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Novel spore-forming species exhibiting intrinsic resistance to third- and fourth-generation cephalosporins and description of *Tigheibacillus jepli* gen. nov., sp. nov.

Georgios Miliotis,[1,2] Pratyay Sengupta,[3,4,5] Asif Hameed,[6] Maria Chuvochina,[7] Francesca McDonagh,[1] Anna C. Simpson,[8] Ceth W. Parker,[8] Nitin K. Singh,[8] Punchappady D. Rekha,[6] Dearbháile Morris,[1,2] Karthik Raman,[3,4,5] Nikos C. Kyrpides,[9,10] Philip Hugenholtz,[7] Kasthuri Venkateswaran[8]

AUTHOR AFFILIATIONS See affiliation list on p. 16.

ABSTRACT A comprehensive microbial surveillance was conducted at NASA's Mars 2020 spacecraft assembly facility (SAF), where whole-genome sequencing (WGS) of 110 bacterial strains was performed. One isolate, designated 179-BFC-A-HS^T, exhibited less than 80% average nucleotide identity (ANI) to known species, suggesting a novel organism. This strain demonstrated high-level resistance [minimum inhibitory concentration (MIC) >256 mg/L] to third-generation cephalosporins, including ceftazidime, cefpodoxime, combination ceftazidime/avibactam, and the fourth-generation cephalosporin cefepime. The results of a comparative genomic analysis revealed that 179-BFC-A-HS^T is most closely related to *Virgibacillus halophilus* 5B73C^T, sharing an ANI of 78.7% and a digital DNA-DNA hybridization (dDDH) value of 23.5%, while their 16S rRNA gene sequences shared 97.7% nucleotide identity. Based on these results and the recent recognition that the genus *Virgibacillus* is polyphyletic, strain 179-BFC-A-HS^T is proposed as a novel species of a novel genus, *Tigheibacillus jepli* gen. nov., sp. nov (type strain 179-BFC-A-HS^T = DSM 115946^T = NRRL B-65666^T), and its closest neighbor, *V. halophilus*, is proposed to be reassigned to this genus as *Tigheibacillus halophilus* comb. nov. (type strain 5B73C^T = DSM 21623^T = JCM 21758^T = KCTC 13935^T). It was also necessary to reclassify its second closest neighbor *Virgibacillus soli,* as a member of a novel genus *Paracerasibacillus*, reflecting its phylogenetic position relative to the genus *Cerasibacillus*, for which we propose *Paracerasibacillus soli* comb. nov. (type strain CC-YMP-6^T = DSM 22952^T = CCM 7714^T). Within *Amphibacillaceae* (*n* = 64), *P. soli* exhibited 11 antibiotic resistance genes (ARG), while *T. jepli* encoded for 3, lacking any known β-lactamases, suggesting resistance from variant penicillin-binding proteins, disrupting cephalosporin efficacy. *P. soli* was highly resistant to azithromycin (MIC >64 mg/L) yet susceptible to cephalosporins and penicillins.

IMPORTANCE The significance of this research extends to understanding microbial survival and adaptation in oligotrophic environments, such as those found in SAF. Whole-genome sequencing of several strains isolated from Mars 2020 mission assembly cleanroom facilities, including the discovery of the novel species *Tigheibacillus jepli*, highlights the resilience and antimicrobial resistance (AMR) in clinically relevant antibiotic classes of microbes in nutrient-scarce settings. The study also redefines the taxonomic classifications within the *Amphibacillaceae* family, aligning genetic identities with phylogenetic data. Investigating ARG and virulence factors (VF) across these strains illuminates the microbial capability for resistance under resource-limited conditions while emphasizing the role of human-associated VF in microbial survival, informing sterilization practices and microbial management in similar oligotrophic settings beyond

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Address correspondence to Kasthuri Venkateswaran, kjvenkat1955@gmail.com, or Georgios Miliotis, georgios.miliotis@universityofgalway.ie.

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spacecraft assembly cleanrooms such as pharmaceutical and medical industry cleanrooms.

KEYWORDS cephalosporin, phylogenetic analysis, *Virgibacillus* taxonomy

Phylogenetic investigations into the *Bacillaceae* family have revealed a polyphyletic structure, signaling the necessity for taxonomic re-evaluation to accurately depict evolutionary lineages (1). This has been partially addressed by the recently proposed family *Amphibacillaceae* (2), encompassing halophilic genera like *Virgibacillus* (3), renowned for its robustness in saline environments (4–7). This family, which includes 16 genera, contains many bacterial taxa that have halophilic or halotolerant traits, with members commonly isolated from marine habitats, salt lakes, saline soils, and seafood, suggesting significance in food safety (8, 9).

Aerobic spore-forming bacteria, with their diverse ecological adaptations, are instrumental in biotechnological applications and have implications for human health due to their secondary metabolite production. These metabolites range from antimicrobial agents, such as bacitracin produced by *Bacillus licheniformis* (10) and polymyxin E produced by *Paenibacillus polymyxa* (11). *Amphibacillaceae* members like *Oceanobacillus* (6) not only demonstrate antimicrobial compound production but also present a fertile ground for discovering novel metabolites (12).

While typically non-pathogenic, some *Bacillaceae* members, such as *Bacillus cereus* and *Bacillus anthracis*, possess VF enabling opportunistic infections (13, 14). Conversely, *Amphibacillaceae* pathogens are rare; however, any bacterium can become opportunistic, especially in immunocompromised hosts. For example, a recent publication identified an *Oceanobacillus* spp. infection in an immunocompetent patient, with the pathogenesis mechanisms still largely unidentified, highlighting the knowledge gaps in the virulence of halophilic species (15).

Environmental *Amphibacillaceae* strains also harbor ARG, indicating that resistance can evolve even without direct clinically associated antibiotic exposure. The discovery of the β-lactamase OIH-1 in *Oceanobacillus iheyensis* that confers resistance to several penicillins but no other β-lactams exemplifies the independent evolution of AMR in remote environments like the oceanic depths (16, 17). *Virgibacillus* species have shown resistance to aminoglycosides and other antibiotics, with cephalosporins maintaining their clinical relevance against Gram-positive spore formers (18). Despite the availability of first-line treatments for Gram-positive spore-former-induced infections, such as vancomycin, erythromycin, and ciprofloxacin (19), the role of cephalosporins in treating such infections remains clinically relevant. These findings underscore the underappreciated role of natural environments in shaping AMR and necessitate vigilance against emerging ARG threats.

As part of a microbial surveillance study (6 months) associated with the NASA Mars 2020 mission, spacecraft assembly environments were assessed for microbial contamination (20). Bacterial strains (n = 110) were isolated from cleanroom samples that were subjected to a heat-shock (80°C; 15 min) procedure and cultured aerobically as per the NASA standard spore assay (21), and their genomes were sequenced (22).

The primary aim of this study was to characterize a bacterial isolate retrieved from the Mars 2020 mission assembly facility, identified as belonging to *Amphibacillaceae* through traditional microbiological methods and genomic analyses. Conserved marker genes were analyzed, and the phylogenetic affiliations of the isolate were delineated. A secondary objective was to determine the relative abundance of the novel isolate on the floors of the SAF cleanrooms (n = 236 samples) where the Mars 2020 mission rover had been assembled. Detailed genetic profiles of the species described herein were created, including ARG and VF profiles, and their distinct phenotypic traits were predicted. Their AMR phenotypes were assayed, and MIC were defined. Lastly, a predictive functional analysis was conducted, revealing genes encoding for putative bioactive compounds. This approach provides insights into the survival mechanisms of spore-forming bacteria

in the harsh and nutrient-deficient environments of SAF cleanrooms, which could pose challenges to future NASA life detection missions.

RESULTS

Genome assembly

The genome size of the novel strain 179-BFC-A-HS^T is 3,877,640 bp, and G+C content is 41.64%. Strain 5B73C^T has a 4,199,006-bp genome with 41.59% G+C content, and the genome of CC-YMP-6^T strain is 3,876,281 bp with 36.13% G+C value. Other genome statistics are summarized in Table 1 and File S1.

Genotypic characterization of ARG and VF

In the *Amphibacillaceae* family (n = 64 species), 52 distinct ARG across 11 genera (Fig. 1A and B) were identified, with over 80% coverage and 70% identity to the NCBI's ARG database. These ARG confer resistance to multiple antibiotic classes. Strain 5B73C^T harbors 11 ARG, resistant to aminoglycosides, diaminopyrimidines, macrolide-lincosamide-streptogramin B (MLSB), tetracyclines, and glycopeptides. Fifteen species possess a single ARG, with strain 179-BFC-A-HS^T exhibiting three ARG related to vancomycin resistance.

Following a bacteremia case in South Korea caused by an *Oceanobacillus* species, a VF gene analysis across related genera revealed 17 human-associated VF genes (Fig. 2), aligning with the virulence factor database (VFDB) (23). *Pseudogracilibacillus* contained the most VF (n = 8), while *Tigheibacillus* had the fewest (n = 3). EF-Tu- and ATP-dependent Clp protease genes appeared in all 64 tested genomes. In contrast, *hasC*, *bspD*/*E*/*F*, and *lspA* were unique to single genomes: *Cerasibacillus terrae* (*hasC*), *Oceanobacillus zhagkaii* (*bspE*/*F*), and *Ornithinibacillus scapharcae* (*lspA*), respectively. These results underscore the varied resistance and virulence potentials within *Amphibacillaceae*, indicative of an environmental influence on emerging AMR and virulence.

Phenotypic characterization of antimicrobial resistance properties

Cultivation-based analysis revealed that the novel strain 179-BFC-A-HS^T exhibited resistance to multiple antibiotics, including third-generation cephalosporins, ceftazidime, and cefpodoxime; fourth-generation cephalosporin cefepime; third-generation cephalosporin-β-lactamase inhibitor combination ceftazidime/avibactam and monobactam aztreonam (all with MIC >256 mg/L) but remained susceptible to penicillins [ampicillin and ampicillin/sulbactam (MIC <0.047 mg/L) (Fig. 1C). The strain CC-YMP-6^T exhibited high-level resistance to macrolide azithromycin (MIC >64 mg/L) and monobactam aztreonam (MIC >256 mg/L) while being susceptible to all tested penicillins, cephalosporins, and cephamycins.

Phylogeny and genomic relatedness

The comparative genomic analysis indicates that the strain 179-BFC-A-HS^T shares considerable genomic similarity with strain 5B73C^T, with an ANI of 78.7% (File S1) and a dDDH value of 23.5%. A heat map of ANI, including representative genomes of type species of the family *Amphibacillaceae* (n = 64), is shown in File S2. The *gyrB* sequence similarity between these strains is 79.13%, and the 16S rRNA gene sequence of strain 179-BFC-A-HS^T shares a 97.7% similarity with that of 5B73C^T. The next closest genetic relatives based on 16S rRNA gene identity are *Virgibacillus marismortui* (95.77%) and *Virgibacillus salaries* (95.71%). Phylogenetic trees, including representative genomes of all validly published species of the family *Amphibacillaceae*, were reconstructed using 16S rRNA and *gyrB* gene sequences. These trees reveal a distinct clade, uniquely identifying the new strain 179-BFC-A-HS^T (File S3). Further average amino acid identity (AAI) comparisons indicate that strains 179-BFC-A-HS^T and 5B73C^T share a 69.82% AAI, substantiating their classification within the same genus. In contrast, the AAI values

TABLE 1 Genomic information of sequenced isolates[b]

Species	Strain	NCBI accession no.	Isolation location	No. of contigs	Genome size (bp)	N50 (bp)	Average coverage	G + C content (%)	Filtered short reads used for assembly	Filtered long reads used for assembly	Coding sequences	16S rRNA genes[a]
Tigheibacillus jepli	179-BFCA-HS	JAROCA000000000.2	Clean room floor, JPL spacecraft assembly facility	9	3,939,646	2,866,002	1,121	41.79	19,068,144	176,000	4,401	16
Tigheibacillus halophilus	5B73C(T)	JAWDIP000000000.1	Field soil, Kakegawa, Shizuoka, Japan	4	4,199,006	2,848,454	309	41.59	N/A	261,000	5,963	7
Paraceracibacillus soli	CC-YMP-6	JAWDIQ000000000.1	Yang-Ming Mountain, Taiwan	7	3,876,281	1,950,473	714	36.13	8,485,365	152,000	5,112	7
Virgibacillus pantothenticus	179-F W3.5 NHS	JAYDXX000000000	Clean room floor, JPL spacecraft assembly facility	89	4,850,066	115,791	316	37.16	10,376,860	N/A	4,342	1
Virgibacillus pantothenticus	179-I 4A3 NHS	JAYDXW000000000	Clean room floor, JPL spacecraft assembly facility	83	4,852,173	115,791	239	37.16	7,840,196	N/A	4,343	1
Virgibacillus pantothenticus	179-I 6B2 NHS	JAYDXV000000000	Clean room floor, JPL spacecraft assembly facility	84	4,851,827	118,784	204	37.16	6,708,850	N/A	4,345	1
Virgibacillus pantothenticus	179_I 6B3 NHS	JAYDXU000000000	Clean room floor, JPL spacecraft assembly facility	82	4,850,152	129,457	249	37.16	8,174,846	N/A	4,345	1
Virgibacillus pantothenticus	179-I 6B4 NHS	JAYDXT000000000	Clean room floor, JPL spacecraft assembly facility	90	4,849,929	116,033	190	37.16	6,257,450	N/A	4,342	1

[a]Owing to the draft status of these genomes, only 16S rRNA gene sequences >1,400 base pairs were counted. For *V. pantothenticus* genomes, the actual count of 16S rRNA genes likely surpasses the single copy indicated in this table due to genome incompleteness.
[b]Assembly statistics for *T. jepli* 179-BFC-A-HS^T and the genomes of type strains of the reclassified *T. halophilus* 5B73C^T and *P. soli* CC-YMP-6^T described in this study. The genomic information of *V. pantothenticus* (n = 5), also isolated from JPL-SAF, sequenced and characterized for the purposes of this study is also included.

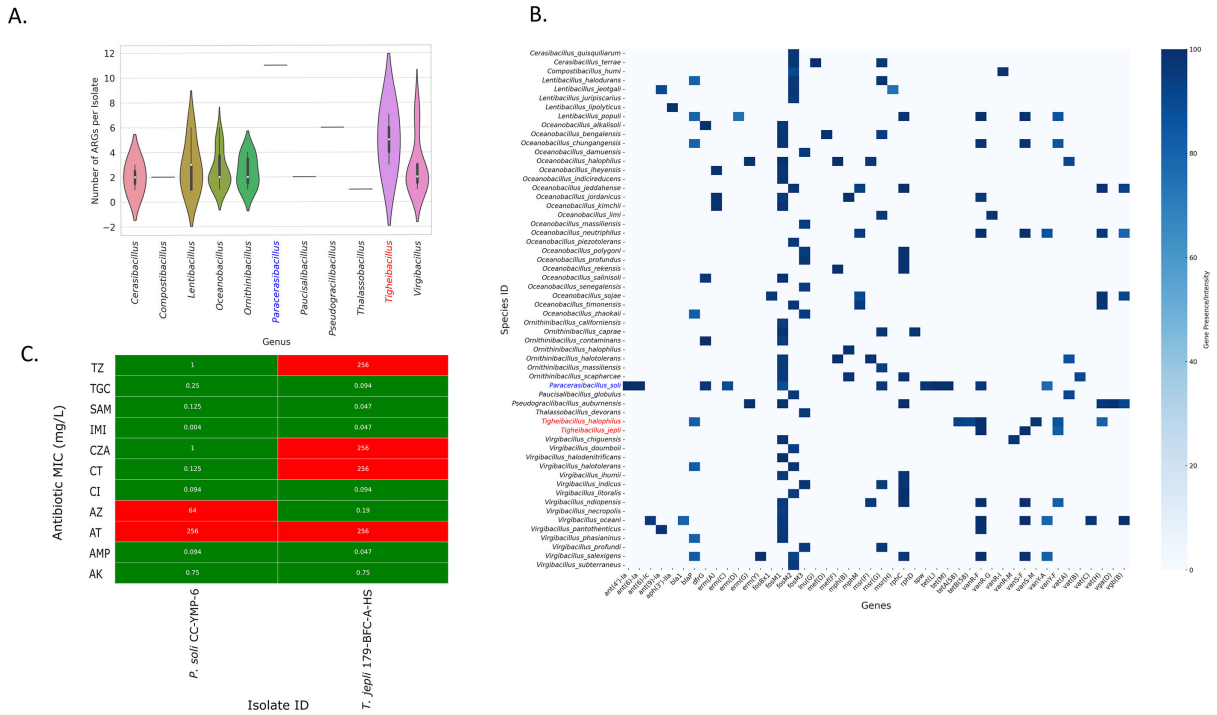


FIG 1 ARG and phenotypic resistance to antibiotics: the mean resistome (± stdev) of the newly described *T. jepli* 179-BFC-A-HS^T and representative genomes of type species of the family *Amphibacillaceae*. (A) Number of ARG per genus analyzed (n = 11). (B) Breakdown of ARG per genome. Color gradient reflects percentage identity with respective gene in the NCBI database. Only hits with >70% identity and coverage are depicted. White color indicates absence of the gene. *Tigheibacillus* spp. is highlighted in red text, and *Paracerasibacillus soli* is highlighted in blue text. (C) MIC (mg/L) values for *T. jepli* 179-BFC-A-HS^T and *P. soli* CC-YMP-3^T for 11 antibiotics interpreted with EUCAST clinical breakpoints under the "non-species" category: imipenem (IMI), ampicillin (AMP), ceftazidime-avibactam (CZA), ampicillin-sulbactam (SAM), cefotaxime (CT), tigecycline (TGC), ceftazidime (TZ), ciprofloxacin (CI), azithromycin (AZ), amikacin (AK), and aztreonam (AT).

between 179-BFC-A-HS^T and CC-YMP-6^T, and between 5B73C^T and CC-YMP-6^T, are 58.53% and 55.88%, respectively, which support their categorization in different genera from CC-YMP-6^T.

Recent analysis based on the Genome Taxonomy Database (GTDB) has reclassified many genera within *Bacillaceae*, including *Virgibacillus* into the new family *Amphibacillaceae* (2). Genomic analysis reveals *Virgibacillus* is polyphyletic, interspersed with genera like *Oceanobacillus*, *Lentibacillus*, *Paucisalibacillus*, and *Cerasibacillus* (Fig. 3). Consequently, strain 179-BFC-A-HS^T is proposed as a new genus and species, *Tigheibacillus jepli* gen. nov., sp. nov. *V. halophilus* 5B73C^T is reclassified as *Tigheibacillus halophilus* comb. nov. and *V. soli* CC-YMP-6^T as *Paracerasibacillus soli* comb. nov., positioned between *Cerasibacillus* and *Tigheibacillus*. The phylogenetic analysis indicates the genus *Virgibacillus* within *Amphibacillaceae* differentiates into clades E, F, and G, suggesting the need for further taxonomic studies to determine if these should be distinct genera.

Biochemical and chemotaxonomic characteristics

Cell growth for *T. jepli* type strain 179-BFC-A-HS^T was measured at temperatures ranging from 4°C to 40°C, with an optimum at 35°C, and in 0.5%−15% NaCl concentrations, optimally at 4%−5%. Biochemical test results using the BioLog GNIII MicroPlate system and the Vitek 2 GP card are detailed in File S4. Fatty acid analysis revealed C 15:0 iso (37.8%) as the predominant component, followed by C 15:0 anteiso (24.2%), C 17:0 anteiso (13.3%), C 16:0 (9.0%), and C 16:0 iso (7.1%) (File S5). This contrasts with *Virgibacillus pantothenticus* CN8028^T, which predominantly produces C 15:0 anteiso (47.4%). The polar lipid profile of strain 179-BFC-A-HS^T includes diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine

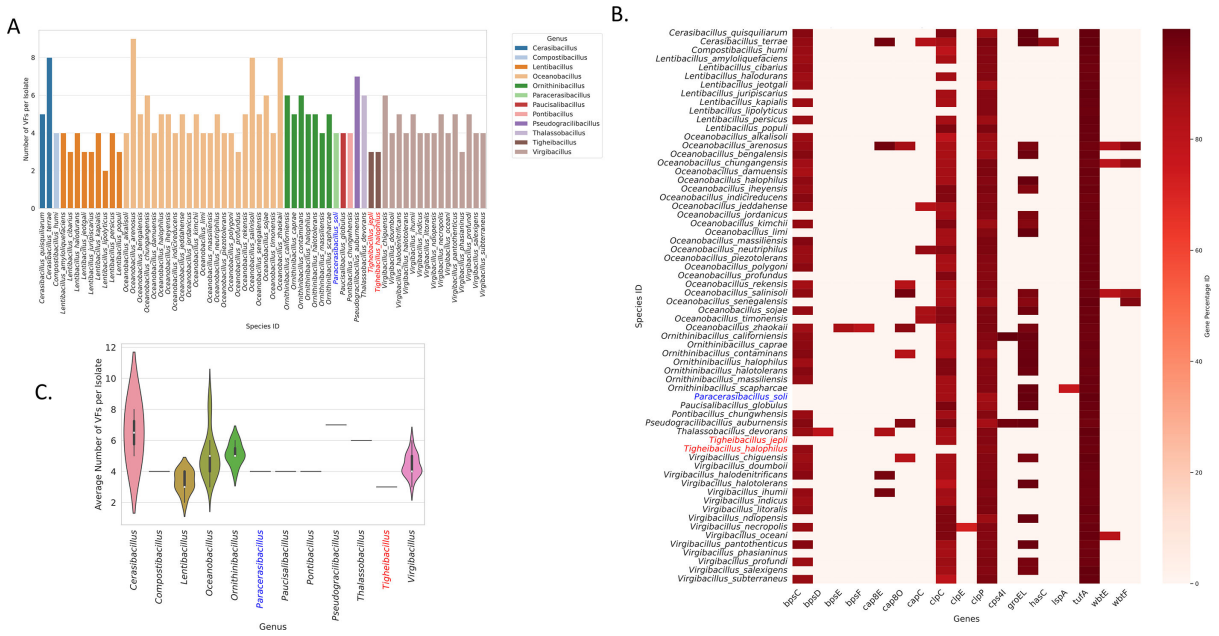


FIG 2 Virulence factors. (A) The virulome of the newly described *T. jepli* 179-BFC-A-HS^T and genomes from the type species of associated genera (n = 64). The number of VF per genome is colored by associated genera. (B) Breakdown of VF per genome. Color gradient reflects percentage identity with respective gene in the VFDB. Only hits >70% identity are depicted. White color indicates absence of the gene. (C) An overview of mean number (± stdev) of identified VF per genus. *Tigheibacillus* spp. is highlighted in red text, and *Paracerasibacillus soli* is highlighted in blue text.

(PC), phosphatidylmonomethylethanolamine (PME), phosphatidylethanolamine (PE), phosphatidylserine (PS), two unidentified aminolipids (AL1−2), an unidentified phospholipid (PL1), and an unidentified aminophospholipid (APL1), as shown in File S6. Menaquinone MK-7 is the primary respiratory quinone, and A1γ with meso 2,6-diaminopimelic acid is the cell wall peptidoglycan's diagnostic diamino acid.

Differential characteristics of *Tigheibacillus* genus

Table 2 highlights distinctive features of *Tigheibacillus*, differentiating it from other *Amphibacillaceae* genera strains. Our study compared phenotypes of type strains from five genera: *Tigheibacillus, Virgibacillus, Oceanobacillus, Ornithinibacillus*, and *Lentibacillus*, comprising 96 recognized species. *T. jepli* 179-BFC-A-HS^T grows between 4°C and 40°C (optimal at 35°C) and tolerates up to 15% NaCl, indicating moderate heat and high salt tolerance. This contrasts with *Lentibacillus*, which prefers cooler temperatures (30°C) and tolerates up to 23% salt. *T. jepli* 179-BFC-A-HS^T can produce acid from D-mannose and D-trehalose, a trait shared with *Virgibacillus* but not *Ornithinibacillus* or *Lentibacillus*. Its iso-C15:0/anteiso-C15:0 fatty acid ratio is 1.6, differing from *Virgibacillus* and *Lentibacillus*, implying variations in cell membrane structure. *Tigheibacillus* has a unique A1γ peptidoglycan type with meso 2,6-DAP, distinct from other genera's peptidoglycan types. While sharing phosphatidylglycerol as a common lipid with other genera, its specific MK-7 quinone and DNA G+C content of 41.6% further set it apart biochemically.

Description of *Tigheibacillus* gen. nov.

Tigheibacillus (Tig.he.i.ba.cil'lus. L. masc. n. *bacillus*, a small rod; N.L. masc. n. *Tigheibacillus* a rod named to honor Scott Tighe, an American microbiologist, for his contribution in all areas of microbiology and extremophiles).

Cells are Gram-staining positive, strictly aerobic, non-spore forming, chemoheterotrophic, and mesophilic. They exhibit positive oxidase reaction and are catalase negative. The cells are flagellated, thick, slender rods with rounded ends, and endospores (Fig. 4). Their major fatty acids include 15:0 iso, 15:0 anteiso, and 17:0 anteiso. The predominant

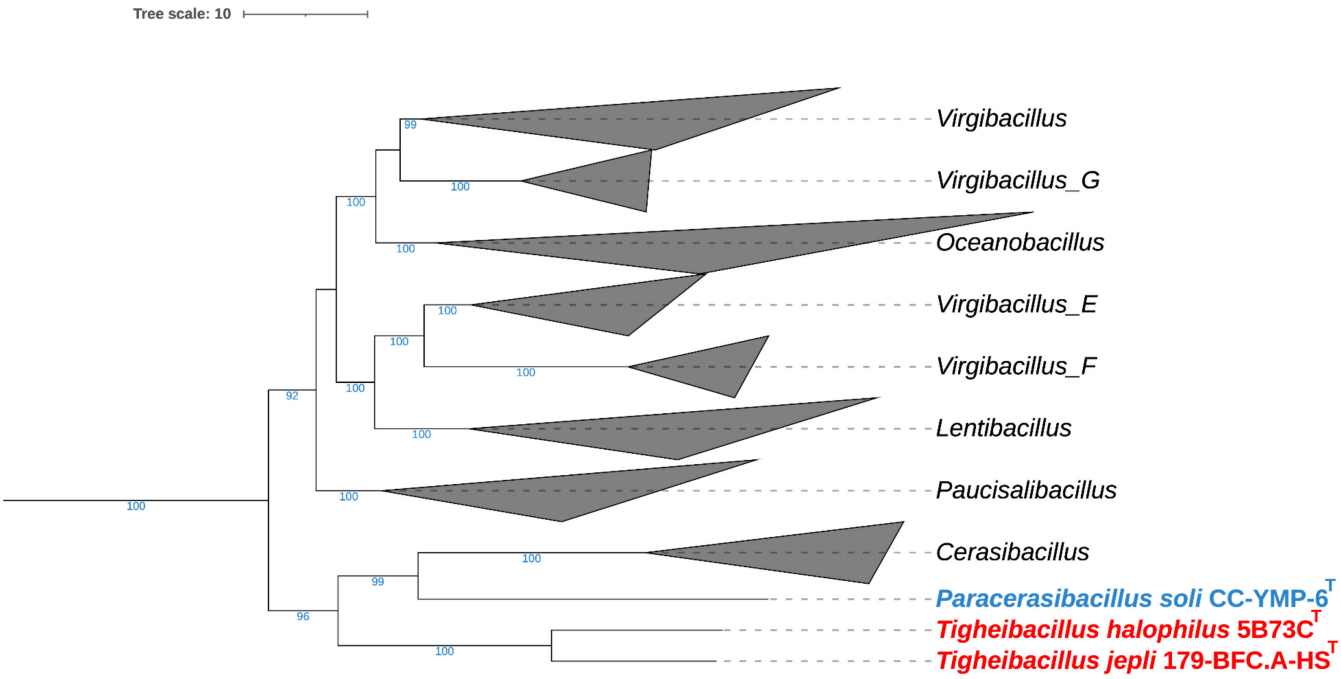


FIG 3 Maximum-likehood phylogenomic tree based on 120 conserved marker genes showing the placement of 179-BFC-A-HS^T with the closely related members of *Amphibacillaceae* family. The tree was rooted on *Thalassobacillus devorans* CECT 7046 and *Pontibacillus chungwhensis* BH030062 as outgroup (not shown). Ultrafast bootstrap values >90% are indicated below the branches.

polar lipids are DPG, PG, PME, PE, and PS, with MK-7 being the major isoprenoid quinone. A1γ with meso 2,6-diaminopimelic acid characterizes the cell wall peptidoglycans. Based on the phylogenetic analyses the genus is placed within the family *Amphibacillaceae*, with *Tigheibacillus jepli* as the type species.

Description of *Tigheibacillus jepli* sp. nov.

Tigheibacillus jepli (jep'li. N.L. gen. n. *jepli*, arbitrary name derived from the abbreviation JPL, meaning of or pertaining to the NASA's Jet Propulsion Laboratory, where the type strain was isolated).

The following properties are observed in addition to those given for the genus description. Cells are 2.4–3.8 µm in length and 0.3–0.4 mm in width. On trypticase soy agar (TSA), after 1–2 days of incubation at 30°C, colonies are circular with regular margins, convex, 0.5–1.0 mm in diameter, and motile. Cell growth occurs at 4°C−40°C (35°C optimum), 0.5%−15% NaCl (4%−5% optimum), and at pH 6.0–9.0 (optimum, pH 7.5). In addition to the major polar lipids listed in the genus description, PC, AL1−2, PL1, and APL1 are produced in moderate-to-minor amounts.

The type strain of *T. jepli* 179-BFC-A-HS^T (DSM 115946^T = NRRL B-65666^T), isolated from spacecraft assembly cleanrooms.

Description of *Tigheibacillus halophilus* comb. nov.

Tigheibacillus halophilus (ha.lo.phi'lus. Gr. masc. n. *hals*, salt; Gr. masc. adj. *philos*, loving; N.L. masc. adj. *halophilus*, salt-loving).

Basonym: *Virgibacillus halophilus* An et al. (32).

The description is identical to that given by An et al. (32). The type strain, 5B73C^T = DSM 21623^T = JCM 21758^T = KCTC 13935^T, was isolated from field soil in Kakegawa, Shizuoka, Japan.

TABLE 2 Differential characteristics of *T. jepli* 179-BFC-A-HS^T and closely related type strains of the type species of the family *Amphibacillaceae*[j]

	1	2	3	4[e]	5
	Tigheibacillus T. jepli	*Virgibacillus* (*n* = 34)	*Oceanobacillus* (*n* = 29)	*Ornithinibacillus* (*n* = 10)	*Lentibacillus* (*n* = 19)
Characteristic	**179-BFC-A HS^T**	***V. pantothenticus***	***O. iheyensis***	***O. bavariensis***	***L. salicampi***
Origin	SAF/JPL, USA	Soil, UK[a]	Deep-sea sediment, Japan[b]	Pasteurized milk, Germany	Salt field, Korea
Growth temperature range (°C)	4–40 (24)	37[c]	15–42[b]	15–45 (25)	15–40 (26)
NaCl tolerance (%)	15 (4–5)	4[c]	0–21[b]	10 (0.5–4)	2–23
pH range for growth	6–9 (7.5)	7[c]	6.5–10[b]	7–10	6–8
Acid production from:					
D-mannose	+	+[c]	+[b]	–	–
D-trehalose	+	+[c]	–[b]	+	–
iso-$C_{15:0}$/anteiso-$C_{15:0}$ ratio	1.6	0.3[d]	<1[e]	1.96	0.8
Peptidoglycan type	A1γ with meso 2,6-DAP	meso DAP direct[f]	meso DAP direct[e]	A4β (L-Orn←D-Asp)	meso DAP
Polar lipids					
Identified	DPG, PG, PME, PE, PC, PS	DPG[d], PG[d], PE[d]	DPG[g], PG[g], PE[g], PC[g]	DPG, PG	DPG, PL
Unidentified	PL1, AL1–2, APL1	APL[d], PL[d], GL[d]	PL[g], L[g], AL[g]	PL, L, APL	UPL
Phosphatidylglycerol content	Major	Major	Major to moderate	Minor	Unknown
Major quinone	MK-7	MK-7[d]	MK-7[b]	MK-7	MK-7
gDNA G+C content (%)	41.6[i]	37.1[d]	35.8[b]	36.4	44

[a]Proom and Knight (27).
[b]Lu et al. (6).
[c]Heyndrickx et al. (26).
[d]Wainø et al. (28).
[e]Mayr et al. (9).
[f]Claus and Berkeley (29).
[g]Long et al. (data were not from type species) (30).
[h]Yoon et al. (31).
[i]Deduced from genome sequence data; SAF, Spacecraft Assembly Facility; JPL, Jet Propulsion Laboratory; DAP, Diaminopimelic Acid; +, Positive; –, Negative; ND, Not Determined.
[j]Taxa: 1: *T. jepli* 179-BFC-A-HS^T; 2, *Virgibacillus pantothenticus* CN8028^T (26); 3, *Oceanobacillus iheyensis* HTE831^T (6); 4, *Ornithinibacillus bavariensis* WSBC 24001^T (9); 5, *Lentibacillus salicampi* SF-20^T (31).

Description of *Paracerasibacillus* gen. nov.

Paracerasibacillus (Pa.ra.ce.ra.si.ba.cil'lus. Gr. prep. *para*, beside, near; N.L. masc. n. *Cerasibacillus*, a bacterial genus name; N.L. masc. n. *Paracerasibacillus* beside the genus *Cerasibacillus*, referring to the close but distinct relationship to the genus *Cerasibacillus*).

The reclassification of *Virgibacillus soli* to *Paracerasibacillus soli* is based on the phylogenetic analyses of 120 conserved protein genes. The description of the genus is identical to that given by Kämpfer et al. (33). The key properties of this species, now representing the genus *Paracerasibacillus*, include Gram-positive bacilli, predominant isoprenoid quinone of menaquinone MK-7, a polar lipid profile featuring diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine, and a polyamine pattern dominated by spermidine. Additionally, physiological and biochemical tests differentiate this strain from other *Virgibacillus* species. These distinguishing features support the reclassification of *Virgibacillus soli* as the type species of the new genus *Paracerasibacillus*, named *Paracerasibacillus soli*.

Description of *Paracerasibacillus soli* comb. nov.

Paracerasibacillus soli (so'li. L. gen. n. *soli*, of soil).

Basonym: *Virgibacillus soli* Kämpfer et al. (33).

The description is identical to that given by Kämpfer et al. (33). The type strain, CC-YMP-6^T = DSM 22952^T = CCM 7714^T, was isolated from soil samples collected from Yang-Ming Mountain, Taiwan.

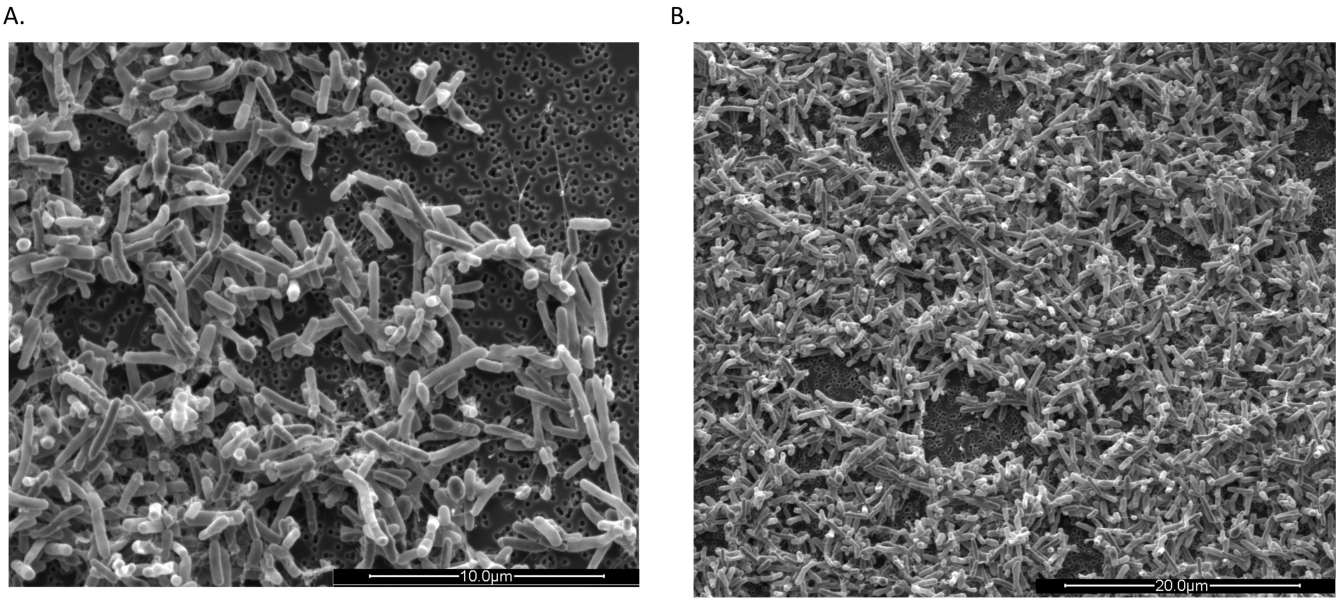


FIG 4 Scanning electron microscopy (SEM) images ([A] 10.0 µm and [B] 20 µm zoom) showcasing cellular structure and overall morphology, including presence of flagellum structure and spatial arrangement of *T. jepli* 179-BFC-A-HS^T.

In silico metabolic predictions

In silico metabolic predictions for *T. jepli, T. halophilus, P. soli,* and 61 other genomes corresponding to type species representing genera within the *Amphibacillaceae* were conducted using the distilled and refined annotation of metabolism (DRAM) software (Fig. 5). The genome of *T. jepli* 179-BFC-A-HS^T encoded for parts 2 and 3 of the acetate to methane pathway, nitrate to nitrite and nitrite to nitrate metabolic pathways, arsenic and mercury reduction pathways, and as part 2 and part 1 of the butyrate and acetate pathways, respectively.

The presence and characteristics of various biosynthetic gene clusters (BGCs) within the genome of *T. jepli* 179-BFC-A-HS^T were predicted using antiSMASH v.7.0.0 with a "strict" detection system. A loosely related (8% identity) BGC corresponding to a type III polyketide synthase cluster and specifically to legonindolizidine A6 was identified (Table S3). Furthermore, the production of a yet-to-be-characterized terpene cluster-associated metabolite was also predicted.

Metagenomics-based mapping to reads generated from JPL-SAF

The genome of the newly characterized *T. jepli* was tracked in metagenomic data sets gathered from the JPL-SAF during a period spanning 6 months in 2016, coinciding with the preparation phase for the Mars 2020 rover's components. Analysis of 236 paired-end shotgun metagenomic data sets, with a threshold of greater than 1% genome coverage, revealed genomic evidence of *T. jepli* in 15 samples as depicted in Fig. 6. These samples were collected over six different dates ranging from 15 March to 28 June 2016. The findings suggest that *T. jepli* is present in low relative abundance and demonstrates limited spatial-temporal distribution within the SAF environment.

DISCUSSION

The genomic landscape within the *Bacillaceae* family has undergone substantial revaluation, particularly with the reclassification of numerous genera following the rank normalization process by GTDB (2). Our comparative genomic analysis emphasizes this taxonomic fluidity, revealing that strain 179-BFC-A-HS^T is not closely aligned with the current genus *Virgibacillus*. Although ANI values below the 95%–96% threshold typically

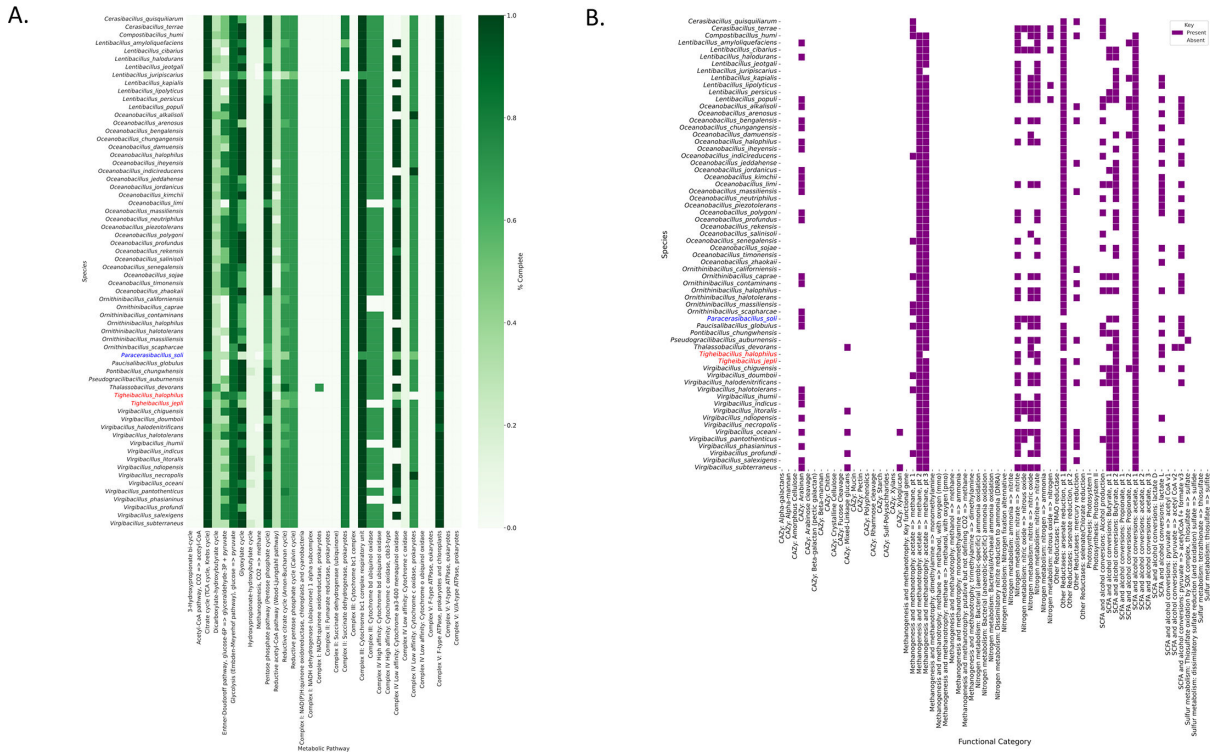
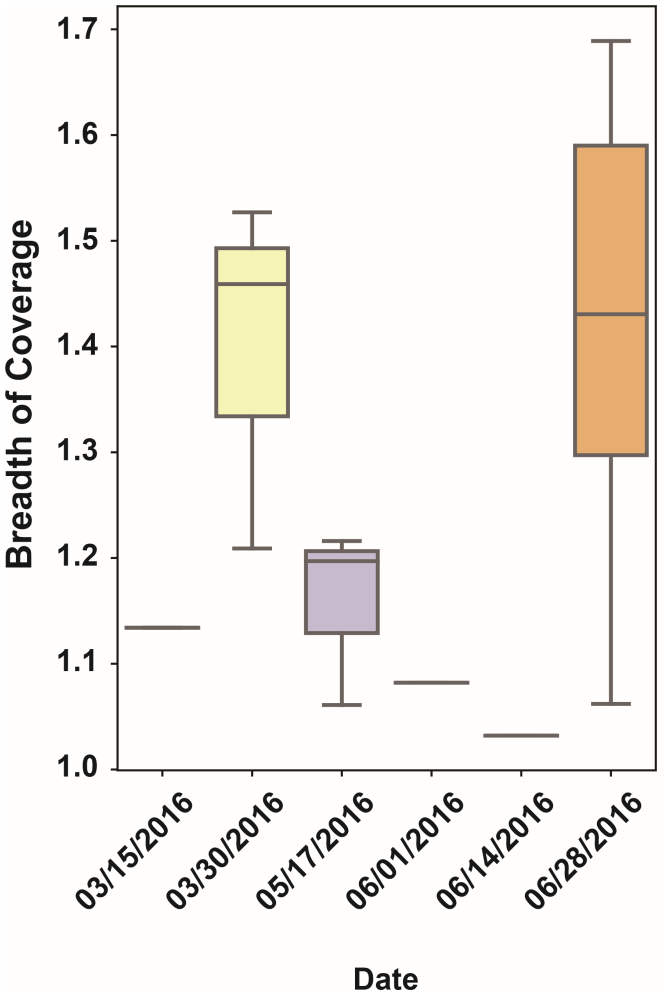


FIG 5 Phenotypic predictions based on genomic data using the DRAM tool. The chart showcases predicted metabolic capabilities and associated pathways, providing insight into the potential physiological roles and adaptations of *T. jepli* 179-BFC-A-HS^T and associated representative genomes of type species within the *Amphibacillaceae* family. (A) Electron transport chain complexes green color-coded gradient based on their completeness, ranging from 0 (absent) to 1 (fully complete). (B) Metabolic predictions are visualized with specific focus on carbohydrate-active enzyme, nitrogen metabolism, sulfur metabolism, various other reductases, photosynthesis, methanogenesis, methanotrophy, short-chain fatty acid conversions, and alcohol conversions. Metabolic features are color coded to indicate whether a function is present (purple) or absent (white).

suggest a novel species (34), a universally accepted threshold for genus-level distinction is still elusive. To confirm our genomic findings, we conducted an in-depth phylogenetic analysis, using marker genes (16S rRNA gene and *gyrB*) and genome-based phylogeny involving 120 conserved protein markers. The average AAI value between *P. soli* and both *T. jepli* and *T. halophilus* genomes is substantially below the 63.43% suggested as a threshold for genus delineation (35). In addition, the dDDH analysis further supports the classification of isolate 179-BFC-A HS^T as a novel genus, with values below the 70%-mark indicative of a distinct species or potentially novel genera (36). While genomic data play a pivotal role in the delineation of species (ANI) and genera (AAI), the core attributes of an organism are frequently manifested through its phenotypic traits and the makeup of its cell wall. Consequently, we adopted an integrative methodology, combining genomic insights with phenotypic characteristics and chemotaxonomic data to achieve a comprehensive understanding. Based on both phenotypic and genotypic evidence and adhering to the rules of the International Code of Nomenclature of Prokaryotes (ICNP) (37), we propose the establishment of a new species within a new genus *Tigheibacillus* for the strain 179-BFC-A-HS^T.

Our phylogenetic analysis reinforces the polyphyletic nature of *Virgibacillus* (26), as members of this genus are separated by other distinct genera such as *Oceanobacillus* and *Lentibacillus*, suggesting a need for re-evaluation of its genus boundaries (6, 38). In contemporary research, genome-based phylogeny is widely recognized as the primary reference point for genus delineation despite the lack of uniform standards in this area. However, the absence of universally applicable thresholds presents significant challenges in making comparative analyses across taxa within the same hierarchical rank (39). This has been addressed by the introduction of the relative evolutionary divergence



Collection date (MM/DD/YYYY)	Number of samples
03/15/2016	1
03/30/2016	3
05/17/2016	3
06/01/2016	1
06/14/2016	1
06/28/2016	6

FIG 6 Mapping of the novel species to SAF metagenomic reads: box plots showing the breadth of coverage of the consensus genome were constructed from mapped reads aligned to *T. jepli* 179-BFC-A-HS^T (reported percentage breadth of coverage cutoff >1%). Reads mapping to the genome of *T. jepli* 179-BFC-A-HS^T were identified for 15 samples retrieved from 6 different collection dates.

approach in GTDB (40), resulting in rank-normalized taxonomy for bacterial and archaeal taxa. The divergence of strains 179-BFC-A-HS^T and 5B73C^T, leading to their reassignment to the newly proposed genus *Tigheibacillus*, reflects their genetic distinction within the *Amphibacillaceae* family. Moreover, based on the phylogenetic placement and relative evolutionary divergence, we suggest to place *V. soli* into its own genus *Paracerasibacillus,* to reflect its distinction from *Virgibacillus* (41). Furthermore, our study calls for more investigation into the clades E, F, and G within *Virgibacillus*, identified as potentially distinct genera.

Species within the *Amphibacillaceae*, including *Virgibacillus* and related genera, are generally not associated with human diseases. Yet, *O. oncorhynchi* subsp. *incaldanensis* has been reported to infect immunocompetent individuals (15) showing growth preference for alkaline environments like the male reproductive system (42). Furthermore, there are documented instances of *Virgibacillus senegalensis* and *Lentibacillus* sp. CBA3610 being isolated from the human gut (4, 24), indicating possible transient colonization with unknown systemic effects. Our VF analysis of the *Amphibacillaceae* family revealed 17 human-associated VF gene homologs. Notably, the *hasC* gene homolog in *C. terrae*'s genome is associated with biofilm formation and commonly found in pathogens like group B streptococci (43). The type IV secretion system effector BspE homolog in *O. zhagkaii* and the signal peptidase II encoding *lspA* homolog in *O.*

scapharcae are associated with the human pathogens *Brucella* species and *Rickettsia typhi,* respectively (44, 45), highlighting a diverse range of VF. This range of human-associated VF in the genomes of environmental microorganisms signifies the importance of further understanding the ecological role of VF genes as well as the need for an in vitro evaluation of the pathogenic potential of such microorganisms.

T. jepli 179-BFC-A-HS^T exhibits an unusual antibiotic resistance phenotypic profile, showing high-level resistance to third- and fourth-generation cephalosporins (MIC >256 mg/L), even when these are combined with the β-lactamase inhibitor avibactam while remaining susceptible to ampicillin. This selective resistance pattern is atypical and not fully explained by the known type resistome as no known β-lactamases were identified. Albeit atypical, similar resistance patterns have been observed in *Bacillus* species within the *Bacillaceae* family. Adamski et al. (46) reported susceptibility to ampicillin and resistance to cefotaxime among a subset of isolates of *B. cereus, B. pumilus,* and *B. licheniformis* isolated from raw milk.

β-Lactam antibiotics function by binding to penicillin-binding proteins (PBPs), inhibiting cell wall synthesis (47). In Gram-positive bacteria, PBPs are more accessible due to the lack of an outer membrane (48), and resistance often involves PBPs with low β-lactam affinity (25, 49). The resistance phenotype in *T. jepli* 179-BFC-A-HS^T may be attributed to modifications in PBPs, diminishing their affinity for cephalosporins relative to penicillin antibiotics. The β-lactam-inducible PBPs of *T. jepli* 179-BFC-A-HS^T show substancial divergence (<63.7% amino acid identity) from those in closely related species, suggesting a distinct resistance profile. The isolation of cephalosporin-resistant bacteria like *T. jepli* 179-BFC-A-HS^T in cleanrooms signals a need for the pharmaceutical and medical industries to strengthen microbial reduction protocols and invest in new decontamination technologies. Such measures are vital to safeguard product integrity, comply with stricter regulatory standards, and mitigate health risks, ensuring the reliability of the global supply chain and maintaining industry reputation against the backdrop of rising antibiotic resistance (50).

Conversely, *P. soli* CC-YMP-6^T presented high-level resistance to azithromycin (MIC > 64 mg/L), a clinically significant macrolide antibiotic for treating infections caused by Gram-positive bacteria (51). This resistance is likely due to the presence of plasmid-mediated erythromycin methyltransferase genes (*ermG/Y/C*), which confer resistance to MLSB antibiotics (52). Isolated from mountainous soil in Yang-Ming Mountain, Taiwan, *P. soli* CC-YMP-6^T's possession of *erm* might offer uncharacterized adaptive advantages or indicate anthropogenic environmental contamination. The detection of *erm* genes in soil samples, particularly in urban areas with high anthropogenic activity, varies widely (53), underscoring the need to understand the environmental and evolutionary factors influencing AMR in natural settings.

T. jepli 179-BFC-A-HS^T genomic analysis predicts a metabolic profile involved in methanogenesis, nitrogen cycling, and reductive reactions, notably in the latter stages of acetate-to-methane conversion. It also exhibits pathways for nitrogen utilization, reducing heavy metals like arsenate and mercury, while it lacks pathways for carbohydrate-active enzymes (CAZy) and short-chain fatty acid (SCFA)/alcohol transformations. The predicted heavy metal metabolic capacity of *T. jepli* 179-BFC-A-HS^T aligns with the members of the genus *Bacillus* known for their bioremediation potential in reducing environmental concentrations of heavy metals (54). The metabolic traits of *T. jepli* indicate its putative capacity to utilize energy and nutrients from limited sources, neutralize heavy metals, and participate in biogeochemical cycles, suggesting a resilient phenotype suitable for nutrient-limited environments such as controlled clean rooms. The resilience of *T. jepli* in oligotrophic environments, mirroring traits of related *Amphibacillaceae* genera like *Oceanobacillus* and *Virgibacillus*, highlights their efficient nutrient utilization and stress resistance (6, 33). Such traits are vital for survival in nutrient-poor habitats, suggesting a possible role in nutrient cycling and ecosystem stability (55). Studying these related genera enhances our understanding about the ecological role of *T. jepli*.

Metagenomic analysis in JPL-SAF revealed the infrequent occurrence of *T. jepli* 179-BFC-A-HS^T during the assembly phase of the Mars 2020 rover subsystems. Over 6 months in 2016, genomic signatures of *T. jepli* were identified in only 15 of 236 samples. This intermittent presence implies that *T. jepli* may have a niche-specific ecology or be a transient organism in the SAF environment. Generally, clean room microbiomes are dominated by gram-positive, spore-forming species, with *Bacillus* species comprising 10%–13% of isolates (56). Previous culture-based analyses in the JPL-SAF have identified 16 bacterial genera, frequently detecting *Bacillus subtilis* and *V. pantothenticus* (20). *Bacillaceae* and *Amphibacillaceae* species are notable for their persistence in such environments. Understanding the low prevalence of certain *Amphibacillaceae* species, including *T. jepli*, can inform contamination control strategies for space missions (57) and contribute to planetary protection efforts.

The presence of a high 16S rRNA gene copy number (16S GCN) in *T. jepli* is an intriguing aspect of the findings that merits further investigation. The 16S GCN in bacteria usually ranges from 1 to 15 and is often correlated with ecological strategies: oligotrophs, which thrive in nutrient-poor environments, usually have a lower 16S GCN, while copiotrophs, which flourish in nutrient-rich settings, have a higher 16S GCN, reflecting their adaptive strategies to their respective environment (58). The high full length 16S GCN in *T. jepli* (n = 16), found in the oligotrophic environment of the SAF, suggests it might confer an advantage for growth when conditions become favorable (59). The observed high 16S GCN copy number may also confer ecological versatility, allowing *T. jepli* to rapidly adjust to sporadic nutrient availability, which could be a result of evolutionary adaptations (60). While a high 16S GCN is advantageous for initial growth, it is suggested that the ability to endure extended periods of starvation involves more complex survival mechanisms that go beyond just the number of 16S GCN (61). Furthermore, the presence of 27 dormancy, sporulation, and spore germination-associated genes in the genome of *T. jepli* indicates their role in enabling survival in nutrient-poor environments, employing spore formation as an adaptive mechanism for survival.

The robustness of *T. jepli* in oligotrophic environments, coupled with its potential for heavy metal detoxification, mirrors similar capabilities observed in *Bacillus* species (54), indicating its potential suitability for bioremediation in nutrient-scarce, contaminated areas. Additionally, atypical resistance profile of *T. jepli*, alongside its occurrence in human-associated settings, necessitates further investigation into its pathogenicity. Studies focusing on its interactions with human cells and serum resistance are essential for evaluating potential health risks in medical and industrial contexts.

Conclusion

In conclusion, microbial surveillance conducted as part of the Mars 2020 mission revealed a novel bacterial strain, 179-BFC-A-HS^T, classified as *T. jepli* which represents the type strain of a novel species of a novel genus named herein. The high-level resistance of *T. jepli* to third- and fourth-generation cephalosporins, contrasted with its susceptibility to penicillins, along with its unique genetic profile, underscores the importance of characterizing resistance patterns, even in the absence of clinically associated environmental pressures. Furthermore, several human pathogenicity-associated VF were identified in the genome of *T. jepli*. The metabolic versatility of *T. jepli*, particularly its involvement in pathways such as methanogenesis, nitrogen cycling, reductive reactions, and heavy metal detoxification, highlights the resilience and adaptability of microorganisms in nutrient-limited environments. These findings are not only crucial for space exploration but also have significant implications for maintaining stringent microbial control standards in other sensitive industries, such as semiconductor manufacturing, pharmaceutical production, and medical device fabrication, where microbial contamination can have profound impacts.

MATERIALS AND METHODS

Sample collection, isolation, and preliminary identification of the strain 179-BFC-A-HS^T using 16S rRNA gene phylogeny were presented elsewhere (20). Additionally, type strains of CC-YMP-6^T (formerly *Virgibacillus soli* DSM 22952^T) and 5B73C^T (formerly *Virgibacillus halophilus* DSM 21623^T) were purchased from the Leibniz Institute DSMZ—the German Collection of Microorganisms and Cell Cultures GmbH. Their genomes were sequenced, and extensive AMR phenotypic analyses were performed.

DNA extraction: second- and third-generation sequencing

DNA from overnight-grown (30°C, TSA) cultures was extracted using a GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA). Samples with >10 ng/µL concentration, A_{260}/A_{280} ratios of 1.8–2.0, and A_{260}/A_{230} ratios of 1.8–2.2 were selected for sequencing, and libraries prepared using the SQK-RBK114.24 Kit (Oxford Nanopore Technologies, UK) were sequenced on a MinION Mk1C system. Base calling was completedusing MinKnow/Guppy, adapter trimming with Porechop (v.0.2.4) (62), and read filtering by Filtlong v.0.2.1 with parameters --min_length 1000, --keep_percent 90. Hybrid genome assemblies for CC-YMP-6^T (ASM3400633v1) and 179-BFC-A-HS^T (ASM3073200v2) used Unicycler (v.0.5.0) (63), while strain 5B73C^T (ASM3400637v1) had a long-read assembly via Flye v.2.9.1 (64). Assembly quality was assessed using Quast (v.5.2.0) (65). For *V. pantothenticus* isolates 179-FW3.5N-HS, 179-I4A3N-HS, 179-I6B2N-HS, 179-I6B3N-HS, and 179-6B4N-HS, DNA was extracted, sequenced, and assembled as previously described (22).

Phenotypic and genotypic assessment of antimicrobial resistance properties and genotypic prediction of virulence potential

ARG and VF-encoding genes were classified using Abricate v. 1.0.0 against National Center for Biotechnology Information (NCBI) (66) and VFDB (23) databases, considering only hits with >80% nucleotide coverage and >70% identity. Data analysis and visualization were carried out using R programming. Antibiotic susceptibility testing was conducted on 11 antibiotics (imipenem, ampicillin, ceftazidime-avibactam, ampicillin-sulbactam, cefotaxime, tigecycline, ceftazidime, ciprofloxacin, azithromycin, amikacin, and aztreonam) using MIC Test Strips (Liofilchem, Italy) on Mueller-Hinton II agar (Fannin Ltd., Ireland). Susceptibility categories were assigned based on the EUCAST clinical breakpoints (v. 13.0, 2023) using PK-PD interpretive criteria (67).

Phylogeny and genome annotations

A two-step phylogenomic analysis was conducted on isolate 179-BFC-A-HS^T using 120 conserved single-copy marker proteins (40) as follows: initially using GTDB-Tk v2.3.0 (68) by running (i) the ani_rep command to compare genome sequence from strain 179-BFC-A-HS^T against all GTDB representative genomes (release 08-RS214) and (ii) classify_wf to find out the maximum-likelihood placement in the GTDB reference tree. Based on the output from the classify workflow (classify_wf), we selected a total of 61 genome sequences representing closely related type strains of all genera from the family *Amphibacillaceae* according to GTDB taxonomy (08-RS214), including genomes sequenced as part of this study from *P. soli* CC-YMP-6^T and *T. halophilus* 5B73C^T strains. Second, we inferred genome tree from the multiple sequence alignment of 61 selected genomes including the genome of strain 179-BFC-A-HS^T and two genomes representing an outgroup using FastTree v.2.1.11 (69) with the WAG model executed via GTDB-Tk v2.3.0. The inferred tree was further used as a starting tree in the IQ-TREE v.2.1.2 (70) executed via the following command: iqtree -s < alignment > m LG + C10+F + G -ft < starting_tree> -bb 1000. The tree was visualized, rooted, and pruned for illustration purposes in ARB v.6.0.6 and beautified in iTOL (71).

We assessed the genetic relatedness of 64 genomes in this study by creating an ANI heatmap with ANIclustermap (v1.2.0), which utilizes fastANI and seaborn algorithms.

Genomic annotations were performed using PROKKA v1.14.5 with the --compliant extension (72). AAI values were determined using protein sequences as input based on the AAI calculator software developed by Kostas Lab. We extracted the *gyrB* and 16S rRNA gene sequences of *T. jepli* 179-BFC-A-HS^T and compared their percentage identities with their three closest sequences using BLASTn. Additionally, the dDDH value was estimated using formula 2 of the Genome-to-Genome Distance Calculator v.3.0 and the BLAST+ alignment tool (73). For the 61 species of *Amphibacillaceae* family, we extracted their 16S rRNA gene sequences from NCBI and aligned them with *P. soli* CC-YMP-6^T, *T. jepli* 179-BFC-A-HS^T, and *T. halophilus* 5B73C^T using MUSCLE v3.8.1551. A phylogenetic tree was constructed using IQTREE v.2.2.0.3 (74) with the GTR + G model and 1,000 ultrafast bootstrap replicates, while trees were visualized in iTOL v.6.7 (75).

Light microscopy and scanning electron microscopy

Spore formation was checked using malachite green and safranin followed by light microscopy using a DP25 camera and cellSens software (76). SEM analyses were carried out as previously published (77), and images were collected with an FEI Quanta 200F electron microscope at the California Institute of Technology's Kavli Nanoscience Institute.

Biochemical and phenotypic characteristics

We used the Vitek 2 GP ID (bioMérieux) card for biochemical testing as per the manufacturer's guidelines. Colonies from TSA were aseptically transferred into saline (0.45%–0.50% NaCl, pH 4.5–7.0) to create a 0.5-McFarland suspension (~1.5 × 10^8 cfu/mL), verified with VITEK 2 DensiCHEK Plus. This suspension, along with the Vitek 2 GP ID card, was incubated in a cassette at 37°C. Cassette loading, data entry, and retrieval of results (within 10 hours of inoculation) followed the VITEK instrument manual.

For phenotypic fingerprinting, a GNIII MicroPlate (BioLog) was used, preparing inoculum from TSA colonies in solution A (Cat # 72401; BioLog) to achieve a McFarland of 0.5. A volume of 100 µL of this bacterial suspension was loaded into each well of a GNIII MicroPlate and incubated at 37°C for 24 hours. OmniLog readings (A_{590}–A_{750}) were taken with a FLUO star Omega MicroPlate reader (BMG Labtech, Germany) within 24 hours of incubation.

Temperature tolerance and optimal growth temperatures were tested by incubating cells in tryptic soy broth at various temperatures (4°C, 10°C, 20°C, 27°C, 35°C, 40°C, 50°C, 70°C, 100°C). Salt tolerance and optimal concentration were assessed by growing cells in R2A broth with varying NaCl concentrations (0%–18% [wt/vol], in increments of 0.5% up to 6% and larger increments thereafter). For pH tolerance and optimal pH, cells were cultured across a pH range of 4.0–10.0 (in 1.0 pH unit intervals) using specific buffer systems: 0.1 M citric acid/0.1 M trisodium citrate for pH 4.0–5.0; 0.2 M Na_2HPO_4/0.2 M NaH_2PO_4 for pH 6.0–8.0; and 0.1 M $NaHCO_3$/0.1 M Na_2CO_3 for pH 9.0–10.0. The pH was verified and adjusted post-sterilization of the media (78).

Chemotaxonomic analysis

Cells grown on TSA at 30°C for 48 hours to mid-exponential phase were used for fatty acid methyl esters (FAMEs) analysis. FAMEs were extracted from cell biomass via sequential saponification, methylation, and extraction (79) and then separated using a gas chromatograph (Agilent 7890A) with a flame ionization detector. Identification of FAMEs utilized the Microbial Identification System (MIDI) (80) and the Aerobe database (RTSBA6) in Sherlock version 6.0, adhering to the standard protocol (81).

Cells grown on TSA for 3 days at 30°C were used for extracting polar lipids, quinones, and peptidoglycans. Polar lipids and quinones were analyzed using 2D thin-layer chromatography (TLC) (82). TLC plates were sprayed with various reagents for lipid identification: 10% ethanolic phosphomolybdic acid for total lipids, 0.2% ninhydrin in butanol for aminolipids, Dittmer and Lester's Zinzadze reagent for phospholipids,

and alpha-naphthol for glycolipids. Peptidoglycans characterization followed established protocols by Staneck and Roberts (83).

Genotypic prediction of metabolic traits

The DRAM software v.1.4.6 (84) was used to analyze the genomic assemblies of all three strains tested during this study and 61 associated representative genomes of type species within the *Amphibacillaceae* family, for electron transport chain complexes and key metabolic functions, assigning a completeness scale from 0 (absent) to 1 (fully complete). This analysis included CAZy profiles, nitrogen and sulfur metabolism, reductases, photosynthesis pathways, methanogenesis, methanotrophy, and SCFA and alcohol conversions. Features were color coded for presence or absence in the genomic data. For strain 179-BFC-A-HS^T, antiSMASH v.7.0.1 was additionally used with a "strict" detection method (85) to predict BGCs associated with secondary metabolites, aligning them with the MIBiG v.3.1 database for functional annotations (85).

Mapping of the novel species to SAF metagenomic reads

Shotgun metagenomics sequence data sets (236 samples) from the NASA's-JPL SAF were tested to evaluate the prevalence of strain 179-BFC-A-HS^T (20). Quality control and adapter trimming were conducted using Fastp v.0.23.4 with default settings. To map and quantify the metagenome reads relative to the novel species, MetaCompass v.2.0 was used with a maximum read length of 150, employing a reference-guided assembly approach. This included using Bowtie2 for read alignment and MEGAHIT for consensus genome construction. Samples without sequence data mapping the reference genome were excluded from further analysis.

AUTHOR AFFILIATIONS

[1]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, University of Galway, Galway, Ireland
[2]Centre for One Health, Ryan Institute, University of Galway, Galway, Ireland
[3]Department of Biotechnology, Bhupat and Jyoti Mehta School of Biosciences, Indian Institute of Technology Madras, Chennai, Tamil Nadu, India
[4]Center for Integrative Biology and Systems mEdicine (IBSE), Indian Institute of Technology Madras, Chennai, Tamil Nadu, India
[5]Robert Bosch Centre for Data Science and Artificial Intelligence (RBCDSAI), Indian Institute of Technology Madras, Chennai, Tamil Nadu, India
[6]Division of Microbiology and Biotechnology, Yenepoya Research Centre, Yenepoya (Deemed to be University), Mangalore, Karnataka, India
[7]The University of Queensland, School of Chemistry and Molecular Biosciences, Australian Centre for Ecogenomics, Brisbane, Australia
[8]Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA
[9]US Department of Energy Joint Genome Institute, Berkeley, California, USA
[10]Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA

AUTHOR ORCIDs

Georgios Miliotis http://orcid.org/0000-0002-0944-2206
Pratyay Sengupta http://orcid.org/0000-0002-0184-9335
Asif Hameed http://orcid.org/0000-0003-4445-7080
Maria Chuvochina http://orcid.org/0000-0002-3977-6012
Francesca McDonagh http://orcid.org/0000-0002-0830-4899
Punchappady D. Rekha http://orcid.org/0000-0002-9187-6395
Karthik Raman http://orcid.org/0000-0002-9311-7093

Nikos C. Kyrpides http://orcid.org/0000-0002-6131-0462
Philip Hugenholtz http://orcid.org/0000-0001-5386-7925
Kasthuri Venkateswaran http://orcid.org/0000-0002-6742-0873

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AUTHOR CONTRIBUTIONS

Georgios Miliotis, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing | Pratyay Sengupta, Data curation, Formal analysis, Investigation, Software, Validation, Visualization, Writing – original draft, Writing – review and editing | Asif Hameed, Formal analysis, Resources, Validation, Visualization, Writing – original draft, Writing – review and editing | Maria Chuvochina, Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review and editing | Francesca McDonagh, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review and editing | Anna C. Simpson, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review and editing | Ceth W. Parker, Validation, Visualization, Writing – original draft, Writing – review and editing | Nitin K. Singh, Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review and editing | Punchappady D. Rekha, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review and editing | Dearbháile Morris, Investigation, Supervision, Writing – original draft, Writing – review and editing | Karthik Raman, Investigation, Validation, Visualization, Writing – original draft, Writing – review and editing | Nikos C. Kyrpides, Investigation, Methodology, Writing – original draft, Writing – review and editing | Philip Hugenholtz, Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review and editing | Kasthuri Venkateswaran, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing

DIRECT CONTRIBUTION

This article is a direct contribution from Nikos C. Kyrpides, a Fellow of the American Academy of Microbiology, who arranged for and secured reviews by Fumito Maruyama, Office of Academic Research and Industry-Government Collaboration, Academy of Hiroshima University, and Alexandre Rosado, KAUST, King Abdullah University of Science and Technology.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental material (Supplemental file.docx). Supplemental figures and tables.

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