Draft Genome Sequence of *Bacillus pergaminensis* sp. nov. strain Bva_UNVM-123: A Promising Candidate for Bioremediation.

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Abstract

The *Bacillus* genus comprises physiologically versatile, endospore-forming bacteria widely distributed in natural environments. In this study, we report the isolation and genomic characterization of strain Bva_UNVM-123, recovered from agricultural soil in Pergamino, Argentina. Whole-genome sequencing using Illumina technology yielded a 5.1 Mbp draft genome assembled in 67 contigs with a GC content of 36%. Comparative genomic analyses using the TYGS server and digital DNA–DNA hybridization (dDDH) values supported its classification as a novel species within the genus *Bacillus*. We propose the name *Bacillus pergaminensis* sp. nov. for this taxon, with strain Bva_UNVM-123 as the type strain. Genome annotation revealed 4,866 protein-coding genes, including multiple determinants conferring resistance to antibiotics (e.g., fosfomycin, tetracycline, beta-lactams) and toxic heavy metals (e.g., arsenic, cadmium, mercury), supporting its potential application in bioremediation. Additionally, PathogenFinder predicted a low probability of human pathogenicity (0.207), reinforcing its safety for environmental use. This study adds to the growing knowledge of environmental *Bacillus* species and their biotechnological potential.

Keywords: *Bacillus* spp.; genomic sequence; antibiotic resistance; heavy metal resistance; soil microbiome; NCBI; genome assembly; Firmicutes; endospore-forming bacteria; bioremediation.

Introduction

Species belonging to the *Bacillus* genus are known for their remarkable natural properties, which range from the synthesis of various toxins (e.g., cereulide) to the production of proteins and enzymes with numerous biotechnological applications, including fermentation, biodegradation, and the production of secondary metabolites. Many of these organisms can synthesize a wide range of bioactive compounds, including antibiotics (e.g., bacitracina), enzymes (amylases, proteases, lipases, etc.), and biopolymers, which can be used in industrial applications [1].

The genes responsible for these biosynthetic pathways are often located on extrachromosomal DNA elements, such as plasmids or bacteriophages, which can harbor additional virulence factors including toxins and antibiotic resistance genes [2].

These plasmids are typically transferable, and through horizontal gene transfer (HGT), they can be transmitted to related bacterial species [3]. Horizontal transfer of genetic material between bacteria plays a crucial role in the spread of antibiotic resistance, as it allows for rapid dissemination of resistance traits across different bacterial populations [4, 5]. This transfer can occur between pathogenic and non-pathogenic bacteria, and in the case of non-pathogenic species, it can result in the presence of antibiotic resistance genes even in bacteria that do not cause disease [6]. Notably, recent studies emphasize the role of non-pathogenic soil bacteria in harboring mobile resistance elements that may be horizontally transferred to clinical pathogens under environmental stress conditions. Such non-pathogenic bacteria, isolated from various ecosystems like soil, water, or animal manure, are of great interest because they can serve as reservoirs for resistance genes that may eventually be transferred to pathogenic organisms under selective pressure [7].

Antibiotic resistance genes are essential mechanisms used by pathogenic bacteria to evade the effects of antibiotics, a challenge that has become a major global health concern. However, they are not only found in pathogenic bacteria but are also commonly detected in non-pathogenic environmental microorganisms, including species used in biotechnology and environmental applications [8]. This characteristic is particularly interesting from an environmental management perspective because non-pathogenic, antibiotic-resistant bacteria could potentially be utilized in bioremediation strategies. For example, certain *Bacillus* strains with intrinsic resistance to antibiotics can be employed in bioremediation efforts to clean up environments contaminated by antibiotics, particularly those originating from hospitals, dairy farms, and livestock operations, where residues of antibiotics often persist in soil and water [9].

This provides a valuable opportunity for using these bacteria in the development of new biotechnological tools aimed at mitigating the environmental impact of antibiotic contamination, which is a growing concern in public health and environmental science [10].

In addition to antibiotic resistance, several Bacillus species are known to tolerate or resist toxic heavy metals such as arsenic, cadmium, lead, copper, and mercury. This resistance is often mediated by efflux systems, metal-binding proteins, and enzymatic detoxification pathways encoded in their genomes [11, 12]. These properties make them promising candidates for bioremediation strategies aimed at decontaminating polluted environments. The capacity to thrive in metal-contaminated soils, together with their ability to form resilient endospores, enhances their potential in long-term or large-scale remediation projects [13].

The capacity of *Bacillus* species to adapt to diverse environments, combined with their resistance to antibiotics and heavy metals, makes them ideal candidates for further research in the field of bioremediation. By leveraging their natural resistance mechanisms, it may be possible to engineer more efficient systems for cleaning up contaminated environments, particularly those impacted by human activity.

In this work, we aimed to sequence and analyze the genome of strain Bva_UNVM-123, a newly isolated *Bacillus* strain from soil, to explore its taxonomic identity and biotechnological potential. The assembled draft genome sequence of Bva_UNVM-123 provides insights into its genomic makeup, revealing potential resistance genes against antibiotics and heavy metals, which may contribute to its potential as a bioremediation agent. This study will contribute to a deeper understanding of the genetic factors underlying the resistance of Bacillus species, furthering their application in environmental remediation efforts.

Materials and Methods

Isolation and Morphological Characterization

The strain Bva_UNVM-123 was isolated from a soil sample collected from soybean field in Pergamino, Buenos Aires Province (Argentina), as part of a screening program aimed at identifying entomopathogenic bacteria (e.g., *Bacillus thuringiensis*) as previously described [14].

Those strains showing a *B. thuringiensis*-like (matte white and with uneven borders) phenotype were reisolated in axenic cultures and observed by phase contrast microscopy (Nikon Eclipse Ti, Japan). Gram staining was performed using a commercial kit (Biopack, Buenos Aires, Argentina), following the manufacturer's instructions. In addition, the strain was also examined using Scanning Electron Microscopy (SEM) at the Comprehensive Center for

106 Electron Microscopy (CONICET-Universidad Nacional de Tucumán, Argentina). The strain is
107 maintained as glycerol suspension (50% v/v) at −80 °C. The strain Bva_UNVM-123 was also
108 deposited in the International Culture Collection of the Institute of Microbiology and
109 Agricultural Zoology at INTA Castelar (IMYZA-INTA) (access number pending).

110 **Biochemical Characterization and Antibiotic Resistance Analyses**

111 NaCl tolerance was evaluated by culturing the strain on TSA medium supplemented with
112 NaCl concentrations ranging from 2.5% to 7.5% (w/v) and incubating for 72 hours. Anaerobic
113 growth was assessed using the candle jar technique. Motility was tested on semisolid agar
114 medium (0.75% w/v). Hemolytic activity was tested on blood agar plates supplemented with
115 5% sheep blood (Britania Laboratories, Argentina) incubated at 37 °C for 48 hours.

116 The sensitivity of strain Bva_UNVM-123 to various antibiotics, chloramphenicol (30 µg),
117 novobiocin (5 µg), ampicillin (10 µg), tetracycline (30 µg), and penicillin (10 U) (Britania
118 Laboratories, Argentina), was assessed on Mueller–Hinton agar using the disc diffusion
119 method. Oxidase and catalase activities were determined using standard microbiological
120 procedures. Additional physiological and biochemical characterizations were conducted using
121 the API 50CHB, API ZYM, and API 20A systems (bioMérieux), following the protocols
122 provided by the manufacturer.

123 Fatty acid profiling of the strain was performed using the MIDI Sherlock Microbial
124 Identification System (version 6.2B) following the methodology described by Ferrari et al.,
125 (2018). The sample was analyzed using the RTSBA6 method, and fatty acids were identified
126 based on their equivalent chain length (ECL) and retention time [15].

127 **DNA isolation, Genome assembly and Annotation and Species Delimitation**

128 For DNA extraction, strain Bva_UNVM-123 was grown in LB broth for 16 hours. Genomic
129 DNA, including chromosomal and plasmid DNA, was extracted using the Wizard Genomic
130 DNA Purification Kit (Promega, Madison, WI, USA), following the manufacturer's protocol
131 for gram-positive bacteria. The extracted DNA was analyzed by electrophoresis in 1% agarose
132 gels, stained with SYBR Safe (ThermoFisher Scientific, Waltham, MA, USA), and quantified
133 using a PICODROP PICO 100 µL spectrophotometer.

134 The extracted DNA was subjected to PCR to confirm its belonging to the *Bacillus* genus
135 [16]. The PCR assay was performed using the methodology described by Wu et al. (2006),
136 which involves a group-specific primer set designed for Bacillus species identification.

137 Then, the purified DNA was used to construct a pooled Illumina sequencing library, which
138 was sequenced at the Genomics Unit of the National Institute of Agricultural Technology

(INTA, Argentina) using Illumina high-throughput sequencing technology. Raw sequencing reads were processed to remove low-quality regions and assembled into contigs using Velvet within Geneious version R11 software (www.geneious.com, accessed on 10 November 2023) with default parameters for de novo assembly. Assembly statistics were evaluated using QUAST v4.5 [17], and genome completeness and contamination were assessed using CheckM [18]. Gene predictions and functional annotations were done via PGAP [16] and validated through the RAST server [19].

Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (2023 release) and the RAST server [19]. Species delimitation was carried out using the Type (Strain) Genome Server [20] and PubMLST [21]. Secondary metabolites searches were performed using antiSmash server [22].

Results

Morphological Characterization

The strain formed opaque colonies with a matte texture and creamy white color after overnight growth on TSA medium at 37 °C during 72 h. Microscopic examination revealed rod-shaped vegetative cells with terminal deforming spores (Figure 1A and B). The terminal positioning of the endospore is consistent with known morphological traits in environmental *Bacillus* species [23].

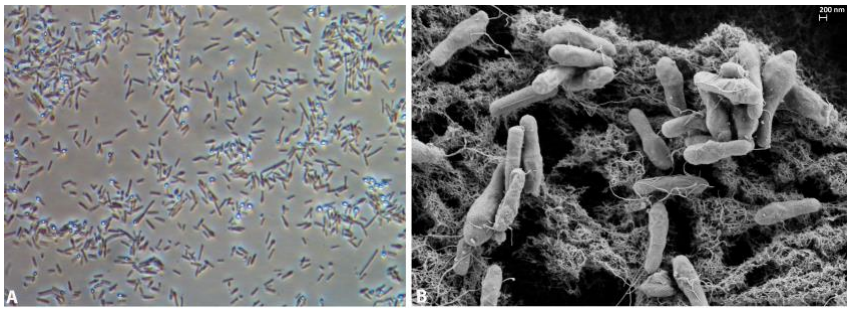

Figure 1. Microscopic analysis of strain Bva_UNVM-123 grown on TSA medium (72 h/37 °C). A) Morphological characterization using phase-contrast microscopy and B) Scanning electron microscopy. A scale bar (200 nm) was included in SEM images to reflect structural dimensions.

Biochemical Characterization

Cells are Gram-stain-positive, facultatively anaerobic, non-motile, and form terminal endospores. The strain was able to grow on nutrient agar (NA), casein–collagen–yeast extract agar (CCY), tryptic soy agar (TSA), and brain heart infusion agar. Optimal growth was observed on TSA at 37 °C and the strain was non-hemolytic. The strain tested positive for both

catalase and oxidase activities and when cultured in the presence of chloramphenicol, did not exhibit measurable inhibition zones.

Additional physiological and enzymatic tests were carried out using the API 50CHB, API ZYM, and API 20A systems. However, the results obtained were inconclusive due to limited growth under assay conditions, including incubation at 30 °C and 37 °C.

The fatty acid profile of strain Bva_UNVM-123 consisted primarily of branched-chain saturated fatty acids, a chemotaxonomic trait typical of gram-positive bacteria, particularly members of the genus Bacillus or related taxa. The major components included iso- and anteiso-fatty acids such as iso-C15:0, anteiso-C15:0, iso-C16:0, and iso-C17:0. A total of 61.21% of the detected fatty acids were identified, with iso-C15:0 representing the most abundant component (32.46%). The complete fatty acid profile is presented in Table 1.

Table 1. Key Major fatty acid components of strain Bva_UNVM-123. Only those representing ≥3% of the total identified fatty acids are shown. Summed Feature 4 includes fatty acids that could not be resolved individually by the system.

Fatty Acid	Composition (%)	Other Characteristics
iso-C15:0	32.46	Most abundant component
anteiso-C15:0	7.28	-
iso-C16:0	5.21	-
C16:0	4.39	Also known as palmitic acid
iso-C17:0	6.16	-
anteiso-C17:0	3.69	-
Summed Feature 4	5.20	Includes C17:1 iso I / anteiso B isomers

Genome Characterization and Species Identification

The Illumina sequencing produced 14,532,725 high-quality paired end reads, resulting in 67 assembled contigs with a total genome size of 5,119,019 bp in size and 36% G+C content (Table 2).

Table 2. Key features statistics and quality analysis of the draft genome assembly of Bva_UNVM-123 strain obtained with QUAST [17], CheckM [18] and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [24].

Feature statistics	Value
Assembled Genome Size (bp)	5,119,099
Contig Number	67
Largest contig (bp)	583,230
N50 (bp)	341,242
N's	5074
GC content (%)	36
Suspected Contamination (%)*	3.85
Estimated Completeness (%)*	95.85
Estimated Coverage (×)	400
PGAP Predicted Genes	5139
PGAP Predicted Protein Coding Genes	4866

*CheckM determinations.

Table 3 highlights multiple genes potentially related to antibiotic resistance, identified using the RAST annotation server. The presence of genes conferring resistance to fosfomycin (fosB), tetracyclines, chloramphenicol, fosmidomycin, kanamycin, oxetanocin A, and various beta-lactams (e.g., beta-lactamase class A-like and penicillin-binding proteins) suggests that strain Bva_UNVM-123 possesses a broad resistance profile.

Table 3. Putative annotated genes related to antibiotic resistance found at the genome sequence of Bva_UNVM-123 strain using RAST server [19].

Product	Type	Copies
Efflux ABC transporter (ATP-binding/permease)	Multidrug resistance	45
Fosmidomycin resistance protein	Fosfomycin resistance	2
Similar to tetracycline resistance protein	Tetracyclin resistance	1
Similar to tetracycline resistance, MFS efflux pump	Tetracyclin resistance	1
Chloramphenicol/florfenicol resistance efflux pump	Chloramphenicol resistance	1
Zwittermicin A resistance protein	Zwittermicin A resistance	1
Kanamycin kinase	Kanamycin resistance	1

Oxetanocin A resistance protein	Oxetanocin A resistance	1
Beta-lactamase (Class A/C-like)	Beta-lactam resistance	12
Vancomycin response proteins (VanR/VanS)	Glycopeptide resistance	5
Penicillin-binding protein 1A/1B	Penicillin resistance	1
Penicillin acylase	Penicillin resistance	1
Penicillin-binding protein 4B	Penicillin resistance	1

Table 4 lists genes potentially associated with resistance to toxic heavy metals, including arsenic, cadmium, zinc, cobalt, lead, mercury, and tellurite. These genes encode various efflux pumps, ATPases, and regulatory proteins, such as TehB, ArsD, ACR3, and CzcD, which are known to facilitate resistance and detoxification [25, 26].

Table 4. Putative heavy-metal resistance genes found at the genome sequence of Bva_UNVM-123 strain using RAST server.

Product	Metal	Copies
Tellurium resistance proteins (TerD, TerB)	Tellurium	5
Arsenical resistance proteins (Acr3, ArsR, ATPase)	Arsenic	9
Cadmium/Zinc/Cobalt transporters (P-type ATPase, CzcD)	Cd/Zn/Co	7
Copper chaperones and transporters (CopZ, CopA)	Copper	4
Zinc ABC transporter systems (ZnuA/B/C, Zur)	Zinc	5
Manganese efflux pump (MntP)	Manganese	1
Lead, Mercury, and other heavy-metal transporters	Pb/Hg/Cd/Zn	6
Multicopper oxidases and polyphenol oxidases	Copper	1
Aluminum resistance protein	Aluminum	1

Concerning species identification, preliminary PCR analysis showed that isolate 123 belongs to the genus *Bacillus* (Figure 2).

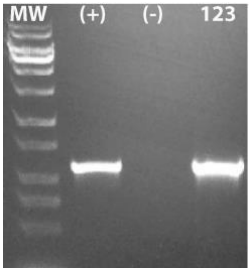

Figure 2. PCR analysis of strain Bva_UNVM-123 for the Bacillus genus showing a positive PCR amplicon (positive control: *B. thuringiensis* HD1, 123: Bva_UNVM-123 strain).

The low average branch support observed in the GBDP phylogenetic tree generated by the TYGS server likely reflects the high genomic divergence of strain Bva_UNVM-123 and the lack of closely related reference genomes. Despite this, digital DNA–DNA hybridization (dDDH) values support its classification as a potentially novel species (Figure 3).

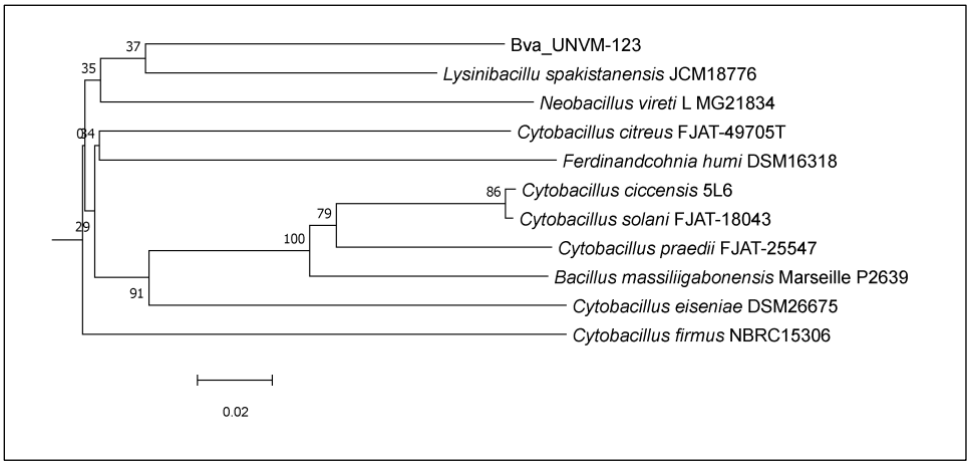

Figure 3. Genome-based phylogenetic tree of Bacillus sp. strain Bva_UNVM-123 and related taxa, constructed using the GBDP method via the TYGS server. Branch lengths are scaled in terms of GBDP distance. Bootstrap values indicate the average support for each branch, based on 100 pseudoreplicates.

Based on PathogenFinder 1.1, the strain was predicted as non-pathogenic, with a human pathogenicity probability of 0.207, indicating its safety for environmental applications [27].

Discussion

After a comprehensive genomic and phenotypic analyses, including phylogenomic reconstruction and digital DNA-DNA hybridization (dDDH) values below the 70% species threshold, we propose that strain Bva_UNVM-123 represents a novel species within the genus *Bacillus*, for which the name *Bacillus pergaminensis* sp. nov. is proposed, with strain Bva_UNVM-123 as the type strain.

The genome of *B. pergaminensis* sp. nov. Bva_UNVM-123 reveals multiple adaptive traits that support its role in bioremediation. In line with this, preliminary phenotypic assays showed that strain Bva_UNVM-123 exhibited no measurable inhibition zones against chloramphenicol and novobiocin, suggesting intrinsic tolerance that may correspond to the resistance genes found in the genome. Nonetheless, further MIC assays are necessary to validate this resistance.

In addition, the presence of antibiotic resistance genes such as fosB, tetA, bla-like beta-lactamases, and multidrug efflux systems suggests long-term exposure to antibiotic residues in soil environments. This aligns with findings that environmental *Bacillus* species often act as reservoirs for antibiotic resistance genes due to selective pressure in natural and agricultural soils [6, 7]. This resistance could be determinant and particularly relevant for bioremediation applications, where bacteria must survive in antibiotic-contaminated environments.

The use of API-based systems for metabolic profiling did not yield conclusive results. This outcome is frequently observed with environmental isolates, which often exhibit poor growth or atypical metabolic patterns when tested in commercial platforms originally designed for clinical or fast-growing laboratory strains. These limitations highlight the need to optimize test conditions or employ alternative phenotypic strategies when characterizing environmentally adapted bacteria. For instance, even in industrial contexts such as dairy processing environments, phenotypic microbiological techniques have shown limited reliability for environmental strains like *Pseudomonas* spp. and *Bacillus* spp., often resulting in inconclusive species-level identifications [28]. Consequently, in this study, species identification relied primarily on genome-based approaches, which provided higher taxonomic resolution and reliability.

Additionally, the fatty acid profile of strain Bva_UNVM-123 did not match any entry in the RTSBA6 reference library, which is consistent with the genomic evidence suggesting novelty. However, the total response signal obtained in both replicates was below the recommended threshold for reliable identification. This limitation was most likely due to the poor growth of the strain under the conditions used for biomass collection, rather than to methodological issues. Further optimization of culture conditions is therefore needed to obtain sufficient biomass for accurate chemotaxonomic profiling.

Moreover, the strain harbors a diverse array of heavy metal resistance genes, including arsD, acr3, czcD, and tehB, which confer tolerance to arsenic, cadmium, mercury, and tellurite. These genes are commonly found in metal-contaminated environments and are considered key biomarkers for bacterial adaptation and survival in such niches [11, 25, 26]. The abundance and diversity of these genes suggest that strain Bva_UNVM-123 is well adapted to metal-contaminated environments. This further supports its potential use in bioremediation, particularly in soils impacted by industrial or agricultural pollutants.

The non-pathogenic nature of the strain, as predicted by PathogenFinder, along with the absence of virulence determinants and hemolytic activity, strongly suggests its suitability for

environmental application. This safety profile is crucial for any candidate microorganism intended for bioremediation purposes, as environmental deployment requires strict biosafety assessments [27].

Importantly, the genome of *B. pergaminensis* sp. nov. also provides a valuable resource for future biotechnological exploration. Its genetic repertoire could be exploited not only for bioremediation of antibiotic- and metal-polluted sites but also for the potential production of industrial enzymes or bioactive secondary metabolites, as often observed in *Bacillus* spp. [1, 22].

Author contributions

Conceptualization, L.P., methodology, D.H.S, C.P and L.P.; formal analysis, D.H.S, C.P, V.F. and L.P.; investigation, D.H.S; C.P, V.F., E.E.D.V and L.P.; resources, L.P.; data curation, L.P.; writing—original draft preparation, L.P.; writing, review and editing, L.P. and D.H.S; visualization, L.P.; supervision, L.P.; project administration, L.P.; funding acquisition, L.P. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare there are no conflicts of interest.

Funding information

This research was funded by Ministerio de Ciencia, Tecnología e Innovación (FONCYT), Plan Argentina Innovadora 2020, grant number PICT 2017-0087, by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), grant number PIP 2017-2019 GRUPO INVES and the Instituto de Investigación of the Universidad Nacional de Villa María, grant number PIC 2017-2019.

Data availability

The unprocessed genome sequencing data have been deposited in the NCBI database under the BioSample accession SAMN18815149, within the BioProject PRJNA723377. The corresponding genome assembly is accessible through the NCBI Whole Genome Shotgun (WGS) database under the accession number JAGTPU000000000.

Acknowledgements

Leopoldo Palma gratefully acknowledges the Spanish Ministry of Science, Innovation, and Universities, the Spanish State Research Agency, and the European Union for funding his Ramón y Cajal contract (grant ref. RYC2023-043507-I).

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