The finding is consistent with earlier studies [94]. According to ANI-based pan-genome analysis, *B. subtilis* BDSA1 was found to be closely related to other tested isolates with ANI values of 98 % or higher except strain CU1 and CW14 (92.6 %) (Fig. 6C). Functional classification of Pan-genome by COG's distribution indicated that majority of gene clusters were associated with Inorganic ion transport and metabolism (P), Amino acid transport and metabolism (E), Signal transduction mechanisms (T), Energy production and conversion (C) (Fig. 6D). Moreover, phylogenetic tree generated by hierarchical clustering from binary matrix (presence/absence) of accessory genes suggested that BDSA1 occurred in the same clade with strains SG6, PMB102, HY2, GOT9, FY-1 etc (Fig. 6E).

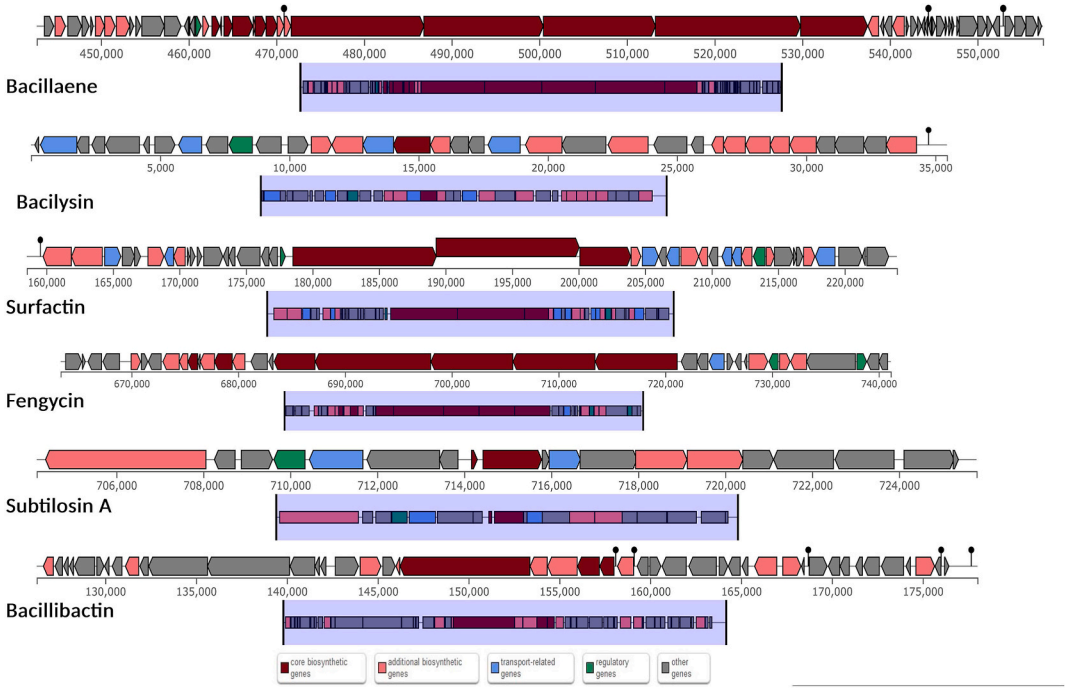

Figure 5. Schematic diagram of secondary metabolites biosynthetic gene clusters in *B. subtilis* BDSA1

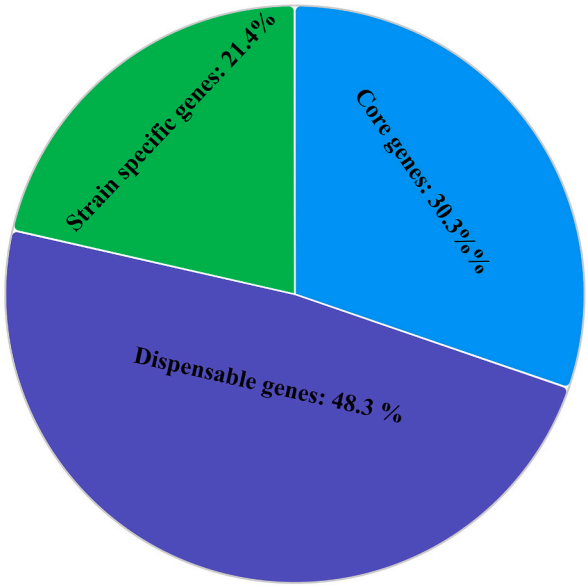

Figure 6. aPan-genome analysis of *B. subtilis* strains: Distribution of core and accessory genes,

4. Conclusion

Our study performed a comprehensive genomic analysis of native *B. subtilis* BDSA1 to uncover the potential biocontrol properties and to assess its safety. The whole genome analysis reveals the presence of an array of genes associated with bioremediation, plant growth promotion, secondary metabolites formation and indicates the diverse biotechnological applications that this isolate might possess. Phenotypic data of chromium reduction and cadmium absorption also supports the potential, which needs further evaluation of optimum experimental parameters. However, future works on experimental validation of *in silico* identified characteristics, production of secondary metabolites, application for plant growth development, and others can determine the suitability of this strain for

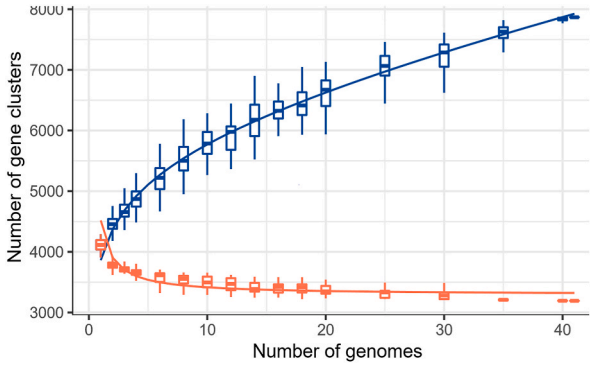

Figure 6. bPan-genome analysis of *B. subtilis* strains: Pan-genome size rarefaction curve

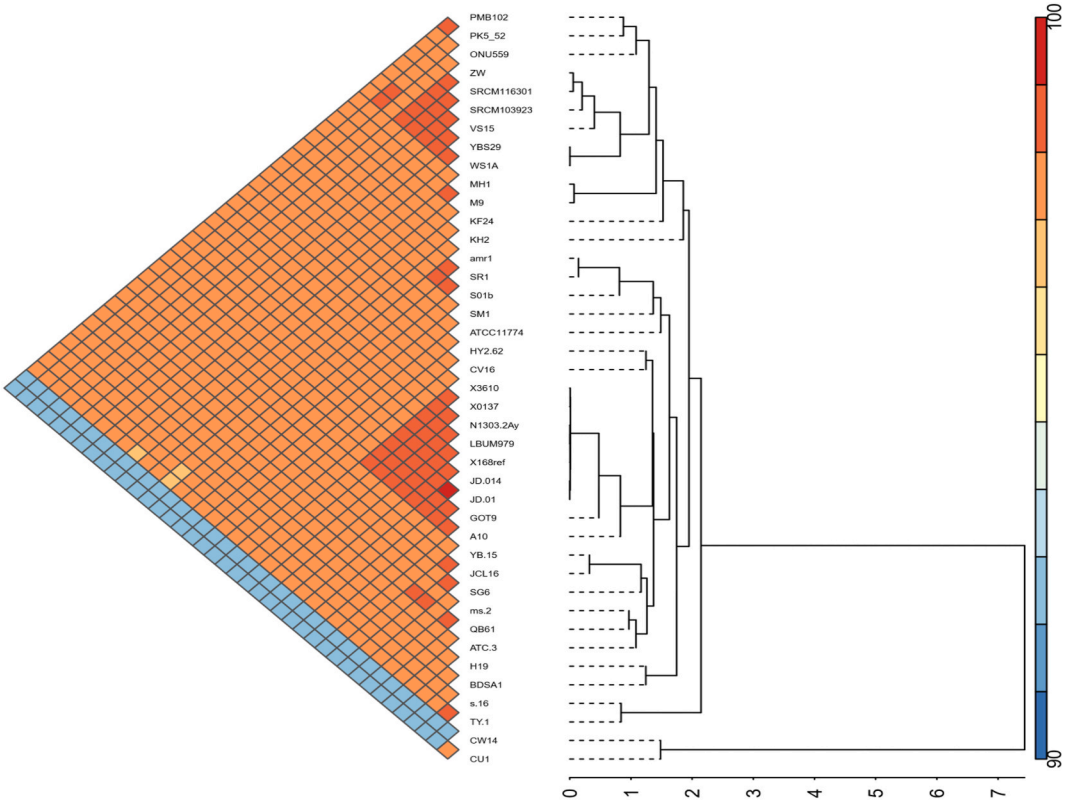

Figure 6. cPan-genome analysis of *B. subtilis* strains: Comparison of the ANI values between strains

industrial-scale use.

Declarations

The authors declared no conflict of interests.

Data availability statement

The genome sequence of *Bacillus subtilis* BDSA1, as discussed in the manuscript, has been deposited in the NCBI GenBank under the accession number JAPFBY000000000.1.

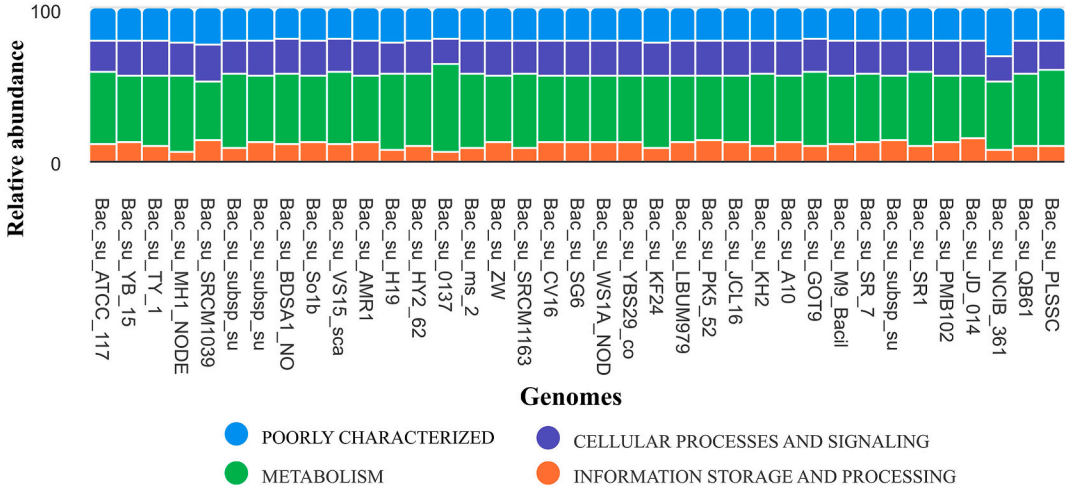

Figure 6. dPan-genome analysis of *B. subtilis* strains: COG's distribution

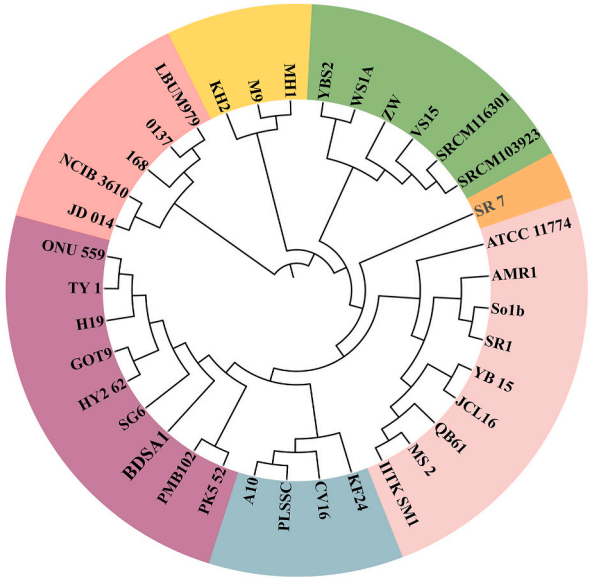

Figure 6. ePan-genome analysis of *B. subtilis* strains: Pan-genome phylogenetic tree *na=not available

CRediT authorship contribution statement

Tanvir Ahmed Saikat: Writing – original draft, Methodology, Formal analysis, Data curation. **Md Abu Sayem Khan:** Writing – original draft, Methodology, Formal analysis, Data curation. **Md Saiful Islam:** Formal analysis. **Zarin Tasnim:** Formal analysis. **Sangita Ahmed:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34369.

References

[1] J. Briffa, E. Sinagra, R. Blundell, Heavy metal pollution in the environment and their toxicological effects on humans, Heliyon 6 (2020) 1183691.
[2] M.J. Uddin, Y.-K. Jeong, Urban river pollution in Bangladesh during last 40 years: potential public health and ecological risk, present policy, and future prospects toward smart water management, Heliyon 7 (2021) 1183691.
[3] P. Vats, U.J. Kaur, P. Rishi, Heavy metal-induced selection and proliferation of antibiotic resistance: a review, J. Appl. Microbiol. 132 (2022) 4058–4076.
[4] S. Siddiquee, K. Rovina, S. Al Azad, L. Naher, S. Suryani, P. Chaikaew, Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: a review, J. Microb. Biochem. Technol. 7 (2015) 384–395.
[5] N.A.A. Qasem, R.H. Mohammed, D.U. Lawal, Removal of heavy metal ions from wastewater: a comprehensive and critical review, Npj Clean Water 4 (2021) 36.
[6] R. Jobby, P. Jha, A.K. Yadav, N. Desai, Biosorption and biotransformation of hexavalent chromium [Cr (VI)]: a comprehensive review, Chemosphere 207 (2018) 255–266.
[7] M.S. Ayilara, O.O. Babalola, Bioremediation of environmental wastes: the role of microorganisms, Front. Agron. 5 (2023) 1183691.
[8] M. Wróbel, W. Śliwakowski, P. Kowalczyk, K. Kramkowski, J. Dobrzyński, Bioremediation of heavy metals by the genus Bacillus, Int. J. Environ. Res. Public Health. 20 (2023) 4964.
[9] W.L. Nicholson, N. Munakata, G. Horneck, H.J. Melosh, P. Setlow, Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments, Microbiol. Mol. Biol. Rev. 64 (2000) 548–572.
[10] Y. Wang, C. Zhang, J. Liang, L. Wang, W. Gao, J. Jiang, R. Chang, Surfactin and fengycin B extracted from Bacillus pumilus W-7 provide protection against potato late blight via distinct and synergistic mechanisms, Appl. Microbiol. Biotechnol. 104 (2020) 7467–7481.
[11] C. Leal, F. Fontaine, A. Aziz, C. Egas, C. Clément, P. Trotel-Aziz, Genome sequence analysis of the beneficial Bacillus subtilis PTA-271 isolated from a Vitis vinifera (cv. Chardonnay) rhizospheric soil: assets for sustainable biocontrol, Environ. Microbiome. 16 (2021) 1–14.
[12] T. Li, Y. He, J. Wang, H. Xiang, X. Xu, C. Li, Z. Wu, Bioreduction of hexavalent chromium via Bacillus subtilis SL-44 enhanced by humic acid: an effective strategy for detoxification and immobilization of chromium, Sci. Total Environ. 888 (2023) 164246.
[13] Y. Xie, N. He, M. Wei, T. Wen, X. Wang, H. Liu, S. Zhong, H. Xu, Cadmium biosorption and mechanism investigation using a novel Bacillus subtilis KC6 isolated from pyrite mine, J. Clean. Prod. 312 (2021) 127749.
[14] M. Mardiyono, S. Sajidan, M. Masykuri, P. Setyono, Bioremediation of nickel heavy metals in electroplating industrial liquid waste with Bacillus subtilis, in: AIP Conf. Proc., AIP Publishing, 2019.
[15] S. Iqbal, F. Begum, A.A. Rabaan, M. Aljeldah, B.R. Al Shammari, A. Alawfi, A. Alshengeti, T. Sulaiman, A. Khan, Classification and multifaceted potential of secondary metabolites produced by Bacillus subtilis group: a comprehensive review, Molecules 28 (2023) 927.
[16] N. Ichikawa, M. Sasagawa, M. Yamamoto, H. Komaki, Y. Yoshida, S. Yamazaki, N. Fujita, DoBISCUIT: a database of secondary metabolite biosynthetic gene clusters, Nucleic Acids Res. 41 (2012) D408–D414.
[17] Z. Su, X. Chen, X. Liu, Q. Guo, S. Li, X. Lu, X. Zhang, P. Wang, L. Dong, W. Zhao, Genome mining and UHPLC–QTOF–MS/MS to identify the potential antimicrobial compounds and determine the specificity of biosynthetic gene clusters in Bacillus subtilis NCD-2, BMC Genom. 21 (2020) 1–16.
[18] N.D. Franco-Sierra, L.F. Posada, G. Santa-María, M. Romero-Tabarez, V. Villegas-Escobar, J.C. Álvarez, Bacillus subtilis EA-CB0575 genome reveals clues for plant growth promotion and potential for sustainable agriculture, Funct. Integr, Genomics 20 (2020) 575–589.
[19] A. Li, M. Wang, Y. Zhang, Z. Lin, M. Xu, L. Wang, M.F.-A. Kulyar, J. Li, Complete genome analysis of Bacillus subtilis derived from yaks and its probiotic characteristics, Front. Vet. Sci. 9 (2023) 1099150.
[20] A.G. Kumar, D. Manisha, N.N. Rajan, K. Sujitha, D.M. Peter, R. Kirubagaran, G. Dharani, Biodegradation of phenanthrene by piezotolerant Bacillus subtilis EB1 and genomic insights for bioremediation, Mar. Pollut. Bull. 194 (2023) 115151.
[21] S. Guo, X. Li, P. He, H. Ho, Y. Wu, Y. He, Whole-genome sequencing of Bacillus subtilis XF-1 reveals mechanisms for biological control and multiple beneficial properties in plants, J. Ind. Microbiol. Biotechnol. 42 (2015) 925–937.
[22] A. Samaras, M. Nikolaidis, M.L. Antequera-Gómez, J. Cámara-Almirón, D. Romero, T. Moschakis, G.D. Amoutzias, G.S. Karaoglanidis, Whole genome sequencing and root colonization studies reveal novel insights in the biocontrol potential and growth promotion by Bacillus subtilis MBI 600 on cucumber, Front. Microbiol. 11 (2021) 600393.
[23] R. Thiruvengadam, K. Gandhi, S. Vaithiyanathan, H. Sankarasubramanian, K. Loganathan, R. Lingan, V.R. Rajagopalan, R. Muthurajan, J. Ebenezer Iyadurai, P. Kuppusami, Complete genome sequence analysis of Bacillus subtilis Bbv57, a promising biocontrol agent against phytopathogens, Int. J. Mol. Sci. 23 (2022) 9732.
[24] T. Shammi, S. Ahmed, Heavy metal tolerance and antibiotic resistance of Bacillus spp. isolated from two major rivers in Bangladesh, Bangladesh, J. Microbiol. 30 (2013) 17–22.
[25] G.M. Garrity, D.J. Brenner, N.R. Krieg, J.R. Staley, B.S. Manual, Systematic bacteriology, Proteobacteria, Part C alpha-, beta-, delta-, epsilonproteobacteria, Bergey's man, Trust. Dep. Microbiol. Mol. Genet. 2 (2005).
[26] A. Montes-Robledo, D. Baena-Baldiris, R. Baldiris-Avila, Reduction of Cr (VI) by planktonic cells and biofilm of Acinetobacter sp.(ADHR1) isolated from electroplating wastewater, Environ. Technol. Innov. 33 (2024) 103521.
[27] M. Ilias, I.M. Rafiqullah, B.C. Debnath, K.S. Bin Mannan, M. Mozammel Hoq, Isolation and characterization of chromium (VI)-reducing bacteria from tannery effluents, Indian J. Microbiol. 51 (2011) 76–81.
[28] S. Syed, P. Chinthala, Heavy metal detoxification by different Bacillus species isolated from solar salterns, Sci. Tech. Rep. (2015) 2015.
[29] H.W. Bauer, W.M. Kirby, J.C. Sherris, M. andTurck, Antibiotic susceptibility testing by standard single disc method. Amec. method. Amec, J. Clin. Pathol. 45 (1966) 449–494.
[30] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing, 28th ed., CLSI supplement M100, Clinical and Laboratory Standards Institute, Wayne, PA, 2018.
[31] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (2014) 2114–2120.
[32] A. Gurevich, V. Saveliev, N. Vyahhi, G. Tesler, QUAST: quality assessment tool for genome assemblies, Bioinformatics 29 (2013) 1072–1075.
[33] J.P. Meier-Kolthoff, M. Göker, TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy, Nat. Commun. 10 (2019) 1–10.
[34] P.-A. Chaumeil, A.J. Mussig, P. Hugenholtz, D.H. Parks, GTDB-tk: a Toolkit to Classify Genomes with the Genome Taxonomy Database, 2020.
[35] A.R. Wattam, J.J. Davis, R. Assaf, S. Boisvert, T. Brettin, C. Bun, N. Conrad, E.M. Dietrich, T. Disz, J.L. Gabbard, Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center, Nucleic Acids Res. 45 (2017) D535–D542.
[36] I. Letunic, P. Bork, Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation, Nucleic Acids Res. 49 (2021) W293–W296.
[37] K.A. Jolley, J.E. Bray, M.C.J. Maiden, Open-access bacterial population genomics: BIGSdb software, the PubMLST. org website and their applications, Wellcome Open Res 3 (2018).
[38] M. V Larsen, S. Cosentino, O. Lukjancenko, D. Saputra, S. Rasmussen, H. Hasman, T. Sicheritz-Pontén, F.M. Aarestrup, D.W. Ussery, O. Lund, Benchmarking of methods for genomic taxonomy, J. Clin. Microbiol. 52 (2014) 1529–1539.

[39] R.K. Aziz, D. Bartels, A.A. Best, M. DeJongh, T. Disz, R.A. Edwards, K. Formsma, S. Gerdes, E.M. Glass, M. Kubal, The RAST Server: rapid annotations using subsystems technology, BMC Genom. 9 (2008) 1–15.
[40] T. Seemann, Prokka: rapid prokaryotic genome annotation, Bioinformatics 30 (2014) 2068–2069.
[41] C.P. Cantalapiedra, A. Hernández-Plaza, I. Letunic, P. Bork, J. Huerta-Cepas, eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale, Mol. Biol. Evol. 38 (2021) 5825–5829.
[42] Y. Moriya, M. Itoh, S. Okuda, A.C. Yoshizawa, M. Kanehisa, KAAS: an automatic genome annotation and pathway reconstruction server, Nucleic Acids Res. 35 (2007) W182–W185.
[43] B.P. Alcock, A.R. Raphenya, T.T.Y. Lau, K.K. Tsang, M. Bouchard, A. Edalatmand, W. Huynh, A.-L. V Nguyen, A.A. Cheng, S. Liu, Card 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database, Nucleic Acids Res. 48 (2020) D517–D525.
[44] V. Bortolaia, R.S. Kaas, E. Ruppe, M.C. Roberts, S. Schwarz, V. Cattoir, A. Philippon, R.L. Allesoe, A.R. Rebelo, A.F. Florensa, ResFinder 4.0 for predictions of phenotypes from genotypes, J. Antimicrob. Chemother. 75 (2020) 3491–3500.
[45] C. Pal, J. Bengtsson-Palme, C. Rensing, E. Kristiansson, D.G.J. Larsson, BacMet: antibacterial biocide and metal resistance genes database, Nucleic Acids Res. 42 (2014) D737–D743.
[46] L. Chen, J. Yang, J. Yu, Z. Yao, L. Sun, Y. Shen, Q. Jin, VFDB: a reference database for bacterial virulence factors, Nucleic Acids Res. 33 (2005) D325–D328.
[47] S. Cosentino, M. Voldby Larsen, F. Møller Aarestrup, O. Lund, PathogenFinder-distinguishing friend from foe using bacterial whole genome sequence data, PLoS One 8 (2013) e77302.
[48] D. Arndt, J.R. Grant, A. Marcu, T. Sajed, A. Pon, Y. Liang, D.S. Wishart, PHASTER: a better, faster version of the PHAST phage search tool, Nucleic Acids Res. 44 (2016) W16–W21.
[49] I. Grissa, G. Vergnaud, C. Pourcel, CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats, Nucleic Acids Res. 35 (2007) W52–W57.
[50] A. Carattoli, H. Hasman, PlasmidFinder and in silico pMLST: identification and typing of plasmid replicons in whole-genome sequencing (WGS), Horiz, Gene Transf. Methods Protoc (2020) 285–294.
[51] K. Blin, S. Shaw, A.M. Kloosterman, Z. Charlop-Powers, G.P. Van Wezel, M.H. Medema, T. Weber, antiSMASH 6.0: improving cluster detection and comparison capabilities, Nucleic Acids Res. 49 (2021) W29–W35.
[52] A.J. van Heel, A. de Jong, C. Song, J.H. Viel, J. Kok, O.P. Kuipers, BAGEL4: a user-friendly web server to thoroughly mine RiPPs and bacteriocins, Nucleic Acids Res. 46 (2018) W278–W281.
[53] D. Liu, Y. Zhang, G. Fan, D. Sun, X. Zhang, Z. Yu, J. Wang, L. Wu, W. Shi, J. Ma, IPGA: a handy integrated prokaryotes genome and pan-genome analysis web service, IMeta 1 (2022) e55.
[54] A. Dereeper, M. Summo, D.F. Meyer, PanExplorer: a web-based tool for exploratory analysis and visualization of bacterial pan-genomes, Bioinformatics 38 (2022) 4412–4414.
[55] A.J. Page, C.A. Cummins, M. Hunt, V.K. Wong, S. Reuter, M.T.G. Holden, M. Fookes, D. Falush, J.A. Keane, J. Parkhill, Roary: rapid large-scale prokaryote pan genome analysis, Bioinformatics 31 (2015) 3691–3693.
[56] U. Thacker, R. Parikh, Y. Shouche, D. Madamwar, Reduction of chromate by cell-free extract of Brucella sp. isolated from Cr (VI) contaminated sites, Bioresour. Technol. 98 (2007) 1541–1547.
[57] M.S.M. Mangaiyarkarasi, S. Vincent, S. Janarthanan, T.S. Rao, B.V.R. Tata, Bioreduction of Cr (VI) by alkaliphilic Bacillus subtilis and interaction of the membrane groups, Saudi J. Biol. Sci. 18 (2011) 157–167.
[58] D.H. Nies, Microbial heavy-metal resistance, Appl. Microbiol. Biotechnol. 51 (1999) 730–750.
[59] A.R. Shakoori, M. Makhdoom, R.U. Haq, Hexavalent chromium reduction by a dichromate-resistant gram-positive bacterium isolated from effluents of tanneries, Appl. Microbiol. Biotechnol. 53 (2000) 348–351.
[60] N. Upadhyay, K. Vishwakarma, J. Singh, M. Mishra, V. Kumar, R. Rani, R.K. Mishra, D.K. Chauhan, D.K. Tripathi, S. Sharma, Tolerance and reduction of chromium (VI) by Bacillus sp. MNU16 isolated from contaminated coal mining soil, Front. Plant Sci. 8 (2017) 778.
[61] Y. Zhu, J. Yan, L. Xia, X. Zhang, L. Luo, Mechanisms of Cr (VI) reduction by Bacillus sp. CRB-1, a novel Cr (VI)-reducing bacterium isolated from tannery activated sludge, Ecotoxicol. Environ. Saf. 186 (2019) 109792.
[62] F. Xu, T. Ma, L. Shi, J. Zhang, Bioreduction of Cr (VI) by Bacillus sp. QH-1 isolated from soil under chromium-containing slag heap in high altitude area, Ann. Microbiol. 64 (2014) 1073–1080.
[63] S. Shameer, Biosorption of lead, copper and cadmium using the extracellular polysaccharides (EPS) of Bacillus sp., from solar salterns, 3 Biotech. 6 (2016) 194.
[64] R. Sultana, M.S. Islam, M.S. Hossain, M.N. Hassan, M.R. Hasan, M.A.A. Shaikh, M.R. Karim, Isolation and characterization of Bacillus subtilis RZS-01 isolate from agricultural soil in Bangladesh with potent antimicrobial activities, Vegetos (2024) 1–8.
[65] A. V Nair, M.L. Antony, N.K. Praveen, P. Sayooj, T.R. Swaminathan, K.K. Vijayan, Evaluation of in vitro and in vivo potential of Bacillus subtilis MBTDCMFRI Ba37 as a candidate probiont in fish health management, Microb. Pathog. 152 (2021) 104610.
[66] D. Dikson, H. Victor, D. Jong, A. Sanjaya, A. Samantha, J. Jo, et al., Whole-genome analysis of Bacillus subtilis G8 isolated from natto, Biodiversitas J Biol Divers 23 (2022).
[67] M. Lefevre, S.M. Racedo, M. Denayrolles, G. Ripert, T. Desfougères, A.R. Lobach, R. Simon, F. Pélerin, P. Jüsten, M.C. Urdaci, Safety assessment of Bacillus subtilis CU1 for use as a probiotic in humans, Regul. Toxicol. Pharmacol. 83 (2017) 54–65.
[68] I.B. Sorokulova, I. V Pinchuk, M. Denayrolles, I.G. Osipova, J.M. Huang, S.M. Cutting, M.C. Urdaci, The safety of two Bacillus probiotic strains for human use, Dig. Dis. Sci. 53 (2008) 954–963.
[69] S.-H. Kim, G.A. Yehuala, W.Y. Bang, J. Yang, Y.H. Jung, M.-K. Park, Safety evaluation of Bacillus subtilis IDCC1101, newly isolated from cheonggukjang, for industrial applications, Microorganisms 10 (2022) 2494.
[70] G. Buron-Moles, A. Chailyan, I. Dolejs, J. Forster, M.H. Mikš, Uncovering carbohydrate metabolism through a genotype-phenotype association study of 56 lactic acid bacteria genomes, Appl. Microbiol. Biotechnol. 103 (2019) 3135–3152.
[71] M. Richter, R. Rosselló-Móra, Shifting the genomic gold standard for the prokaryotic species definition, Proc. Natl. Acad. Sci. 106 (2009) 19126–19131.
[72] S.-W. Li, M.-Y. Liu, R.-Q. Yang, Comparative genome characterization of a petroleum-degrading Bacillus subtilis strain DM2, Int. J. Genomics. 2019 (2019).
[73] B. Kempf, E. Bremer, OpuA, an osmotically regulated binding protein-dependent transport system for the osmoprotectant Glycine betaine in Bacillus subtilis (*), J. Biol. Chem. 270 (1995) 16701–16713.
[74] S. Arnaouteli, N.C. Bamford, N.R. Stanley-Wall, Á.T. Kovács, Bacillus subtilis biofilm formation and social interactions, Nat. Rev. Microbiol. 19 (2021) 600–614.
[75] L. Hobley, A. Ostrowski, F. V Rao, K.M. Bromley, M. Porter, A.R. Prescott, C.E. MacPhee, D.M.F. Van Aalten, N.R. Stanley-Wall, BslA is a self-assembling bacterial hydrophobin that coats the Bacillus subtilis biofilm, Proc. Natl. Acad. Sci. 110 (2013) 13600–13605.
[76] G. Liu, K. Zhang, H. Gong, K. Yang, X. Wang, G. Zhou, W. Cui, Y. Chen, Y. Yang, Whole genome sequencing and the lignocellulose degradation potential of Bacillus subtilis RLI2019 isolated from the intestine of termites, Biotechnol. Biofuels Bioprod 16 (2023) 130.
[77] T. Chen, Z. Zhang, W. Li, J. Chen, X. Chen, B. Wang, J. Ma, Y. Dai, H. Ding, W. Wang, Biocontrol potential of Bacillus subtilis CTXW 7-6-2 against kiwifruit soft rot pathogens revealed by whole-genome sequencing and biochemical characterisation, Front. Microbiol. 13 (2022) 1069109.
[78] S. Iqbal, N. Ullah, H.A. Janjua, In vitro evaluation and genome mining of Bacillus subtilis strain RS10 reveals its biocontrol and plant growth-promoting potential, Agriculture 11 (2021) 1273.
[79] O.S. Olanrewaju, A.S. Ayangbenro, B.R. Glick, O.O. Babalola, Plant health: feedback effect of root exudates-rhizobiome interactions, Appl. Microbiol. Biotechnol. 103 (2019) 1155–1166.
[80] S. Taghavi, D. van der Lelie, A. Hoffman, Y.-B. Zhang, M.D. Walla, J. Vangronsveld, L. Newman, S. Monchy, Genome sequence of the plant growth promoting endophytic bacterium Enterobacter sp, 638, PLoS Genet. 6 (2010) e1000943.
[81] S.-S. Xie, H.-J. Wu, H.-Y. Zang, L.-M. Wu, Q.-Q. Zhu, X.-W. Gao, Plant growth promotion by spermidine-producing Bacillus subtilis OKB105, Mol. Plant Microbe Interact. 27 (2014) 655–663.

[82] C.L. Gupta, R.K. Tiwari, E. Cytryn, Platforms for elucidating antibiotic resistance in single genomes and complex metagenomes, Environ. Int. 138 (2020) 105667.
[83] N. Noguchi, M. Sasatsu, M. Kono, Genetic mapping in Bacillus subtilis 168 of the aadK gene which encodes aminoglycoside 6-adenylyltransferase, FEMS Microbiol. Lett. 114 (1993) 47–52.
[84] C. Rensing, B. Mitra, B.P. Rosen, The zntA gene of Escherichia coli encodes a Zn (II)-translocating P-type ATPase, Proc. Natl. Acad. Sci. 94 (1997) 14326–14331.
[85] J. Guzmán-Moreno, L.F. García-Ortega, L. Torres-Saucedo, P. Rivas-Noriega, R.M. Ramírez-Santoyo, L. Sánchez-Calderón, I.N. Quiroz-Serrano, L.E. Vidales-Rodríguez, Bacillus megaterium HgT21: a promising metal multiresistant plant growth-promoting bacteria for soil biorestoration, Microbiol. Spectr. 10 (2022).
[86] C.M. Moore, A. Gaballa, M. Hui, R.W. Ye, J.D. Helmann, Genetic and physiological responses of Bacillus subtilis to metal ion stress, Mol. Microbiol. 57 (2005) 27–40.
[87] T. Wang, Y. Liang, M. Wu, Z. Chen, J. Lin, L. Yang, Natural products from Bacillus subtilis with antimicrobial properties, Chin. J. Chem. Eng. 23 (2015) 744–754.
[88] L. Zhang, C. Sun, Fengycins, cyclic lipopeptides from marine Bacillus subtilis strains, kill the plant-pathogenic fungus Magnaporthe grisea by inducing reactive oxygen species production and chromatin condensation, Appl. Environ. Microbiol. 84 (2018).
[89] K.R. Meena, S.S. Kanwar, Lipopeptides as the antifungal and antibacterial agents: applications in food safety and therapeutics, BioMed Res. Int. 2015 (2015).
[90] M. Miethke, O. Klotz, U. Linne, J.J. May, C.L. Beckering, M.A. Marahiel, Ferri-bacillibactin uptake and hydrolysis in Bacillus subtilis, Mol. Microbiol. 61 (2006) 1413–1427.
[91] X.-H. Chen, J. Vater, J. Piel, P. Franke, R. Scholz, K. Schneider, A. Koumoutsi, G. Hitzeroth, N. Grammel, A.W. Strittmatter, Structural and functional characterization of three polyketide synthase gene clusters in Bacillus amyloliquefaciens FZB 42, J. Bacteriol. 188 (2006) 4024–4036.
[92] W. Loeffler, J.S. Tschen, N. Vanittanakom, M. Kugler, E. Knorpp, T. Hsieh, T. Wu, Antifungal effects of bacilysin and fengymycin from Bacillus subtilis F-29-3 a comparison with activities of other bacillus antibiotics, J. Phytopathol. 115 (1986) 204–213.
[93] S. Thennarasu, D.-K. Lee, A. Poon, K.E. Kawulka, J.C. Vederas, A. Ramamoorthy, Membrane permeabilization, orientation, and antimicrobial mechanism of subtilosin A, Chem. Phys. Lipids 137 (2005) 38–51.
[94] P.H. Brito, B. Chevreux, C.R. Serra, G. Schyns, A.O. Henriques, J.B. Pereira-Leal, Genetic competence drives genome diversity in Bacillus subtilis, Genome Biol. Evol. 10 (2018) 108–124.