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ORIGINAL PAPER

Priestia veravalensis sp. nov., isolated from coastal sample

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Abstract

The taxonomic position of two isolates, SGD-V-76^T and SGD-M-37, isolated from sediment sample of Veraval coast, India, was examined using the polyphasic taxonomic approach. The morphological and chemotaxonomic characteristics of these two organisms are typical of the genus *Priestia*. The phylogenetic analyses performed using almost complete 16S rRNA gene sequences demonstrated that the isolate belongs to the *Bacillaceae* family, and forms a clade within the cluster containing *Priestia flexus* MTCC 2909^T, *Priestia aryabhattai* B8W22^T and *Priestia megaterium* KCTC 3007^T and both strains showed highest similarity of > 98% with 3–29 nucleotide differences. The cell wall contained *meso*-diaminopimelic acid as the diagnostic diamino acid. The predominant isoprenoid quinone was MK-7 and the G + C content of strains was 37.5–37.7 mol%. However, the DNA–DNA hybridization and the phenotypic characteristics revealed that, the strain SGD-V-76^T and strain SGD-M-37 are similar species but different from any known *Priestia* species with ANI values of 79.2, 79.3 and 79.2 and the dDDH values of 17.7, 17.8 and 18.0% respectively. On the basis of phenotypic characteristics, phylogenetic analysis and the results of biochemical and physiological tests, and genomic data strain SGD-V-76^T was clearly distinguished from closely related members of the *Priestia* genus. Based on the above data analysis strain SGD-V-76^T (= DSM28242^T = KCT C33802^T = CIP111056^T = NCIM5510^T) represents a novel species of the genus *Priestia*, and we propose the name *Priestia veravalensis* sp. nov.

Keywords Priestia · Coastal sediment · Veraval · Genome

Introduction

Marine communities are the most diverse and complex group of organisms. Conventional methods used for identification of *Bacillaceae* is done mainly using biochemical assay and FAME analysis (Heyrman et al. 1999), and is being further confirmed by 16S rRNA gene sequence analysis. This method helped identification of those species that lack distinguishable phenotypic characteristics and is a benchmark for the modern classification of bacteria, including those in the genus *Bacillus*. Many new species i.e., *Shewanella gaetbuli*, *Erythrobacter aquimaris*, *Erythrobacter flavus* and *Bacillus marisflavi*, have recently been isolated from marine sediments in Korea (Yi et al. 2003; Yoon et al. 2003a, b, 2004a; b). Many novel bacteria are frequently isolated from the saline and hyper saline environments, such as saline soils and saline aquatic habitats (Arahal et al. 2002; Sánchez-Porro et al. 2009; Ventosa 2006; Ventosa et al. 1998). Very recently a robust demarcation of 17 distinct Bacillus species clades, were proposed as novel *Bacillaceae* genera, by phylogenomics and comparative genomic analyses (Gupta et al. 2020). In the course of investigation of bacterial diversity in the Veraval coast, Gujarat, India, two isolates namely, SGD-V-76^T and SGD-M-37 were isolated from sediment sample collected from the coast. The aim of this study was to determine the taxonomic position of both the strains SGD-V-76^T and SGD-M-37 by phylogenetic analyses, chemotaxonomic, physiological and genomic properties.

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Materials and method

Isolation and cultivation

Two isolates SGD-V-76^T and SGD-M-37 were isolated from coastal sediment sample which coordinates 20.91° N 70.37° E on marine agar (Hi-Media, Mumbai) by standard serial dilution method and were incubated at 28 ± 2 °C for 3–5 days. Isolated colonies were purified by repeated streaking on fresh marine agar plates, and further maintained on nutrient agar (NA) slants at 4 °C and as well as glycerol suspensions (20%, v/v) at − 80 °C. References strains were obtained from JCM Japan and used as controls in the phenotypic and FAME analysis.

Morphological, physiological and biochemical properties

Morphological and chemotaxonomic studies were performed for both isolates to confirm whether they exhibited properties similar to those of the genus *Priestia*. Morphology was observed under a light microscope and a scanning electron microscope ((Philips XL30; ESEM-TMP) of 72 h old cultures on nutrient agar. Biomass for chemotaxonomy and molecular-systematic analysis was obtained following growth in tryptic soy broth (Hi-media, Mumbai) under shaking condition of 150 rpm at 28 ± 2 °C for 72 h. Gram staining was carried out using the standard Gram reaction (Smibert and Krieg 1994) and cell motility was confirmed by the development of turbidity throughout a tube containing semisolid medium. To test for motility, a well-isolated colony and stab the 0.4% semisolid nutrient agar medium upto 1 cm of the bottom of the tube and incubated at 28 ± 2 °C for 18 h or until growth is evident from the slant. A positive motility test is indicated by a turbid area extending away from the line of inoculation (Leifson 1960). Growth at different temperatures (4, 10, 20, 30, 37, 42, 45, 50 and 55 °C) was tested on nutrient broth by incubating the cultures for 72 h and measured the optical density of growth at 660 nm and the sterile distilled water used as a control. The pH range for growth (pH 4, 5, 6, 7, 8, 9, 10 and 12), was analyzed using the buffer system described by Xu et al. (2005) at an interval 1.0 pH unit and NaCl tolerance (0, 1, 3, 5, 7, 9, 10, 12 and 15% w/v) was tested at 28 ± 2 °C for 72 h by culturing the strains in nutrient broth. Catalase activity was determined by production of bubbles after the addition of a drop of 3% H_2O_2. Oxidase activity was determined using API oxidase reagent. Hydrolysis of urea was determined on peptone-glucose agar (g/L): peptone 1.0, glucose 1.0, NaCl 5.0, KH_2PO_4 2.0, containing 2% (w/v) urea and 0.001% (w/v) phenol red as a indicator. Gelatin liquefaction was determined by incubation on peptone-gelatin medium (g/L) (peptone 5.0, gelatin 120.0) and milk coagulation and peptonization was determined using 20% (w/v) of skimmed milk as medium and incubation for 48–72 h at 28 ± 2 °C. Hydrolysis of starch was determined on peptone-beef extract agar containing 0.2% (w/v) soluble starch by flooding of the plates with iodine solution. Nitrate reduction and urease were assessed as described by Smibert and Krieg (1994). The strain was characterized biochemically using the API CH50 and API ZYM systems (bioMérieux). Nutrient agar plates were used to examine hydrolysis of starch and Tweens 20, 40, 60 and 80 (at a final concentration of 1%; v/v).

Chemotaxonomic characterization

Analysis of cell wall amino acids was performed using the methods of Collins et al (1983). Polar lipids were extracted, examined using two-dimensional TLC and identified by standard procedures of Minnikin et al. (1984). Polar lipids were extracted using a chloroform/methanol system and analyzed using one & two-dimensional TLC, as described previously (Kates 1986). Merck silica gel 60 F254 aluminum-backed thin-layer plates were used in TLC analysis. Menaquinones were isolated according to Minnikin et al. (1984) and were separated using HPLC (Kroppenstedt 1982). For fatty acids analysis, both strains, SGD-V-76^T and SGD-M-37 were cultured on TSA at 28 ± 2 °C for 72 h. Preparation and analysis of fatty acid methyl esters (FAME) were performed as described by Sasser (1990) using the Microbial Identification System software package (Sherlock Version 6.1; TSBA6).

Phylogenetic characterization

Extractions of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene from both strains SGD-V-76^T and SGD-M-37 were performed as described by Li et al. (2007). The resulting 16S rRNA gene sequence was compared with available 16S rRNA gene sequences from Eztaxon-e server database (Kim et al. 2012) to determine the phylogenetic affiliation of strains. Multiple sequence alignments and distance levels of closely related neighbor were calculated using CLUSTAL_X (Thompson et al. 1997). Phylogenetic analyses were performed using three tree-making algorithms like, neighbor joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971). A phylogenetic tree was constructed using the neighbor joining from K*nuc* values using MEGA version 7.0 (Tamura et al. 2013). The topologies of

the phylogenetic tree were evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Genome analysis

For investigation of genome relatedness, whole genome sequencing was attempted for strain SGD-V-76^T, since SGD-M-37 showed closer 16S rRNA similarity with SGD-V-76^T and compared with available closest reference genomes *Priestia flexus* IFO 15715^T, *Priestia megaterium* IAM 13418^T and *Priestia aryabhattai* B8W22^T. Genomic DNA samples were extracted using a Hi-PUR A™ Bacterial genomic DNA purification kit (Hi-Media, Mumbai) and the draft genome of strain SGD-V-76^T was generated at the DOE Joint Genome Institute (JGI) using the Illumina technology (Bennett 2004). An Illumina shotgun library was constructed and sequenced using the Illumina HiSeq 2000 platform, which generated 4,326,908, reads totaling 653.4 Mb. All general aspects of library construction and sequencing performed at the JGI can be found at http://www.jgi.doe.gov. All raw Illumina sequence data was passed through DUK, a filtering program developed at JGI, which removes known Illumina sequencing and library preparation artifacts. Following steps were then performed for assembly: (1) filtered Illumina reads were assembled using Velvet (version 1.2.07) (Zerbino and Birney 2008), (2) 1–3 kb simulated paired end reads were created from Velvet contigs using wgsim (version 0.3.0) (https://github.com/lh3/wgsim), (3) Illumina reads were assembled with simulated read pairs using Allpaths–LG (version r46652) (Gnerre et al. 2011), Parameters for assembly steps were: (1) Velvet (velvet h: 63 –short Paired and velvet g: –very clean yes –export Filtered yes –min contig length 500–scaffolding no –cov cutoff 10) (2) wgsim (–e 0 –1 100 –2 100 –r 0 –R 0 –X 0) (3) Allpaths–LG (Prepare Allpaths In puts:, Run Allpaths LG:) The final draft assembly contained 158 contigs in 146 scaffolds, totalling 4.1 Mb in size. The final assembly was based on 285.4 Mb of Illumina data. 57.1X input read coverage was used for the final assembly.

Genome annotation

Genes were identified using Prodigal (Hyatt et al. 2010), followed by a round of manual curation using Gene PRIMP (Pati et al. 2010) for finished genomes and Draft genomes in fewer than 20 scaffolds. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) non-redundant database, UniProt, TIGRFam, Pfam, KEGG, COG, and InterPro databases. The tRNAScanSE tool (Lowe and Eddy 1997) was used to find tRNA genes, whereas ribosomal RNA genes were found by searches against models of the ribosomal RNA genes built from SILVA (Pruesse et al. 2007). Other non–coding RNAs such as the RNA components of the protein secretion complex and the RNase P were identified by searching the genome for the corresponding Rfam profiles using INFERNAL (http://infernal.janelia.org). Additional gene prediction analysis and manual functional annotation was performed within the Integrated Microbial Genomes (IMG) platform (Markowitz et al. 2014) developed by the Joint Genome Institute, Walnut Creek, CA, USA (Markowitz et al. 2009). The degree of pairwise genome-based relatedness was estimated by both an average nucleotide identity (ANI) value following the BLAST-based ANI calculation method described by Goris et al. (2007), and the genome-to-genome distance calculation (GGDC) method described by Alexander et al. (2010). The genomic DNA G + C content was calculated directly from the genome sequence. The draft genome sequence of *Priestia veravalensis* NIO-V-76^T was retrieved from the shotgun assembly sequences. GenBank accession number for draft genome is LNQJ01000000.

Further wet lab experiments were performed for the DNA G + C content (mol%) analysis according to Gonzalez and Saiz-Jimenez (2005). ΔTm values between homologous and hybrid DNA is analyzed by Real-Time PCR (Saitou and Nei 1987). For DNA–DNA hybridization experiments, genomic DNA was extracted and purified according to the method of Marmur (1961) with additional RNAse treatment. DNA–DNA hybridization was carried out to reconfirm the ANI and digital DDH analysis as described by De Ley et al. (1970) under the consideration of modifications done by Huss et al. (1983) with optimized fluorimetric procedure evaluated by Loveland-Curtze et al. (2011), using a Step One Plus Real-Time PCR system (Applied Biosystems) fitted with 96 well thermal cycling blocks. DNA suspended in the 2X SSC is used for the analysis in triplicates. The reassociation was carried out at optimum renaturation temperature 70 °C. The relatedness values are expressed by the means of three independent values.

To further study the genetic relatedness of SGD-V-76^T, SGD-M-37 with the three closest type strains, *Priestia flexus* IFO 15715^T, *Priestia megaterium* IAM 13418^T and *Priestia aryabhattai* B8W22^T were analyzed by AP-PCR. In this method, arbitrarily selected primers are annealed to template DNA under low stringency conditions for the initial cycles of DNA amplifications, which allows interactions between the primers and target DNA in regions containing base mismatches. The AP-PCR fingerprinting were performed using the M13F primer [(– 20): GTAAAACGACGGCCAGT] and the following PCR program: two cycles of 94 °C for 5 min, 40 °C for 5 min, and 72 °C for 5 min; followed by 40 high-stringency cycles of 94 °C for 1 min, 60 °C for 1 min and 72° for 2 min. Amplified DNA product were resolved by electrophoresis using 2% (w/v) agarose gels.

Results and discussion

Morphological and physiological characteristics

The colonies of strains SGD-V-76^T and SGD-M-37 were aerobic, Gram positive, motile rods of 0.7–0.8 × 1.2–1.9 µm in diameter (Supplementary Fig. 1). A detailed species description is presented below. A phenotypic comparison of strain SGD-V76^T and related species of the genus bacillus is presented in Table 1, and in the species description. It is evident from Table 1 that there were similar phenotypic properties observed between strains SGD-V76^T with SGD-M-37, but signature difference were observed *Priestia flexus* IFO 15715^T, *Priestia megaterium* IAM 13418^T and *Priestia aryabhattai* B8W22^T.

Phylogenetic and genomic analysis

The nearly complete 16S rRNA gene sequence of strain SGD-V-76^T and SGD-M-37 was determined and compared with the corresponding sequences of other bacterial strains in the GenBank database. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strains SGD-V-76^T and SGD-M-37 are having 99% similarity with three nucleotide variation and confirms their assignment to the genus *Priestia*. The phylogenetic tree, based on 16S rRNA gene sequence data from strains SGD-V-76^T and SGD-M-37 and corresponding sequences from the type strains of the genus *Priestia*, was constructed according to the neighbor-joining algorithm (Fig. 1). The comparative analysis of 16S rRNA gene sequences and phylogenetic relationships showed that both strain SGD-V-76^T and SGD-M-37 lies in a subclade in the tree with *Priestia flexus* IFO 15715^T, *Priestia megaterium* IAM 13418^T and *Priestia aryabhattai* B8W22^T and is supported by a high bootstrap value (Fig. 1), with which it shares a 16S rRNA gene sequence similarity, thus suggesting that the strain SGD-V-76^T should be considered as a novel member of the genus *Priestia*. The genomic DNA G + C content of strain SGD-V-76^T was 37.7 mol%.

The ANI values for strains SGD-V-76^T with *Priestia flexus* IFO 15715^T, *Priestia megaterium* IAM 13418^T and *Priestia aryabhattai* B8W22^T are 79.2, 79.3 and 79.2 respectively, and the dDDH values are 17.7, 17.8 and 18.0%, (average of three formula) respectively (Table S1), which are both significantly lower than the thresholds (95% for ANI and 70% for dDDH) for prokaryotic species delineation (Wayne et al. 1987; Chun et al. 2018). Genome to

Table 1 Phenotypic traits that differentiate *Priestia* sp SGD-V-76^T and SGD-M-37 from the type strains of closely related members *Priestia* genus

Biochemical characters	*Priestia* sp SGD-V-76^T	*Priestia* sp SGD-M-37	*Priestia. flexus* IFO 15715^T	*Priestia. megaterium* IAM 13418^T	*Priestia. aryabhattai* B8W22^T
Cell shape	Short rods	Short rods	Long rods	Rods	Rods
Endospore position	Terminal	Terminal	Terminal	Central	Central
Optimum temperature (°C)	30	30	35	37	28
NaCl (w/v, %) tolerance	0–7.0	0–7.0	2.0–7.0	0–5.8	0–5.8
Lysine decarboxylase	+	+	−	−	−
Ornithine decarboxylase	+	+	−	−	−
Nitrate reduction	−	−	−	−	−
H_2S production	−	−	+	−	−
Citrate utilization	+	+	+	−	−
Malonate	−	−	−	+	+
Esculin	+	+	−	−	−
Cellobiose	−	−	+	−	−
Melibiose	+	+	+	−	+
Saccharose	+	+	+	−	−
Raffinose	+	+	+	−	−
Trehalose	+	+	+	−	−
Glucose	+	+	+	−	−
Lactose	−	−	+	−	−
Oxidase	+	+	+	−	+
G + C mol (%)	37.7	37.5	37.0–39.0	37.0	38.0
Source of Isolation	Sediment	Sediment	Poultry feces	soil	Upper atmosphere

All five strains tested are negative for β-galactosidase activity, urease activity, phenylalanine deamination, Voges Proskauer's test, methyl red, indole production, utilization of arabinose, adonitol, rhamnose and xylose

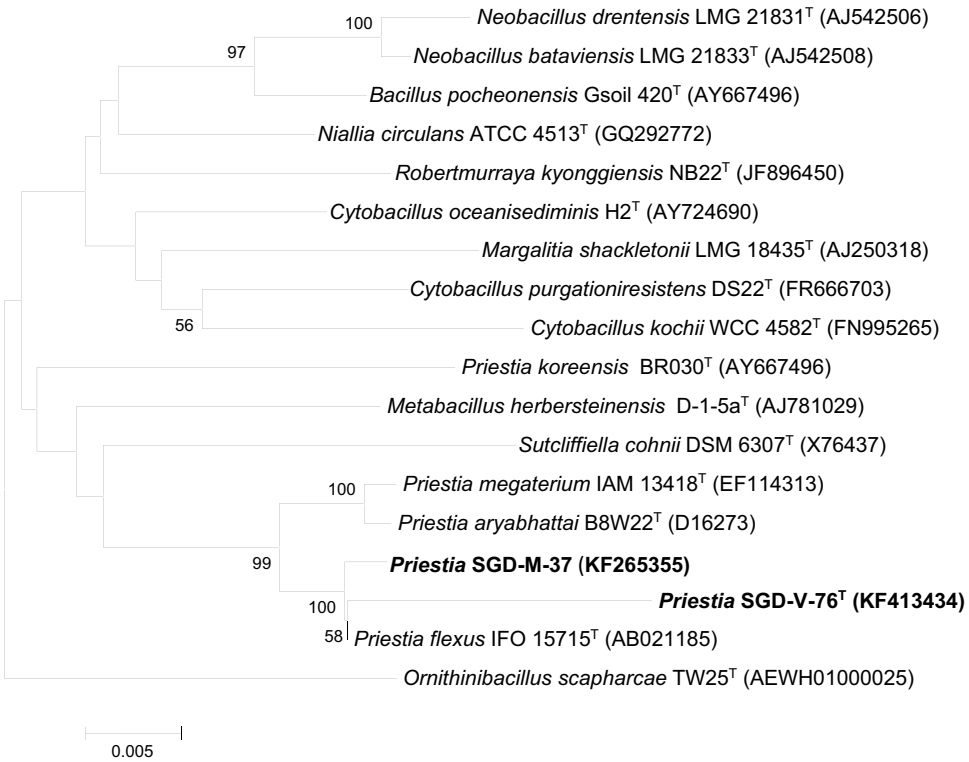

Fig. 1 Neighbor-Joining tree showing the phylogenetic positions of SGD-V-76^T and SGD-M-37 with related species of the genus *Priestia* based on 16S rRNA gene sequences. Bootstrap values (50%) based on 1000 replication are shown at branch points. Ornithinibacillus scaphacae TW25^T (AEWH01000025) is used as out-group

genome-based hybridization demonstrated that strain SGD-V-76^T showed 16.6–20.0% similarity with nearest reference strains by all three formula calculation (Supplementary Table 1). The genomic information of strain SGD-V-76^T is listed in Table S2, and the quality of genome sequences meet the standards for the taxonomy of prokaryotes (Chun et al. 2018). The distribution of proteins into COGs functional categories is shown in Table S3.

Chemotaxonomic characteristics

Chemotaxonomically strain SGD-V-76^T is having meso-diaminopimelic acid (*meso*-Dpm) as the diagnostic diamino acid. Ribose and glucose were major cell wall sugars, while galactose was present in minor quantity. Polar lipid profile contained diphosphatidylglycerol (DPG), phosphatidyl glycerol (PG) phosphatidylethanolamine (PE), and two unidentified aminophospholipids (AL) (Supplementary Fig. 2). MK-7 is a major menaquinone detected. The fatty acids found for strain SGD-V-76^T were $C_{14:0}$ (4.0%), iso-$C_{14:0}$ (2.7%), iso-$C_{15:0}$ (11.9%), anteiso-$C_{15:0}$ (37.9%), $C_{16:1}$ w11c (11.5%), $C_{16:0}$ (16.0%), anteiso-$C_{17:0}$ (6.2%), iso-$C_{16:0}$ (2.1%), iso-$C_{17:0}$ (2.1%), $C_{18:0}$ (1.4%), $C_{18:1}$w7c alcohol (1.1%) and summed feature (iso-$C_{17:1}$ I/anteiso B) 1.13% (Table 2). Arbitrary Prime-PCR fingerprinting were also demonstrated the different amplication banding pattern of the strain SGD-V-76^T with its nearest reference strains (Supplementary Fig. 3).

The observed phenotypic and chemotypic properties of strain SGD-V-76^T, and the 16S rRNA gene sequence comparison results, and genome analysis supports the assignment of strain SGD-V-76^T to the genus *Priestia*. Therefore we propose that this isolate represents a novel species within the genus, for which the name *Priestia veravalensis* sp. nov., is proposed.

Description of *Priestia veravalensis* sp. nov.

Priestia veravalensis (ver.val.en'sis. N.L. masc. adj. *veravalensis* pertaining to Veraval, named thus for being first isolated from the Veraval, Gujarat, India).

Cells are Gram-stain positive, aerobic, motile rods of 0.7–0.8 × 1.2–1.9 µm in diameter. Species grows well on marine agar media (MA) and nutrient agar (NA). Species formed opaque, cream, circular colonies with entire margins after incubation on NA (pH 7.0) at 30 °C for 48–72 h. Strain was catalase-positive, but oxidase-negative. It grew between 25 and 45 °C (optimum at 30 °C), and in the presence of 0–7% (w/v) NaCl. NaCl is not required for its growth. Strain hydrolyzed starch, but not casein, carboxy methyl cellulose (CMC), urea, Tweens 20, 40, 60 or 80. Negative for H_2S production and nitrate reduction. Positive for aesculin, citrate, glucose, melibiose, raffinose, saccharose, trehalose ornithine decarboxylase, lysine decarboxylase and oxidase positive. The fatty acids detected are $C_{14:0}$, iso-$C_{14:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:1}$ w11c, $C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, $C_{18:0}$, $C_{18:1}$w7c

Table 2 Cellular fatty acid profiles of *Priestia* sp SGD-V-76^T with related strain on TSB agar

Fatty acids	*Priestia* sp SGD-V-76^T	*Priestia. flexus* IFO 15715^T	*Priestia. megaterium* IAM 13418^T	*Priestia. aryabhattai* B8W22^T
$C_{12:0}$	ND	1.2		1.4
i-$C_{14:0}$	2.7	3.4	4.3	3.3
$C_{14:0}$	4.0	2.8	ND	1.8
i-$C_{15:0}$	11.9	25.1	27.8	22.0
ai-$C_{15:0}$	37.9	27.2	52.5	36.6
$C_{16:1}$ w7c alcohol	1.1	–	ND	–
i-$C_{16:0}$	2.1	5.2	1.4	2.7
$C_{16:1}$ w11c	11.5	2.4	ND	1.8
$C_{16:0}$	16.0	3.1	8.5	11.3
i-$C_{17:1}$ w10c	–	1.3	ND	–
i-$C_{17:0}$	2.1	8.3	1.5	3.7
ai-$C_{17:0}$	6.2	4.2	3.2	6.8
$C_{18:1}$ w9c	–	1.3	ND	1.0
$C_{18:0}$	1.4	5.2	ND	2.5
Summed feature 4	1.1	ND	ND	–
Summed feature 8	–	–	–	1.4

Summed feature 4 consisted of i-$C_{17:1}$ I/anteiso B and summed feature 8 consisted of $C_{18:1}$ w6c/w7c. Fatty acids representing less than 1.0% are not included

ND not detected

alcohol and summed feature (iso-$C_{17:1}$ I/ anteiso B). The species contained MK-7 as a predominant quinone. Polar lipid profile contained diphosphatidylglycerol (DPG), phosphatidyl glycerol (PG) phosphatidylethanolamine (PE), and two unidentified aminophospholipids (AL). Genome based DNA–DNA hybridization demonstrated that strain showed 16.6–20.0% similarity with nearest reference strains. The DNA G+C content is 37.7 mol%. SGD-V-76^T (=DSM28242^T=KCTC33802^T=CIP111056^T=NCIM5510^T) was isolated from coastal sediment sample in Veraval coast, Gujarat Province of India.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains SGD-V-76^T and SGD-M-37 are KF413434 and KF265355. The NCBI accession numbers for the draft genome sequences of the strains SGD-V-76^{T}is LNPQ00000000. The version described in this paper is version LNPQ01000000.

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Declarations

Conflict of interest The authors declare that they have no direct or indirect conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals.

References

Alexander FA, von Jan M, Klenk H-P, Göker M (2010) Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand Genomic Sci 2(1):117–134

Arahal D, Ludwig W, Schleifer K, Ventosa A (2002) Phylogeny of the family *Halomonadaceae* based on 23S and 16S rDNA sequence analyses. Int J Syst Evol Microbiol 52:241–249

Bennett S (2004) Solexa Ltd. Pharmacogenomics 5(4):433–438

Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu X-W, Meyer SD, Trujillo ME (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–546

Collins MD, Jones D, Kroppenstedt RM (1983) Reclassification of *Brevibacterium imperiale* (Steinhaus) and "*Corynebacterium laevaniformans*" (Dias and Bhat) in a redefined genus *Microbacterium* (Orla-Jensen), as *Microbacterium imperialecomb*. nov. and *Microbacterium laevaniformans* nom. rev.; comb. nov. Syst Appl Microbiol 4:65–78

De Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1985) Confidence limits on phylogenesis: an approach using the bootstrap. Evolution 39:783–791

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416

Gnerre S, Maccallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, Berlin AM, Aird D, Costello M, Daza R, Williams L, Nicol R, Gnirke A, Nusbaum C, Lander ES, Jaffe DB (2011) High-quality draft assemblies of mammalian genomes from mas-sively parallel sequence data. Proc Natl Acad Sci USA 108:1513–1518

Gonzalez JM, Saiz-Jimenez C (2005) A simple fluorimetric method for the estimation of DNA–DNA relatedness between closely related microorganisms by thermal denaturation temperature. Extremophiles 9:75–79

Goris J, Konstantinidis TK, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91

Gupta RS, Patel S, Saini N, Chen S (2020) Robust demarcation of 17 distinct *Bacillus* species clades, proposed as novel *Bacillaceae* genera, by phylogenomics and comparative genomic analyses: description of *Robertmurraya kyonggiensis* sp. nov. and proposal for an emended genus Bacillus limiting it only to the members of the Subtilis and Cereus clades of species. Int J Syst Evol Microbiol 70(11):5753–5798

Heyrman J, Mergaert J, Denys R, Swings J (1999) The use of fatty acid methyl ester analysis (FAME) for the identification of heterotrophic bacteria present on three mural paintings showing severe damage by microorganisms. FEMS Microbiol Lett 181(1):55–62

Huss VA, Festl H, Schleifer KH (1983) Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. Syst Appl Microbiol 4:184–192

Hyatt D, Chen GL, Lacascio PF, Land ML, Larimer FW, Hauser LJ (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinform 11:119

Kates M (1986) Techniques of lipidology, 2nd edn. Elsevier, Amsterdam, pp 106–107 (**241–246**)

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e:a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kroppenstedt RM (1982) Separation of bacterial menaquinones by HPLC using reverse phase (RP 18) and a silver loaded ion exchanger. J Liq Chromatogr 5:2359–2367

Leifson E (1960) Atlas of bacterial flagellation. Academic Press, New York

Li WJ, Xu P, Schumann P, Zhang YQ, Pukall R, Xu LH, Stackebrandt E, Jiang CL (2007) *Georgenia ruanii* sp. nov., anovel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. Int J Syst Evol Microbiol 57:1424–1428

Loveland-Curtze J, Miteva VI, Brenchley JE (2011) Evaluation of a new fluorimetric DNA-DNA hybridization method. Can J Microbiol 57:250–255

Lowe TM, Eddy SR (1997) tRNAscan–SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25:955–964

Markowitz VM, Mavromatis K, Ivanova NN, Chen IMA, Chu K, Kyrpides NC (2009) IMG ER: a system for microbial genome annotation expert review and curation. Bioinformatics 25:2271–2278

Markowitz VM, Chen I-MA, Palaniappan K, Chu K, Szeto E, Pillay M, Ratner A, Huang J, Woyke T, Huntemann M, Anderson I, Billis K, Varghese N, Mavromatis K, Pati A, Ivanova NN, Kyrpides NC (2014) IMG 4 version of the integrated microbial genomes comparative analysis system. Nucleic Acids Res 42:D560–D567

Marmur J (1961) A procedure for isolation of deoxyribonucleic acid from micro-organisms. J Mol Biol 3:208–218

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integratedprocedure for the extraction of bacterial isoprenoid quinones andpolar lipids. J Microbiol Methods 2:233–241

Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyrpides NC (2010) GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. Nat Methods 7:455–457

Pruesse E, Quast C, Knittel FB, Ludwig W, Peplies J, Glöckner FO (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 35:2188–7196

Saitou N, Nei M (1987) The neighbour joining method; a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sánchez-Porro C, Rafael R, Soto-Ramírez N, Márquez MC, Montalvo-Rodríguez R, Ventosa A (2009) Description of *Kushneria aurantia* gen. nov., sp. nov., a novel member of the family *Halomonadaceae*, and a proposal for reclassification of *Halomonas marisflavi* as *Kushneria marisflavi* comb. nov., of *Halomonas indalinina* as *Kushneria indalinina* comb. nov. and of *Halomonas avicenniae* as *Kushneria avicenniae* comb. nov. Int J Syst Evol Microbiol 59:397–405

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI technical note 101. MIDI Inc, Newark

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P (ed) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, pp 607–654

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysistools. Nucleic Acids Res 25:4876–4882

Ventosa A (2006) Unusual micro-organisms from unusual habitats: hypersaline environments. In: Logan NA, Lappin-Scott HM, Oyston PCF (eds) Prokaryotic diversity: mechanisms and significance. Cambridge University Press, Cambridge, pp 223–253

Ventosa A, Joaquín JN, Oren A (1998) Biology of moderately halophilic aerobic bacteria. Microbiol Mol Biol Rev 62(2):504–544

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murry RGE, Stackebrandt E, Starr MP, Trüper HG (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37(4):463–464

Xu P, Li WJ, Tang SK, Zhang YQ, Chen G-Z, Chen H-H, Xu LH, Jiang CL (2005) *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family '*Oxalobacteracea*e' isolated from China. Int J Syst Evol Microbiol 55:1149–1153

Yi H, Chang Y-H, Hyun Woo Oh, Bae KS, Chun J (2003) *Zooshikella ganghwensisgen* nov., sp. nov., isolated from tidal flat sediments. Int J Syst Evol Microbiol 53:1013–1018

Yoon J-H, Kim H, Kim I-G, Kang KH, Park Y-H (2003a) *Erythrobacter flavus* sp. nov., a slight halophile from the East Sea in Korea. Int J Syst Evol Microbiol 53:1169–1174

Yoon J-H, Kim I-G, Kang KH, Oh T-K, Park Y-H (2003b) *Bacillus marisflavi* sp. nov. and *Bacillus aquimaris* sp. nov., isolatedfrom sea water of a tidal flat of the Yellow Sea in Korea. Int J Syst Evol Microbiol 53:1297–1303

Yoon J-H, Kang KH, Oh T-K, Park Y-H (2004a) *Shewanella gaetbuli* sp. nov., a slight halophile isolated from a tidal flat in Korea. Int J Syst Evol Microbiol 54:487–491

Yoon J-H, Kang KH, Oh T-K, Park Y-H (2004b) *Erythrobacter aquimaris* sp. nov., isolated from sea water of a tidal flat ofthe Yellow Sea in Korea. Int J Syst Evol Microbiol 54:1981–1985

Zerbino DR, Birney E (2008) Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18:821–829