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Vibrio rubrogenes sp. nov., prodigiosin producing halophilic bacterium from seaweed *Sargassum wightii* of Mandapam coast, India

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Seaweeds are known for their rich bioactive compounds with potential applications in biotechnological and industrial fields. However, seaweed associated bacteria are least explored for bioactive and other functional capabilities. A study aimed to bio-prospect seaweed associated bacteria for bioactive potential, a Gram-negative, rod shaped red pigmented strain SW71 was isolated from the seaweed thallus of *Sargassum wightii*, which exhibit unique biochemical and genomic characteristics. The strain was observed to be oxidase negative, and catalase positive. The isolate was positioned biochemically under *Gazogenes* clade of *Vibrio*, however the species delineation difficulties further went for molecular phylogenetic analysis. 16S rRNA analysis placed the isolate position to *V. gazogenes* by 97.94%. Whole genome sequence analysis showed that the proposed novel strain is closely identical to *V. ruber* and *V. gazogenes* with phylogenomic tree and the overall genome relatedness indices such as average nucleotide identity (ANIu) (89.95% and 87.92%), and digital DNA-DNA hybridization (dDDH) (38.5% and 34.4%) values. The value for dDDH against *V. rhizosphaerae* was 33%. However, the difficulty in species delineation further supported by ribosomal Multi-Locus Sequence Typing (MLST) analysis where the strain predicted as *V. gazogenes* with only 88% identity. The predominant fatty acids in the cellular fraction was C16:0 (23.47%), followed by C18:1 (17.23%), C22:1 (8.19%), C18:0 (7.64%), and C16:1 (4.91%). The major lipid classes detected were Phosphatidylethanolamine (34:2), Phosphatidylethanolamine (32:1), Galactosylceramide (d18:2/21:0), N-lauroylglycine, Phosphatidic acid (32:2), Diphosphatidyl glycerol (Cardiolipin) (68:4), and Phosphatidylserine (34:0). Gene mining and metabolomic profiling confirmed the red pigmentation by the presence of prodigiosin in the strain genome. Based on the phenotypic, genotypic and chemotypic analyses, the strain proposed as *Vibrio rubrogenes* sp. nov. strain smcift-1^T = MTCC 14081) here.

Vibrios form the natural part of microflora in the fresh, brackish and marine environments[1]. The genus *Vibrio* was first defined using modern taxonomy [2], and it belongs to the family *Vibrionaceae* of phylum *Pseudomonadota* (formerly *Proteobacteria)*[3]. This family includes 24 genera as per the List of Prokaryotic names with Standing in Nomenclature (https://lpsn.dsmz.de/family/vibrionaceae)[4]. Most of the *Vibrio* species are associated with marine organisms including marine animals and plants[5]. There are more than 190 recognized species in the Genus *Vibrio* (Jiang et al., 2014)[8]. *Vibrio* species plays important role in mineral and biogeochemical cycles in the aquatic ecosystem and few of them are known for their pathogenic characteristics[6]. Many of the *Vibrio* species shows higher phenotypic and genomic similarities unlike other bacteria[5]. It was reported that many of the sister *Vibrio* species has nearly identical phenotypes with different evolutionary lineages[7]. Based on genomic similarities, *Vibrio* species are grouped under 51 clade or lineage such as Cholerae clade, Gazogenes clade, Splendidus clade etc[8]. Presently, *Gazogenes* clade consist of seven *Vibrio* species namely *V. gazogenes*, *V. ruber*, *V. quintilis*, *V. mangrovi*, *V. aerogenes*, *V. spartinae, and V. rhizosphaerae*[8]. Marine Vibrios capable of producing bioactive red pigment called prodigiosin, include *Vibrio gazogenes, V. rhizosphaerae* and *V. ruber*[9,10]. *Vibrio* species under Gazogenes clade is primarily isolated from estuarine or saline waters, mangrove, aquatic

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plants and sediments[11]. The red pigment, prodigiosin is primarily produced and characterized from *Serratia marcescens,* a Gram-negative pathogenic bacterium of nosocomial importance[12]. This pigment is known for the bioactive properties such as antibacterial, antifungal, and anticancerous properties[13]. Since then, research has been focusing on the search of novel non-pathogenic strains for prodigiosin pigment production, developing new bio prospecting methods and exploring future perspectives of microbial pigment research to counter compete the use of synthetic alternatives. Further, the identification of non-pathogenic producers of prodigiosin becomes a vital step to overcome the biosafety, regulatory and technical limitations of the pathogenic alternatives for industrial applications.[14] Characterization of prodigiosin pigment from *Streptomyces coelicolor*, a non-pathogenic source by unravelling its complex regulatory systems and suggested that *S. coelicolor* has the excellent potential for the production of prodigiosin [[14]]. Several other red pigmented bacteria such as *Pseudoalteromonas rubra, Hahella chejuensi, Pseudovibrio denitrificans,*

V. psychroerryhreus, Zooshikella rubdius have been isolated and their prodigiosins were characterized[15,16]. Marine environment possesses highly diverse microbial communities with wide metabolic processes to adapt to the changing environmental conditions[17,18,19]. Seaweed in particular harbor several endophytic and epiphytic bacteria and several researchers have reported for the diverse set of secondary metabolites from seaweed associated bacteria[20,21]. Despite the ecological importance of Mandapam coast of India for seaweeds[22,23], its seaweed associated bacterial communities are least explored. This study addresses this knowledge gap by focusing on the uncharacterized bacterial isolate with bioactive potential recovered from the endemic macroalgae, *Sargassum wightii*. The novelty of the study lies in the integration of the data generated by employing comprehensive polyphasic approaches such as phenotypic, genotypic and chemotypic methods to identify the unresolved taxonomical status of the seaweed associated bacterial isolate. In this study, we aimed to characterize the unclassified bacterial isolate (SW71) through a comprehensive polyphasic approach using phenotypic, chemotypic and genomic methods.

Materials and methods

Bacterial isolation

Brown seaweed (*S. wightii)* was collected from Mandapam coast, India (Latitude 9.2886N; Longitude 79.1329 E). The seaweed sample was packed in sterile plastic bags and transported to the Microbiology laboratory of ICAR-Central Institute of Fisheries Technology, Kochi, Kerala, India for the isolation of cultivable marine bacteria. Isolation protocol was used according to the procedure[24] with minor modifications [[24]]. Briefly, the seaweed sample (25 gm) was weighed and suspended in sterile seawater (225 mL). Before washing, the sample was kept for shaking incubator for 15 min at 25 °C at 120 rpm to effectively remove the epiphytic bacteria. Five washes were repeated with same condition and a subsequent wash with ethanol (95%) for 15 min and final three additional washes with sterile sea water for 15 min each at 120 rpm was done to remove the surface contaminants. Then sample was homogenized using mortar and pestle and further by stomacher by suspending the sample in sterile seawater (225 mL). Serial dilution of this was plated on marine agar and kept for 1-2 days at 25 °C. The single well-defined colonies appeared as red color was further streaked on to marine agar plates and sub cultured in marine agar (BD, Difco, USA).

Biochemical and phenotypic characterization

The colony morphology of the isolate was determined on marine agar plates. The biochemical characterization was carried out using conventional biochemical tests. Gram staining, catalase, and oxidase test were carried out by growing the bacterial cells in marine broth (MB) (BD Difco, USA) for 24 h at 37 °C as per the protocol described previously[25,26,27]. Motility of the bacterial cell was assessed using motility test medium (Himedia, India). The growth of the bacterial strain was tested in different pH (4.0 to 11.0), NaCl (0 to 34%), and temperature (4 to 50 ℃) range for 4-5 days in marine broth tubes. Sugar utilization pattern was determined in the basal media containing 2% NaCl supplemented with respective sugars and sugar alcohols such as Adonitol, D-Mannitol, L- Arabinose, L- Salicilin, Sucrose, D- Ribose, D-Melibiose, D-Xylose, D-Fructose, D-Mannose, Lactose, D-Cellobiose, D-Maltose, Trehalose, D-Galactose, D-Raffinose, Dulcitol, Inositol, D-Arabinose, D-Melezitose, D- Rhamnose, Glycerol, D-Arabitol, Erythritol, and L-Sorbose. Extracellular enzyme production such as lipase, protease, lecithnase, cellulase and urease production was carried out by spotting the overnight grown culture on Trypticase soya agar (TSA) containing 2% NaCl supplemented with 1% (v/v) Tween 80, 1% (w/v) skim milk, 1% (v/v) egg yolk emulsion, 1% cellulose, and 1% urea solution, respectively[28,29] (Prittijarvi et al., 2000) [73]. Then the agar plates were observed for zone formation by incubating at 30 ℃ for 24-48 h. Haemolytic test was carried out using sheep blood agar (Himedia, India) and clear zone around the colony was recorded as positive haemolysis[30]. Gelatine liquefaction test was carried out in gelatin medium supplemented with 12% gelatin[31]. Starch hydrolysis was determined by growing the culture on starch agar (1.5% soluble starch) with no glucose followed by flooding the plate with iodine solution (Himedia, India)[32]. Decarboxylation of different aminoacids such as alanine, valine, leucine, arginine, ornithine, and lysine were carried out as per the protocol described previously[33]. The automated bacterial identification system (VITEK-2 system), (BioMerieux, USA) was used for the identification of the strain SW71.

16S rRNA sequencing and phylogenetic analysis

The overnight culture was subjected to DNA extraction using Qiagen DNA extraction kit (Qiagen, Germany). The concentration was determined using Qubit fluorometer (Invitrogen, India) and the integrity and purity were assessed using 1% agarose gel electrophoresis and nanodrop (Nanodrop one, Thermofisher Scientific, India) and then dissolved in sterile water and adjusted to a concentration of 10 ng/µL. The 16S rRNA gene was amplified by PCR using universal primers 27F and 1492R as per the protocol described previously[34]. The PCR conditions followed were: initial denaturation at 95 °C for 5 min, followed by 30 cycles of 45 s at 95 °C, 1 min at 65 °C and

1 min at 72 °C, and a final extension at 72 °C for 10 min. The PCR product thus obtained was purified using PCR product clean up kit and sequenced (GeneSpec Pvt. Ltd, India). The sequences for each isolate were checked for quality issues and then trimmed, assembled sequences were submitted to the NCBI databases. The phylogenetic analysis of the isolate was carried out using neighbour-joining phylogenetic tree which was constructed using the Tamura3-parameter model in MEGA7 with 1000 bootstrap replicates. 16S rRNA phylogeny were inferred by the GGDC web server available at http://ggdc.dsmz.de/ using the DSMZ phylogenomics pipeline adapted to single genes [35].

Whole genome sequencing and analysis

The bacterial DNA extracted was sequenced using Illumina HiSeq2500 sequencing platform. Sequence library preparation was constructed following manufacturers instruction (NEBNext® Ultra™II DNA Library Prep Kit for Illumina®, Illumina, San Diego, CA, USA). Sequencing was carried out using a 2 X 150 paired end configuration, image analysis and base calling were conducted by HiSeq Control Software (HCS) + OLB + GA pipeline-1.6 (Illumina) on the HiSeq Instrument HiSeq2500. First, sequences were uploaded for read processing and read quality assessment was achieved using FastQC (version 0.11.5). Then, the quality of the reads obtained from FASTQC tool (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) were checked. The low-quality bases were trimmed using custom made perl script, and the adapter sequences were further removed from the reads using cutadapt (version2.0)[36]. The sequence reads were then assembled using SPAdes (v 1.13.4) and the quality of the assembled reads were examined using quality assessment tool (QUAST). Further, the genome completeness and contamination were estimated using CheckM2 tool kit (https://usegalaxy.org/)[37]. The assembled sequences were then finally annotated by implementing RAST tool kit (RASTtk) available in PATRIC (Pathosystems Resource Integration Center), the bacterial bioinformatics database and analysis resource (https://www.patricbrc.org) using the genetic code 11 and assigned a unique genome identifier of 1,889,776.3[38].

Whole genome based taxonomic analysis

For genome based taxonomical analysis, the sequence data was analyzed using type (Strain) genome server platform[39]. The 16S rRNA sequence from the isolate and the type strains were extracted using RNAmmer[40] and Genome BLAST Distance Phylogeny approach (GBDP) with minimum evolution tree were inferred under the distance algorithm 'coverage' and distance formula *d5*[41]. The extended 16S rRNA analysis of the closely related strains using maximum likelihood (ML) and maximum parsimony (MP) trees were inferred using RAxML and TNT[42,43]. The sequence alignment of the genome of closest type strains was carried out using MASH algorithm[44,45]. GBDP minimum evolution tree based on whole genome data was inferred under the distance algorithm 'greedyWithTrimmimg' and distance formula *d5*[46,41]. The digital DNA-DNA hybridization (dDDH) values and the confidence intervals were calculated using default settings available at Genome-to-Genome Distance Calculator (GGDC 4.0 version)[41]. The average nucleotide identity (ANIu) values between strain SW71 and its closely phylogenetic neighbours were calculated using Ezbio Cloud web server (https://www.ezbiocloud.net/tools/ani)[47]. Further, ribosomal MLST was carried out using the alignment of 55 housekeeping core genes in the strain with the closely related genome by using PUBMLST web server with default settings (https://pubmlst.org/species-id). Multilocus sequence analysis based phylogenomic tree was constructed using the genomic data from multiple loci by automated Muli Locus Species Tree (autoMLST) pipeline[48]. The closely related genomes available in the NCBI database based on the sequence alignment of 100 conserved core genes was selected by Denovo mode. Then, the MLST tree was constructed using maximum likelihood method using MEGA version 7.0 software. Antibiotic resistance genes were predicted using Resistance Gene Identifier (RGI 6.0.5)-Comprehensive Antibiotic Resistance Database (CARD 4.0.1) (https://card.mcmaster.ca/analyze/rgi). The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) profiling was predicted using CRISPRCasFinder (https://crisprcas.i2bc.paris-saclay.fr/CrisprCasFinder/). Secondary metabolites were determined by antiSMASH (version 6.0.0) (https://antismash.secondarymetabolites.org).

Chemotaxonomical characterization

The cell biomass of the bacterial strain SW71 after 48 h of incubation at 37 ℃ was harvested by centrifugation, washed with distilled water and freeze-dried (ANM Industries, India). A Vanquish Flex™ UHPLC fitted with Hypersil Gold C18 reversed-phase (100 mm x 2.1mm, 1.9 µm analytical column provided for chromatographic separation, while mass spectrometric determination was performed using Orbitrap Exploris™ 120 high-resolution mass spectrometer with H-ESI ion source (Thermo Scientific, USA) for the analysis of polar lipids. The flow program and MS conditions were followed as per the protocol described previously[49]. For cellular fatty acids analysis, the freeze-dried pellet of the isolate SW71 was saponified and methylated based on AOAC 991.39 methods[50]. The methylated ester extract was then analyzed using Gas chromatography (Varian CP-3800 model, USA,Galaxy software system) equipped with flame ionization detector. Compound separation was carried out using CP-Sil-88 capillary column (100 m x 0.25 mm,film thickness 0.25 µm). The flow rate for nitrogen gas and the column temperature was maintained at 1 mL/min and 140-220 °C, respectively. The volume of the sample injected was 1 µL at the rate of 2-5 °C. Split ratio for the injector and the detector temperature was maintained at 1:50. The output (retention indices) were compared with a standard solution of fatty acid methyl esters standards (C6-C24) purchased from Merck, USA.

Extraction of secondary metabolites and its antibacterial activity

The isolated strain SW71 was cultured in Luria Bertani (LB) broth (Hi Media, India) with 2% NaCl at 30 °C and 140 rpm for 48 h. The culture broth was then extracted with half volume of ethyl acetate for 48 h at 30 °C in shaking incubator (Labwit, China) at 140 rpm. Crude extract thus prepared was concentrated using rotary evaporator (Heidolph, Germany) and used for antimicrobial activity against clinically relevant pathogens such

as *Escherichia coli* (ATCC 25922), Salmonella typhi (ATCC 51812), Enterococcus faecalis (ATCC 29212), Staphylococcus aureus (ATCC 25923), V. cholerae (MTCC 3904), Bacillus cereus (ATCC 11778), Aeromonas hydrophilla (ATCC 35654) using agar well/disc diffusion method on Muller Hinton agar plate[51]. Briefly, the optical density (corresponds to 10^8 CFU/mL) of the overnight culture was adjusted to 0.5 McFarland standard and 100 µL of the inoculum was spread plated on to the MH agar plate to get an uniform lawn of growth. Then, the well of 6 mm^2 were formed aseptically using gel puncture. A 100 µL of different concentration of the crude extract ranging from 100 to 1000 µg/mL were loaded along with appropriate controls and the plates were incubated at 37 ℃ for 24 h. After incubation, the zone of inhibition (mm) was measured to determine the antibacterial activity.

Metabolomic profiling of the ethyl acetate extract of the strain SW71

For metabolomic characterization, the ethyl acetate extract was centrifuged at 12000 rpm for 15 min and the supernatant was used for Liquid Chromatography High Resolution Mass Spectrometry (LC-HRMS) analysis (Orbitrap Exploris™ 120, Thermo Scientific, USA). The raw data files were processed using Compound Discoverer 3.3 (Thermo Scientific, USA) and metabolites were tentatively identified using level-2 identification as per Metabolomics Standard Initiative[52].

Antibiotic susceptibility test (AST) of the strain SW71

AST profiling was carried out using disc diffusion assay against various antibiotics as per the CLSI guidelines[53]. The antibiotics tested includes Imipenem (10 µg), Ciprofloxacin (5 µg), Tobramycin (10 µg),Moxifloxacin (5 µg), Ofloxacin (5 µg), Sparfloxacin (5 µg), Levofloxacin (5 µg), Norfloxacin (10 µg), Co-Trimoxazole (25 µg), Colistin (10 µg), Nalidixic acid (30 µg), Augmentin (30 µg), Kanamycin (30 µg), Gatifloxacin (5 µg), Gentamicin (10 µg), Amikacin (30 µg), Streptomycin (25 µg) and Ceftriaxone (30 µg), Cefpodoxime (10 µg), Ticarcillin (75 µg), Ampicillin (10 µg), Clarithromycin (15 µg), Amoxicillin/Clavulanate 20/10 µg (30 µg), Cephalexin (30 µg), Vancomycin (30 µg), and Teicoplanin (30 µg). The antibiotic disc were procured from Himedia, India.

Statistical analysis

Statistical analysis of the experimental data was carried out using statistical software SPSS version 23. One way analysis of variance (ANOVA) was used to test the significance of differences. Statistical significance level was set at $p < 0.05$ and the difference between treatments means were tested using Duncan's multiple range test (DMRT).

Results and discussion

The present study aimed to characterize the unclassified bacterial isolates of seaweed associated bacteria, a red bacterium from *S. wightii* was isolated and showed antimicrobial activity phenotypically. In this study, this isolated strain SW71 was characterized biochemically, molecularly *via* 16S rRNA gene sequencing, whole genome sequencing and genome mining, and chemo taxonomically.

Biochemical characteristics

The colonies of the strain SW71 on marine agar plate were red colored 1-1.5 mm size, smooth and circular edge. The strain was observed to be Gram-negative, lacked flagella and endospores. The strain was able to grow 0.5% to 20% NaCl and with pH 5 to 10 and temperature 7 to 40 ^{0}C. The strain was found positive for catalase, negative for oxidase. The strain was not able to grow on the Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar. The strain was weakly motile, fermentative with gas production in motility medium. TSI test showed alkaline slant and acidic butt with H_2S production. All biochemical tests were carried out aerobically at 30 ^{0}C. After 48 h of incubation, D-Mannitol, D- Galactose L- Arabinose, L-Salicilin, Sucrose, D-Ribose, D-Melibiose, D- Xylose, D-Fructose, D-Mannose, Lactose, D-Cellobiose, D-Maltose, and trehalose showed positive result. Sugars such as D-Raffinose, Dulcitol, Inositol, D-Arabinose, D-Melezitose, D-Rhamnose, Glycerol, D- Arabitol, Adonitol and Erythritol showed negative result. L-Sorbose showed weakly positive result. The strain was found to possess lipase, phospholipase, caseinase, amylase and cellulase enzymes. The strain was not able to hydrolyse tyrosine. The strain was positive for indole, methyl red and Vogus Proskauer tests. The strain was not able to decarboxylase lyine, ornithine, and arginine.

The strain was grouped under biochemically to the already known red pigmented *Vibrio* species. The isolate showed similar phenotypic test results of Gazogenes clade such as negative oxidase reaction, lack of growth on TCBS medium, acid and gas production from sucrose and positive result for Vogus Proskaeur test. However, distinct biochemical characteristics which differentiate the strain from *V. ruber* VR1 (JCM 11486) isolated from seawater samples from Taiwan has the ability to ferment trehalose by the strain SW71, whereas the *V. ruber* cannot[58]. Similarly, *V. ruber* VR1 (JCM 11486) was able to ferment D-Arabinose, whereas the strain SW71 cannot. Non-fermentation of D- Melibiose in *V. gazogenes* will differentiate between the strain SW71 and *V. gazogenes*. However, clear differences in certain biochemical test exist in all these strains, however many of the test results are overlapping each other, making it difficult to reliable alone on it for an accurate identification. The comparative differences in the biochemical test results for the newly isolated strain (SW71) with other red pigmented *Vibrio* species of Gazogenes clade is given in Table 1.

Microbial identification using VITEK-2 automated system which relies on the fluorescent measurement with respect to metabolic changes resulting reliable determination of the taxonomical relationship of the bacterial isolate. However, the result from VITEK-2 revealed that the instrument could identify the isolate as *Sphingomonas paucimobilis* only to a maximum probability of 89% which is well below the threshold level of identification (>95%). It was reported that the automated microbial identification systems in general have failed to identify many of the environmental isolates because the dataset for comparison is exclusively available only for clinical isolates[54].

Biochemical test	SW71 (This study)	*V. ruber*[1]	*V. gazogenes*[2]	*V. rhizosphaerae*[3]
Motility	+^{W}	+	+	+
Pigment	Pink red	Red	Red	Pink red
Adonitol	-	-	-	NA
D-Melibiose	+	+	-	NA
Trehalose	+	-	+	+
D-Galactose	+	+	+	+
Dulcitol	-	-	-	+
Inositol	-	-	-	+
D-Arabinose	-	+	+	NA
L- Sorbose	+/-	NA	-	NA
Caseinase	+	+	+	+
Indole	+	-	-	-
L-lysin	-	-	-	+
L-arginine	-	-	-	+
L-ornithine	-	-	-	+
Reduction of nitrate to nitrite	+	+	-	-

Table 1. Comparative differences in the biochemical test results of the strain SW71 and the type strains of prodigiosin producing members of Gazogenes clade. NA- Not available; + Positive;- Negative; + /- Weakly positive, w-Weak. [1]Shieh et al., 2003, [2]Schoch et al., 2020, [3]Kumar and Nair 2007.

16S rRNA sequencing analysis

A full length 16S rRNA gene sequence of 1500 bp was amplified from the isolate DNA and the sequence similarity was assessed with their closely related strains *via* evolutionary phylogenetic analysis. Sequence similarities of the isolated DNA with *Vibrio* species ranged from 97.74% (*V. gazogenes*) to 94.12% *V. Metschnnenikovii*. Sequence similarity with closely related species exhibited below the level of relatedness for the species delineation. Phylogenetic analysis using fast minimum evolution method showed a cluster comprising the strain with the *Vibrio* species and formed separate branch with the member of the Genus *Vibrio* (S. Fig. 1). Similarly, the same kind of trend was observed in the phylogenetic tree constructed using maximum likelihood tree and maximum parsimony tree (S. Fig. 2). The input nucleotide matrix comprised 15 operational taxonomic units and 1553 characters, 174 of which were variable and 123 of which were parsimony- informative. The base-frequency check indicated no compositional bias ($p = 1.00$, $\alpha = 0.05$). ML analysis under the GTR + GAMMA model yielded a highest log likelihood of -4108.66, whereas the estimated alpha parameter was 0.02. The ML bootstrapping did not converge; hence 1000 replicates were conducted; the average support was 72.83%. MP analysis yielded a best score of 360 (consistency index 0.67, retention index 0.69) and 2 best trees. The MP bootstrapping average support was 77.17%. The phylogeny based on 16S rRNA gene sequences showed the strain SW71 form a separate branch with other member of the *Vibrio* species such as *V. rhizosphaerae* , *V. ruber, V. mangrovi, V. qunitilis* and *V. aerogenes*.

Whole genome features

The genome size of the isolate was 4,337,400 bp containing 96 contigs with N50 value of 1,48,259. The average GC content was 45.48%. The assembled genome showed high completeness (96.78%) and low contamination (0.31%). The coarse consistency (97.9%) and fine consistency (95.9%) indicated high quality genome from a taxonomic point of view. The quality scores of the assembled genome are in line with the quality standards required for microbial taxonomy[55].

The genome possesses 4,462 protein coding sequences (CDS), 72 transfer RNA (tRNA) genes, and 4 ribosomal RNA (rRNA) genes. Functional analysis of the annotated genome showed 1,463 hypothetical proteins and 2,999 proteins with functional assignments. The proteins with functional assignments included 1,063 proteins with Enzyme Commission (EC) numbers, 879 with Gene Ontology (GO) assignments, and 776 proteins that were mapped to KEGG pathways. PATRIC annotation includes two types of protein families, and this genome has none of the proteins that belong to the genus-specific protein families (PLFams) and 3,902 proteins that belong to the cross-genus protein families (PGFams). The KEGG subsystem analysis of the annotated genome available at the PARTIC service showed that sets of genes involved in various biological process such as metabolism (761), protein processing (227), stress response, defense and virulence (138), energy (206), cellular processes (142), DNA processing (100), RNA processing (76), membrane transport (26), miscellaneous (16), regulation and cell signaling (35), and cell envelope (35). The circular map and subsystems of the annotated genome is given in Fig. 1A & 1B. It was reported that the different clades under *Vibrionaceae* family exhibit similarity in their genome size and the genome content. The genome size and the GC content of the strain SW71 is in line with those under Gazogenes clade such as 4.3 Kbp ± 0.5, and 45.4% ± 0.3 respectively. The GC content of the type strain of *V ruber, V. gazogenes, V. rhizosphaerae* were found 45.8%, 45.1%, and 51.3%, respectively[56,57,58].

Resistome analysis revealed 5 putative antibiotic resistances genes (AdeFG efflux pumb gene (*Ade*F), cAMP receptor protein gene (CRP), Chloramphenicol (*cat*B11), Fosfomycin (*Fos*G), Elfamycin (R234F) in the genome

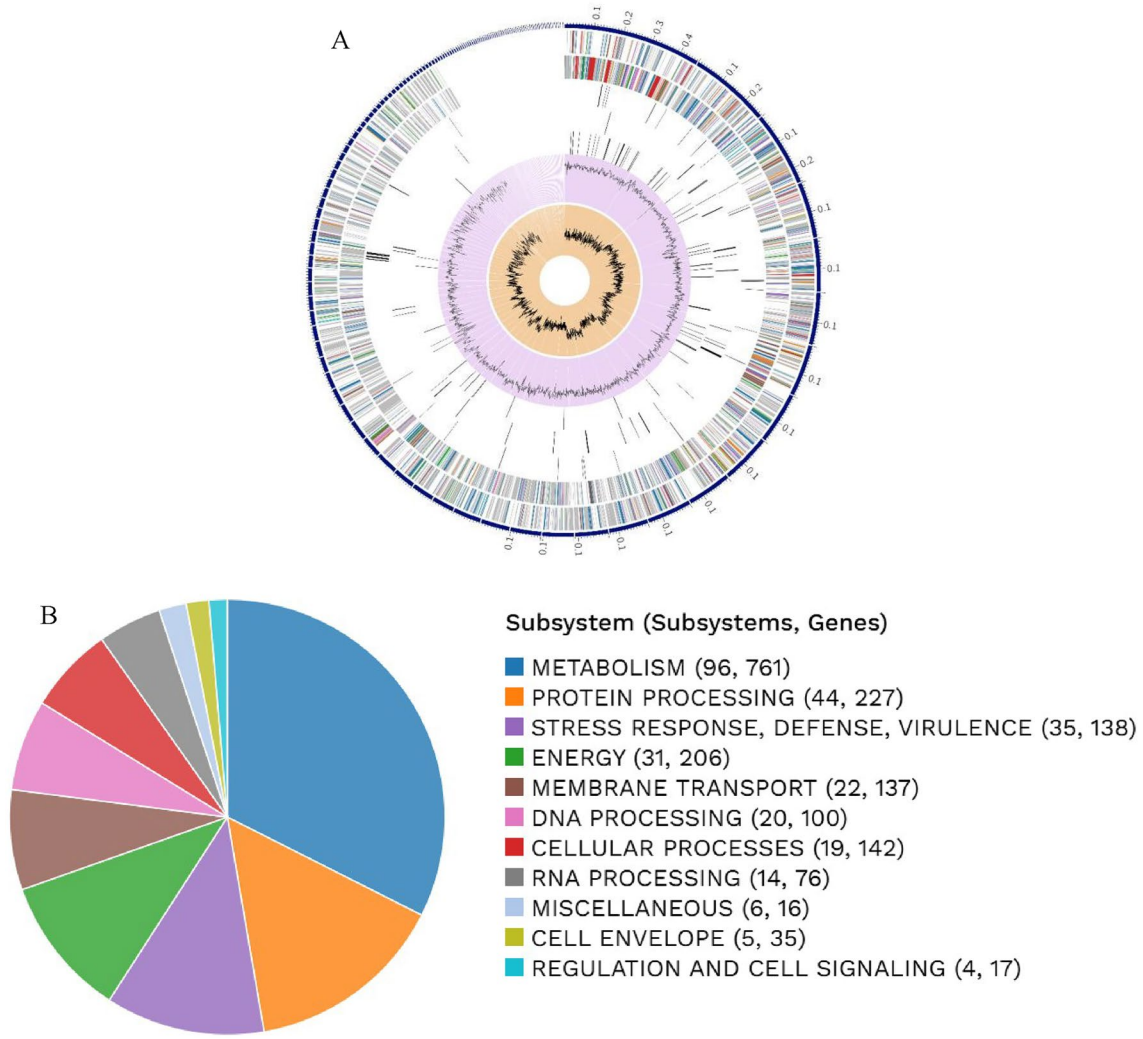

Fig. 1. **A** Circular plot depicting the genome annotations of the strain SW71. The outer to inner genome circles or rings represents the contigs, CDS on the forward strand, CDS on the reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes, CDS with homology to know virulence factors, GC content and GC skew. The colors of the CDS on the forward and reverse strand indicate the subsystem. **B** Subsytem analysis of the genome of the strain SW71.

of the strain SW71. The *Cat*B11, *Fos*G, and *ade*F gene confers resistance to specific antibiotics, the putative genes identified only with strict hits ie the percentage of matching region with the reference genes were less than 100. Resistance genes namely *cat*B11, and CRP were also found in the genome of the other red pigmented *Vibrio* type strains such as *V. ruber*, *V. gazogenes* and *V. rhizosphaerae*[56,57,58]. In addition to this, *Ade*F, and *Fos*G genes were present in the genome of *V. ruber* and *qac*J, *Ade*F, *Fos*G, R234F genes were also present in the genome of *V. gazogenes*.

Six putative CRISPR spacers were identified in the genome of the strain SW71. However, signature genes for CRISPR associated cas system were not found in the genome which is similar to the result from the concatenated sequences of *V. ruber* HNIBRBA4764 (Acc. No. CP134780). Two spacers were only identified in CRISPR system of *V. ruber*. CRISPR associated class 1 cas types belonging to type 1 and type III along with 13 spacers were found in the genome of *V. gazogenes*. Type I cas genes with three putative spacers were identified in the genome of the *V. rhizosphaerae* MSSRF7 strain (Acc. No: JAWRCPO10000001). This result suggested distinct diversity exist in CRISPR cas system among these strains.[59] The CRISPR cas systems were predominant in the mobile genetic elements harboured in the *Vibrionaceae* family which are natural inhabitants of marine waters rich in virus and bacteriophages [[59]]. The findings in the study suggested that members of *Vibrionaceace* family have evolved with diverse set of CRISPR cas systems, mobile genetic elements, and additional sets of defence systems to defend against invading viruses and enabled them to adapt and fit in these environments. [60] The CRISPR cas system plays an important role in understanding the evolutionary lineage of the bacterial species by providing valuable data support for taxonomical and phylogenetic studies [[60]]. They also reported that the environmental adaptability has strong influence on the CRISPR cas system in *Vibrio* and *Photobacterium* species.

Whole genome-based taxonomy and phylogeny

The GBDP phylogeny based on 16S rRNA gene sequence extracted from the whole genome data of the strain SW71 formed separate branch in the subcluster consisting of *V. mangrovi* and *V. rhizosphaerae* (Fig. 2A). A total of 14 closest type or strain genomes was selected based on the smallest MASH distances. The genome blast distance was calculated using the distance formula and greedy With trimming algorithm. The whole genome tree aligned in same branch species cluster with *V. ruber* DSM 16370. However, the branch length of 0.05 and 0.06 in the strain SW71 and the *V. ruber* DSM 16370 indicate genetic distance between the sequences (Fig. 2B). The sequence divergence in the phylogram of the whole genome data further led to the conclusion of the detection of the potential new species and the strains available in the TYGS database failed to match accurately with strain SW71 . It is evident from the phylogenetic result that the strain SW71 genome showed high similarity with Gazogenes clade (*V. mangrovi, V. rhizosphaerae, V. aerogenes, V. qunitilis*, *V. gazogenes,* and *V. ruber*).

Average nucleotide identity (ANIu) value based on orthoANIu algorithm between the strain SW71 and the concatenated sequence of the *V. ruber* HNIBRBA4764 showed 89.95% and with concatenated *V. gazogenes* strain PB1 (NZ_CP092587.1-NZ_CP092588) showed 87.92%. The recommended ANI value for species delineation fall above 95% for considering same species[61]. It was reported that ANI value ranges in the *Vibrio* species varies between 71.9% to 98.9%. In case of Gazogenes clade the ANI values vary between 72.1 to 92.0%[8].

Further to confirm the sequence identity of the strain SW71 genome, we performed (dDDH) analysis. The pairwise comparison of the dDDH (d_4) value of the 14 reference strains with the strain SW71 genome resulted in maximum value with *V. ruber* DSM 16370 (38.5%) followed by *V. gazogenes* DSM 21264 (34.4%) and with *V. rhizosphaerae* DSM 18581 (33%). The difference in G + C content was 0.17 with *V. ruber* DSM 16370 which is well below the permissible G + C content variation within the species (< 1%). In dDDH analysis d_4 was the robust metric compared to d_0 and d_6 values for sequence identity [41].

Ribosomal MLST analysis of the whole genome of the strain SW71 based on 55 *rps* genes revealed that isolate matches only with 9 locus out of 55 *rps* locus and predicted *V. gazogenes* with 88% identity. The mismatching loci could be due to the sequence variation due to horizontal gene transfer or genetic recombination events. Interestingly, the isolate didn't predict *V. ruber* by MLST analysis. Hence, the MLST analysis also suggest that the isolate undergone a significant level of sequence divergence. The maximum likelihood phylogenomic tree constructed based on the multiple sequence alignment of 100 conserved core genes available in autoMLST pipeline (https://automlst.ziemertlab.com/) indicated that the strain SW71 formed within the cluster comprising *V. ruber* DSM 16370, *V. rhizosphaerae*_DSM 18581, and *Vibrio* sp. MEBiC08052 and showed highest similarity with *Vibrio* sp. MEBiC08052 (S. Fig. 3).

Compared to traditional MLST schemes, cgMLST performs superior for the resolution to determine the taxonomical classification of the *Vibrionaceae* where it uses eight housekeeping genes, 34 single copy genes and 11 ribosomal protein genes[62]. However, species delineation using phylogenetic tree based on MLST techniques

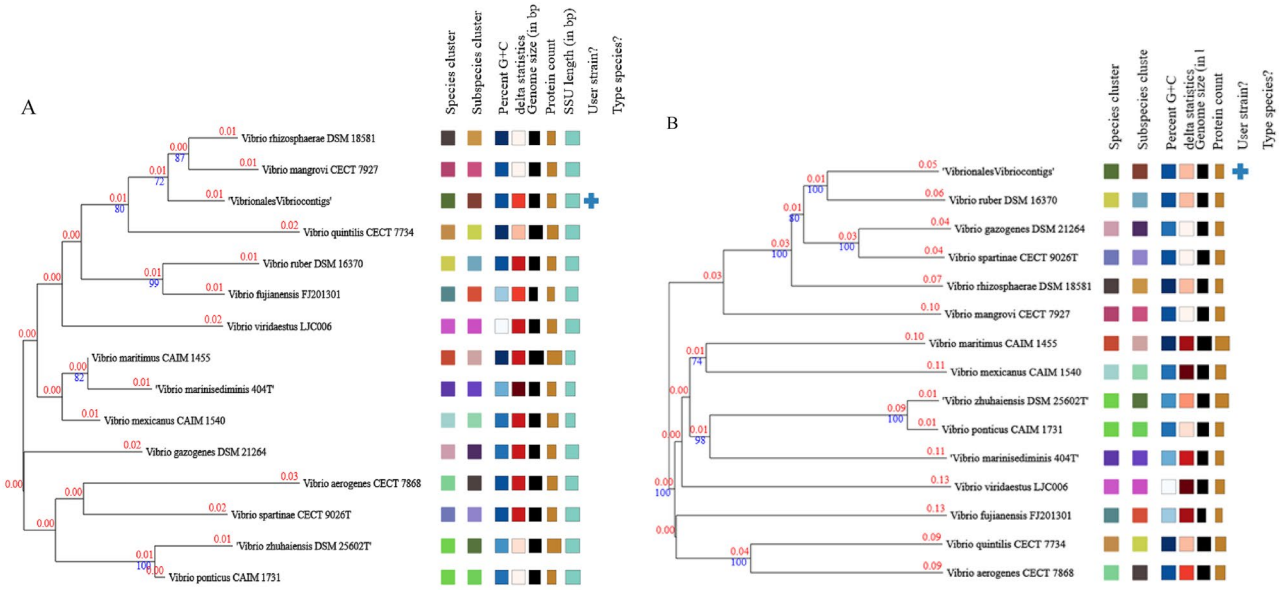

Fig. 2. **A** Minimum evolution tree constructed based on GBDP distance calculated from 16S rRNA gene sequences extracted from whole genome data. GBDP distance formula *d5* was calculated for scaling of branch length. The tree inferred with FastME 2.1.6.1 (Lefort et al., 2015). The numbers above branches are GBDP pseudo-bootstrap support values > 60% from 100 replications, with an average branch support of 74.0%. The tree was rooted at the midpoint. **B** Minimum evolution tree constructed based on GBDP distance calculated using the distance formula and greedy with trimming algorithm from whole genome data. To infer phylogenetic tree FastME 2.1.6.1was used. The GBDP distance formula d5 was used to scale the branch length. The numbers above branches are GBDP pseudo-bootstrap support values > 60% from 100 replications, with an average branch support of 89.6%. The tree was rooted at the midpoint.

still faced difficulty in the placement of strains in some of the clades such as Proteolyticum, Rosenbergii, and Aquae clades[8].

Chemotyping

Cellular fatty acid profiling of the strain SW71 was carried out with a view to identify the various fatty acids in the cellular fraction of the isolate. FAME (fatty acid methyl ester) analysis revealed the presence of 37 fatty acids in the strain SW71. Among these, 17 of them were saturated fatty acids ranging from C4 to C24. The most abundant fatty acid was C16:0 (23.47%), followed by C18:1 (17.23%), C22:1 (8.19%), C18:0 (7.64%), C16:1 (4.91%), C18:3 (3.03%), C14:0 (2.96%), C6:0 (1.74%), C22:6 (1.38%), and C20:1 (1.23%). The unidentified peaks contributed to 17.38% of the total fatty acid composition. The fatty acid profile of other red pigmented Vibrios in the Gazogenes clade namely *V. ruber* (CECT 7878^T), *V. gazogenes* (CECT 5068^T), and *V. rhizosphaerae* (CECT 7877^T) were compared. The qualitative profiling of the dominant fatty acids was similar in all the type strains; however, they exhibited quantitative variations in the amount of different fatty acids. The major fatty acid found in the strain SW71 was C16:0 which was similar to the major fatty acid reported in in the other strains[63]. However, the content of C16:0 was higher in the strain SW71 (23.47%) compared to other strains (21.4% in *V. gazogenes,* 19.6% in *V. ruber* and 20.6% in *V. rhizosphaerae*. The quantitative variations in the content of C18:1 were 17.23% in SW71, 5.0% in *V. gazogenes*, 4.3% in *V. ruber,* and 16.1% in *V. rhizosphaerae.* The comparative difference in the fatty acid profile of the strain along with the other red pigmented reference *Vibrio* strains are listed in Table 2.

Lipidomics

The LC-HRMS employing positive and negative electrospray ionization mode enabled a comprehensive lipid profiling of the strain SW71 (S. Table 1A &1B). The major lipid classes detected in positive mode include 28.9% Phosphatidylethanolamine (34:2), 27.9% Phosphatidylethanolamine (32:1), 10% galactosylceramide (d18:2/21:0), 6.1% Phosphatidylethanolamine (18:1(11Z)/16:0), 6.1% Phosphatidylethanolamine (14:0/18:1(11Z)), 3.6% Phosphatidylethanolamine (15:0/15:0) 2.5% Phosphatidylserine (34:0), 2.6% Diacylglycerol (20:1(11Z)/22:4(7 Z,10Z,13Z,16Z)/0:0), 1.9% phosphatidylserine -N Acyl (36:1). The lipid classes detected in the negative mode includes 33.0% N-lauroylglycine, 14.5% Phosphatidic acid (32:2), ((16:1/16:0), 13.2% Phosphatidylethanolamine (18:1/16:1), 10.38% Diphosphatidyl glycerol (Cardiolipin) (68:4), 5.1% Phophatidic acid (34:2), 5.0% Lysobisphosphatidic acid (34:2), 3.6% Phosphatidylglycerol (16:0/16:1), 3.11% Phosphatidylethanolamine (16:1/16:1), 2.9% Olein, 2.2% Phosphatidylcholine (33:2), 1.49% Phosphatidylethanolamine (30:0), 0.76% Phophatidic acid (32:2), 0.56% Lysophosphatidyl ethanolamine (18:1), 0.52% Lysophosphatidyl ethanolamine (16:1) and 0.2% Lysophosphatidyl ethanolamine (16:0). Research have been shown that the major lipid present in *Vibrio* species are Phosphatidylethanolamine, Phosphatidyl glycerol, Di phosphatidyl glycerol (cardiolipin), Lyso phophatidylethanolamine[64,65,70] . In addition to this, LC–MS based lipidomics also revealed the presence of galactosylceramide (10%) in positive mode. However, the presence of these sphingolipids has not been identified previously in any of the *Vibrio* species. This was reported in four species of *Bacteriodes* using targeted LC–MS analysis[66]. LC–MS have the potential to uncover the species-specific differences in the bacterial lipids unlike the conventional chromatographical techniques.

Biosynthetic gene cluster (BGC) analysis

AntiSMASH database analysis showed that the isolate genome had NRPS, RiPP-like, ectoine, prodigiosin, and other secondary metabolite gene clusters (S. Fig. 4). A total of 15 regions were identified for biosynthetic gene clusters including eight known gene clusters. Among these eight known gene clusters, prodigiosin, ectoine and vanchrobactin gene clusters were displayed significant matches (< 70%) with the reference gene clusters of predicted gene function. The corpeptin, serratiochelin, xenoamicin, and enacycloxin gene clusters lacked significant matches (> 70%) with the reference gene clusters. Interestingly, six gene clusters showed unknown gene function by displaying any similarity with the existing reference database for biosynthetic gene clusters.

Fatty acids	% of fatty acid to total fatty acid			
	SW71 (This study)	*V. ruber*[1]	*V. gazogenes*[2]	*V. rhizosphaerae*[3]
$C_{10:0}$	ND	TR	TR	1.4
$C_{12:0}$	4.4	4.4	5.2	5.8
$C_{14:0}$	2.9	5.6	8.3	6.5
$C_{16:0}$	23.47	19.6	21.4	20.6
$C_{17:0}$	TR	TR	TR	TR
$C_{17:1}$	ND	TR	ND	TR
$C_{18:0}$	7.64	TR	ND	TR
$C_{16:1}$	4.91	3.9	4.2	5.2
$C_{18:1}$	17.23	19.5	8.3	16.1

Table 2. Comparison of major fatty acids in the strain SW71 and other red pigmented *Vibrio* species under Gazogenes clade. *ND -not detected; TR-less than 1% of the total fatty acid. [1]Shieh et al., 2003, [2]Schoch et al., 2020, [3]Kumar and Nair 2007.

One of the polyketide named prodigiosin showed 76% gene similarity with prodigiosin gene cluster of *Serratia* sp. and the red pigmentation and antibacterial activity of the isolate was confirmed due to the presence of the production of these gene cluster. However, there is sequence variation observed in the prodigiosin gene clusters among the prodigiosin producing *Vibrio* species including the strain SW71 (S. Fig. 4). The genes in the ectoine biosynthetic cluster showed 66% sequence similarity with the ectoine BCGS from *Methylomicrobium alcaliphilum 20Z*. The presence of this gene cluster in the strain SW71 confirmed the osmoprotective effect and halophilic nature of the isolate. Evolutionary analysis of the ectoine BGCS reported by several researchers confirmed their role in halophilic bacteria to withstand and survive change in salinity condition. The vanchrobactin a NRP-mettalophore BGCS in the isolate showed the 90% sequence similarity with vanchrobactin biosynthetic gene cluster from *Vibrio anguillarum RV22*. The vanchrobactin is a catecholate siderophore and the presence of this BCGs in the isolate genome facilitate their growth and survivability in the natural environment by competing with other microflora. This BGCS also known for potent antibacterial and anticancerous activity. The sequence variation was also noticed in vanchrobactin gene clusters of the strain with other vanchrobactin gene clusters producing Vibrios.

Prodigiosin pigment identification by LC-HRMS analysis

Untargeted metabolomics analysis of the ethyl acetate extract of the strain SW71 revealed a prodiginine derivative called cycloprodigiosin with a precursor ion at m/z 322.1909 in positive ionization mode. The compound was detected at retention time of 12.79 min and [M + H] + 1 adduct. The MS/MS spectrum showed characteristic peaks at m/z 71.0853, m/z 252.1122, m/z 264.1120, and m/z 279.1352 (Fig. 3). It was reported that the *Serratia marcescens*, *Pseudomonas magneslorubra*, *Vibrio psychroerythrus, Vibrio gazogenes* (ATCC 29988) and *V. ruber* produce a linear derivative called prodigiosin, while *Streptomyces coelicolor, S. lividans, Saccharopolyphora sp* produce undecylprodigiosin[67,13]. The cyclic derivative, cycloprodigiosin is produced by *Pseudoalteromonas denitrificans, V. gazogenes* PB1, *Alteromonas rubra* and *V. spartinae*[68,67]. The LC-HRMS analysis of ethyl acetate extract of the strain SW71 depicted the molecular ion peak (322.1909 m/z) is in accordance with the molecular ion peak of the cycloprodigiosin[68].

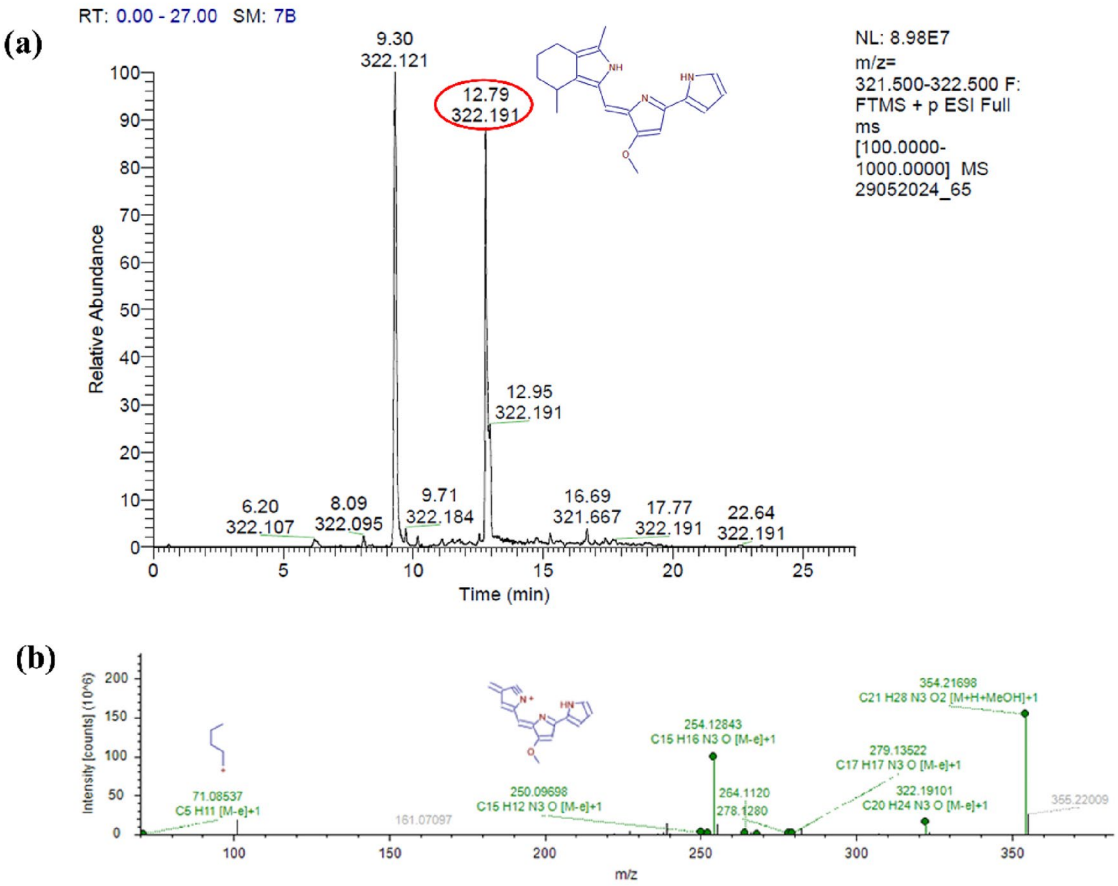

Fig. 3. (**a**) ESI positive mode ion Chromatogram and (**b**) MS2 fragments of cycloprodigiosin (m/z 322.191) putatively identified during the untargeted metabolite profiling of the strain SW71 ethyl acetate extract.

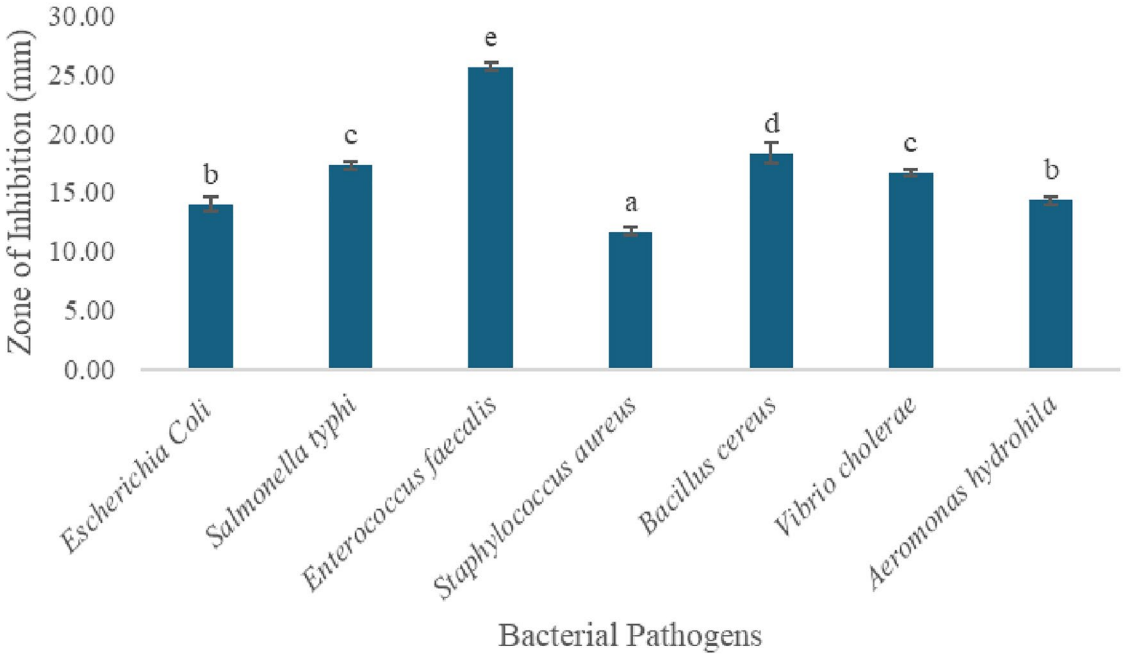

Fig. 4. Antibacterial activity of the ethyl acetate extract of the strain SW71.

Antibacterial activity of the ethyl aetate extract of the strain SW71

The extract showed broad-spectrum antibacterial activity against bacterial pathogens ($p < 0.05$). The zone of inhibition against various test pathogens is illustrated in Fig. 4. The maximum and minimum zone of inhibition (mm) were observed against *E. faecalis* (25.67 ± 0.33) and *S. aureus* (11.67 ± 0.33), respectively. Pigment production and its antibacterial activity in *Serratia marcescens, Streptomyces coelicolor, V. ruber, V. gazogenes,* and *V. rhizospaherae* were well documented previously[69,13,51] Prodigiosin from *S. marcescens* showed significant antibacterial activity against Gram-positive and Gram-negative bacteria, with inhibition zone (mm) ranging from 16.80 to 28.2 in diameter [51].

AST profiling of the strain SW71

The AST results revealed that the strain was sensitive to most of the tested antibiotics including Imipenem, Ciprofloxacin, Tobramycin, Moxifloxacin, Ofloxacin, Sparfloxacin, Levofloxacin, Norfloxacin, Co-Trimoxazole, Colistin, Nalidixic acid, Augmentin, Kanamycin, Gatifloxacin, Gentamicin, Amikacin, Streptomycin and Ceftriaxone. However, it showed resistance to the antibiotics such as Cefpodoxime, Ticarcillin, Ampicillin, Clarithromycin, Amoxicillin/Clavulanate 20/10 µg, Cephalexin, vancomycin, and Teicoplanin.[58] have also studied Similarly, the AST profile of *V. ruber* VR1^T and *V. gazogenes* ATCC 29988^T revealed that both the strains were susceptible to the antibiotics tested.

Conclusion

In conclusion, the microbiological, phylogenetic based on 16S rDNA gene sequences, whole genome sequences, and chemotaxonomic features show that the strain SW71 isolated from brown seaweed, *S. wghtii* from Mandapam coast of India represents a new species under Gazogenes clade of the Genus *Vibrio* for which the name *Vibrio rubrogenes* sp. nov (strain smcift-1 ^{T}), is proposed.

Description of *Vibrio rubrogenes* sp. nov.

Vibrio rubrogenes (L. masc. adj. *ruber*, red; Gr.*v.gennao,* to produce; N.L. part. Adj. *rubrogenes,* red producing referring to the red pigment producing *Vibrio* species).

Strain smcift-1 ^{T} is Gram-negative, aerobic, red colored, 1–1.5 mm size, smooth and circular edge. Growth occurs at 0.5% to 20% NaCl, pH 5 to 10 and temperature 7 to 40 ^{0}C. The strain is weakly motile, fermentative with gas production in motility medium and no swarming on agar medium. Catalase is positive and oxidase is negative. No growth on TCBS medium. Acid and gas are produced from glucose. Sugars such as D-Mannitol, D- Galactose L- Arabinose, L-Salicilin, Sucrose, D-Ribose, D-Melibiose, D- Xylose, D-Fructose, D-Mannose, Lactose, D-Cellobiose, D-Maltose, and Trehalose are fermented. Sugars such as D-Raffinose, Dulcitol, Inositol, D-Arabinose, D-Melezitose, D-Rhamnose, Glycerol, D- Arabitol, Adonitol and Erythritol are not fermented. L-Sorbose is weakly fermented. Lyine, Ornithine, and Arginine decarboxylase tests are negative. The strain possesses lipase, phospholipase, caseinase, amylase, gelatinase and cellulase activity but not tyrosinase. Indole, methyl red and Vogus Proskauer tests are positive. Nitrate is reduced to nitrite. The major fatty acids are C16:0 (23.47%), followed by C18:1 (17.23%), C22:1 (8.19%), C18:0 (7.64%), and C16:1(4.91%). The polar lipid pattern consists of Phosphatidylethanolamine, Galactosylceramide, N-lauroylglycine, Phosphatidic acid, Diphoshatidyl glycerol (cardiolipin) and Phophatidylserine. The strain smcift^T (=MTCC 14081 ^{T}), was isolated from brown

seaweed (*Sargassum wightii*), collected from Mandapam district, Tamil Nadu, India. The DNA G + C content of the type strain is 45.48%. The NCBI SRA Bio project id of whole genome sequences and the gene bank accession number of 16S rRNA gene are PRJNA1240948 and PX526232.

Data availability

The genome sequence of the strain SW71 was submitted in the NCBI genome database. Whole genome sequence data for the strain was deposited in the NCBI database with the bioproject id: PRJNA1240948; BioSample id: SAMN47543877; SRA id: SRS24571658; Metadata (type strain id: *Vibrio* sp. smcift-1, isolation source: seaweed, GPS coordinates: Latitude 9.2886 N; Longitude 79.1329 E; date of collection; 2023) 16S rRNA sequence Accession Number: PX526232.

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References

1. Sampaio, A., Silva, V., Poeta, P. & Aonofriesei, F. *Vibrio* spp.: Life strategies, ecology, and risks in a changing environment. *Diversity* **14**(2), 97. https://doi.org/10.3390/d14020097 (2022).
2. Baumann, P., Baumann, L., Bang, S. S. & Woolkalis, M. J. Reevaluation of the taxonomy of *Vibrio*, *Beneckea*, and *Photobacterium*: Abolition of the genus *Beneckea*. *Curr. Microbiol.* **4**, 127–132. https://doi.org/10.1007/BF02602814 (1980).
3. Butt, M. et al. *Shewanella phaeophyticola* sp. nov. and *Vibrio algarum* sp. nov., isolated from marine brown algae. *Int. J. Syst. Evol. Microbiol.* **74**(5), 006378. https://doi.org/10.1099/ijsem.0.006378 (2024).
4. Parte, A. C. LPSN–List of prokaryotic names with standing in nomenclature (bacterio. net), 20 years on. *Int. J. Syst. Evol. Microbiol.* **68**(6), 1825–1829. https://doi.org/10.1099/ijsem.0.002786 (2018).
5. Romalde, J. L., Diéguez, A. L., Lasa, A. & Balboa, S. New *Vibrio* species associated to molluscan microbiota: A review. *Front. Microbiol.* **4**, 413. https://doi.org/10.3389/fmicb.2013.00413 (2014).
6. Malham, S. K. et al. The interaction of human microbial pathogens, particulate material and nutrients in estuarine environments and their impacts on recreational and shellfish waters. *Environ. Sci. Process. Impacts* **16**(9), 2145–2155. https://doi.org/10.1039/C4EM00031E (2014).
7. Amaral, G. R. S. et al. Genotype to phenotype: Identification of diagnostic *Vibrio* phenotypes using whole genome sequences. *Int. J. Syst. Evol. Microbiol.* **64**(Pt_2), 357–365. https://doi.org/10.1099/ijs.0.057927-0 (2014).
8. Jiang, C. et al. Vibrio Clade 3.0: New Vibrionaceae evolutionary units using genome-based approach. *Curr. Microbiol.* **79**, 10. https://doi.org/10.1007/s00284-021-02725-0 (2022).
9. Ramesh, C. H., Vinithkumar, N. V. & Kirubagaran, R. Marine pigmented bacteria: A prospective source of antibacterial compounds. *J. Nat. Sci. Bio. Med.* https://doi.org/10.4103/jnsbm.JNSBM_201_18 (2019).
10. Srilekha, V., Krishna, G., Sreelatha, B., Jagadeesh Kumar, E. & Rajeshwari, K. V. N. Prodigiosin: A fascinating and the most versatile bioactive pigment with diverse applications. *Syst. Microbiol. Biomanuf.* **4**(1), 66–76. https://doi.org/10.1007/s43393-023-00192-1 (2024).
11. Thompson, F. L., Iida, T. & Swings, J. Biodiversity of vibrios. *Microbiol. Mol. Biol. Rev.* **68**(3), 403–431 https://doi.org/10.1128/mmbr.68.3.403-431 (2004).
12. Hejazi, A. & Falkiner, F. R. *Serratia marcescens*. *J. Med. Microbiol.* **46**(11), 903–912. https://doi.org/10.1099/00222615-46-11-903 (1997).
13. Darshan, N. & Manonmani, H. K. Prodigiosin and its potential applications. *J. Food Sci. Technol.* **52**(9), 5393–5407. https://doi.org/10.1007/s13197-015-1740-4 (2015) (**PMID: 26344956 PMCID: PMC4554646**).
14. Sánchez de la Nieta, R., Santamaría, R. I. & Díaz, M. Two-component systems of *Streptomyces coelicolor*: An intricate network to be unraveled. *Int. J. Mol. Sci.* **23**(23), 15085. https://doi.org/10.3390/ijms232315085 (2022).
15. Kim, D. K. et al. Red to red-the marine bacterium *Hahella chejuensis* and its product prodigiosin for mitigation of harmful algal blooms. *J. Microbiol. Biotechnol.* **18**(10), 1621–1629. https://repository.kopri.re.kr/handle/201206/6617 (2008).
16. Lee, J. S. et al. Exceptional production of both prodigiosin and cycloprodigiosin as major metabolic constituents by a novel marine bacterium, *Zooshikella rubidus* S1-1. *Appl. Environ. Microbiol.* **77**(14), 4967–4973. https://doi.org/10.1128/AEM.01986-10 (2011).
17. De Carvalho, C. C. & Fernandes, P. Production of metabolites as bacterial responses to the marine environment. *Mar. Drugs* **8**(3), 705–727. https://doi.org/10.3390/md8030705 (2010).
18. Follows, M. J. & Dutkiewicz, S. Modeling diverse communities of marine microbes. *Annu. Rev. Mar. Sci.* **3**(1), 427–451. https://doi.org/10.1146/annurev-marine-120709-142848 (2011).
19. Villarreal-Chiu, J. F., Quinn, J. P. & McGrath, J. W. The genes and enzymes of phosphonate metabolism by bacteria, and their distribution in the marine environment. *Front. Microbiol.* **3**, 19. https://doi.org/10.3389/fmicb.2012.00019 (2012).
20. Egan, S. et al. The seaweed holobiont: Understanding seaweed–bacteria interactions. *FEMS Microbiol. Rev.* **37**(3), 462–476. https://doi.org/10.1111/1574-6976.12011 (2013).
21. Menaa, F. et al. Ecological and industrial implications of dynamic seaweed-associated microbiota interactions. *Marine Drugs* **18**(12), 641. https://doi.org/10.3390/md18120641 (2020).
22. Gokulakrishnan, S., Raja, K., Sattanathan, G. & Subramanian, J. Proximate composition of bio potential seaweeds from Mandapam South East coast of India. *Int. Lett. Nat. Sci.* https://doi.org/10.56431/p-916qco (2015).
23. Manivannan, K., Thirumaran, G., Karthikai Devi, G., Hemalatha, A. & Anantharaman, P. Biochemical composition of seaweeds from Mandapam coastal regions along Southeast Coast of India. *Am.-Eurasian J. Bot.* **1**(2), 32–37 (2008).
24. Suji, H. A. et al. Whole genome sequence of seaweed endophyte *Bacillus halotolerans* strain AUPP for antagonistic activity against *Fusarium incarnatum* causing chilli fruit rot. *Sci. Rep.* **14**, 31881. https://doi.org/10.1038/s41598-024-83317-3 (2024).
25. AL-Joda, B. M. S. & Jasim, A. H. Biochemical testing revision for identification several kinds of bacteria. *J. Univ. Babylon Pure Appl. Sci.* **29**(2), 168–176. https://doi.org/10.29196/jubpas.v29i2.3751 (2021).
26. Coico, R. Gram staining. Current Protocols in Immunology, 23(1), pp.A-3O. Reiner, K., 2010. Catalase test protocol. American society for microbiology, 1(1), pp.1–9 (1997).
27. Steel, K. J. The oxidase reaction as a taxonomic tool. *Microbiology* **25**(2), 297–306. https://doi.org/10.1099/00221287-25-2-297 (1961).
28. Esselmann, M. T. & Liu, P. V. Lecithinase production by gram-negative bacteria. *J. Bacteriol.* **81**(6), 939–945. https://doi.org/10.1128/2Fjb.81.6.939-945.1961 (1961).
29. Nakazawa, T., Yamada, Y. & Ishibashi, M. Characterization of hemolysin in extracellular products of *Pseudomonas cepacia*. *J. Clin. Microbiol.* **25**(2), 195–198. https://doi.org/10.1128/jcm.25.2.195-198.1987 (1987).
30. Joseph, S. W., Colwell, R. R. & Kaper, J. B. *Vibrio parahaemolyticus* and related halophilic vibrios. *CRC Crit. Rev. Microbiol.* **10**(1), 77–124. https://doi.org/10.3109/10408418209113506 (1982).

31. Donovan, T. J. & Van Netten, P. Culture media for the isolation and enumeration of pathogenic *Vibrio* species in foods and environmental samples. *Prog. Ind. Microbiol.* **34**, 203–217. https://doi.org/10.1016/S0079-6352(05)80014-1 (1995).
32. Shinagawa, K. Analytical methods for *Bacillus cereus* and other *Bacillus* species. *Int. J. Food Microbiol.* **10**(2), 125–141. https://doi.org/10.1016/0168-1605(90)90061-9 (1990).
33. Moller, V. Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. *Ada path, microbiol. Scand.* **36**, 158.https://doi.org/10.1111/j.1699-0463.1955.tb04583.x (1955).
34. Frank, J. A. et al. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl. Environ. Microbiol.* **74**(8), 2461–2470. https://doi.org/10.1128/AEM.02272-07 (2008).
35. Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P. & Göker, M. GBDP on the grid: A genome-based approach for species delimitation adjusted for an automated and highly parallel processing of large data sets. *Beiträge zu Anwenderprojekten und Infrastruktur im bwGRiD* **2012**, 83. https://doi.org/10.5445/KSP/1000039516 (2014).
36. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnetjournal* https://doi.org/10.14806/ej.17.1.200 (2011).
37. Chklovski, A., Parks, D. H., Woodcroft, B. J. & Tyson, G. W. CheckM2: A rapid, scalable and accurate tool for assessing microbial genome quality using machine learning. *Nat. Methods* **20**(8), 1203–1212. https://doi.org/10.1038/s41592-023-01940-w (2023).
38. Brettin, T. et al. *RASTtk:* A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci. Rep.* **5**, 8365. https://doi.org/10.1038/srep08365 (2015).
39. Meier-Kolthoff, J. P. & Göker, M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat. Commun.* **10**(1), 2182. https://doi.org/10.1038/s41467-019-10210-3 (2019).
40. Lagesen, K. et al. RNAmmer: Consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* **35**(9), 3100–3108. https://doi.org/10.1093/nar/gkm160 (2007).
41. Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P. & Göker, M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* **14**(1), 60. https://doi.org/10.1186/1471-2105-14-60 (2013).
42. Goloboff, P. A., Farris, J. S. & Nixon, K. C. TNT, a free program for phylogenetic analysis. *Cladistics* **24**(5), 774–786. https://doi.org/10.1111/j.1096-0031.2008.00217.x (2008).
43. Stamatakis A. Parsimonator: A fast open-source parsimony program. https://cme.h-its.org/exelixis/software.html. (2014).
44. Edgar, R. C. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**(5), 1792–1797. https://doi.org/10.1093/nar/gkh340 (2004).
45. Ondov, B. D. et al. Mash: Fast genome and metagenome distance estimation using MinHash. *Genome Biol.* **17**, 132. https://doi.org/10.1186/s13059-016-0997-x (2016).
46. Farris, J. S. Estimating phylogenetic trees from distance matrices. *Am. Nat.* **106**, 645–667. https://doi.org/10.1086/282802 (1972).
47. Yoon, S. H. et al. Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int. J. Syst. Evol. Microbiol.* **67**(5), 1613–1617. https://doi.org/10.1099/ijsem.0.001755 (2017).
48. Alanjary, M., Steinke, K. & Ziemert, N. AutoMLST: An automated web server for generating multi-locus species trees highlighting natural product potential. *Nucleic Acids Res.* **47**(W1), W276–W282. https://doi.org/10.1093/nar/gkz282 (2019).
49. Haridas, P. C. et al. Authentication of the species identity of squid rings using UHPLC-Q-Orbitrap MS/MS-based lipidome fingerprinting and chemoinformatics. *Food Chem.* **442**, 138525. https://doi.org/10.1016/j.foodchem.2024.138525 (2024).
50. Official methods of analysis of AOAC International 16th edn, AOAC International, Rockville. (1995).
51. Rafiq, M. et al. Microbial prodigiosin shows broad-spectrum bioactivity confirmed by experimental and computational analyses. *Front. Microbiol.* **16**, 1676959. https://doi.org/10.3389/fmicb.2025.1676959 (2025).
52. Salek, R. M., Steinbeck, C., Viant, M. R., Goodacre, R. & Dunn, W. B. The role of reporting standards for metabolite annotation and identification in metabolomic studies. *Gigascience* **2**(1), 2047–2217. https://doi.org/10.1186/2047-217X-2-13 (2013).
53. Humphries, R. M. et al. CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. *J. Clin. Microbiol.* **56**(4), 10–1128. https://doi.org/10.1128/jcm.01934-17 (2018).
54. Al Bulushi, I. M., Al Kharousi, Z. S. & Rahman, M. S. Vitek: A Platform for a Better Understanding of Microbes. In *Techniques to measure food safety and quality: microbial, chemical, and sensory* 117–136 (Springer International Publishing, Cham). https://doi.org/10.1128/jcm.01934-17 (2021).
55. Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P. & Tyson, G. W. CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* **25**(7), 1043–1055. http://www.genome.org/cgi/doi/10.1101/gr.186072.114 (2015).
56. Baby, B. et al. Complete genome sequence of *Vibrio gazogenes* PB1: An estuarine bacterium capable of producing prodigiosin from starch or cellulose. *Front. Mar. Sci.* **10**, 1028319. https://doi.org/10.3389/fmars.2023.1028319 (2023).
57. Kumar, N. R. & Nair, S. *Vibrio rhizosphaerae* sp. nov., a red-pigmented bacterium that antagonizes phytopathogenic bacteria. *Int. J. Syst. Evol. Microbiol.* **57**(Pt 10), 2241–2246. https://doi.org/10.1099/ijs.0.65017-0 (2007).
58. Shieh, W. Y., Chen, Y. W., Chaw, S. M. & Chiu, H. H. *Vibrio ruber* sp. nov., a red, facultatively anaerobic, marine bacterium isolated from sea water. *Int. J. Syst. Evol. Microbiol.* **53**(Pt 2), 479–484. https://doi.org/10.1099/ijs.0.02307-0 (2003) (**PMID: 12710616**).
59. McDonald, N. D. et al. CRISPR-Cas systems are present predominantly on mobile genetic elements in *Vibrio* species. *BMC Genomics* **20**, 105. https://doi.org/10.1186/s12864-019-5439-1 (2019).
60. Parra, Á. et al. Comparative analysis of CRISPR- Cas systems in *Vibrio* and *Photobacterium* genomes of high influence in aquaculture production. *Biointerface Res. Appl. Chem.* **11**, 9513–9529. https://doi.org/10.33263/BRIAC112.95139529 (2021).
61. Jain, C., Rodriguez-R, L. M., Phillippy, A. M., Konstantinidis, K. T. & Aluru, S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat. Commun.* **9**(1), 5114. https://doi.org/10.1038/s41467-018-07641-9 (2018).
62. Liang, K. Y. et al. A *Vibrio cholerae* core genome multilocus sequence typing scheme to facilitate the epidemiological study of cholera. *J. Bacteriol.* **202**(24), 10–1128. https://doi.org/10.1128/jb.00086-20 (2020).
63. Lucena, T. et al. *Vibrio palustris* sp. nov. and *Vibrio spartinae* sp. nov., two novel members of the Gazogenes clade, isolated from salt-marsh plants (*Arthrocnemum macrostachyum* and *Spartina maritima*). *Int. J. Syst. Evol. Microbiol.* **67**(9), 3506–3512. https://doi.org/10.1099/ijsem.0.002155 (2017).
64. Adams, R. L. & Russell, N. J. Interactive effects of salt concentration and temperature on growth and lipid composition in the moderately halophilic bacterium *Vibrio costicola*. *Can. J. Microbiol.* **38**(8), 823–827. https://doi.org/10.1139/m92-134 (1992).
65. Hanna, K. I. M., Bengis-Garber, C., Kushner, D. J., Kogut, M. & Kates, M. The effect of salt concentration on the phospholipid and fatty acid composition of the moderate halophile *Vibrio costicola*. *Can. J. Microbiol.* **30**(5), 669–675. https://doi.org/10.1139/m84-10 (1984).
66. Ryan, E., Gonzalez Pastor, B., Gethings, L. A., Clarke, D. J. & Joyce, S. A. Lipidomic analysis reveals differences in *Bacteroides* species driven largely by plasmalogens, glycerophosphoinositols and certain sphingolipids. *Metabolites* **13**(3), 360. https://doi.org/10.3390/metabo13030360 (2023).
67. Williamson, N. R., Fineran, P. C., Leeper, F. J. & Salmond, G. P. C. The biosynthesis and regulation of bacterial prodiginines. *Nat. Rev. Microbiol.* **4**, 887–899. https://doi.org/10.1038/nrmicro1531 (2006).
68. Vitale, G. A. et al. Genomics–metabolomics profiling disclosed marine *Vibrio spartinae* 3.6 as a producer of a new branched side chain prodigiosin. *J. Nat. Prod.* **83**(5), 1495–1504. https://doi.org/10.1021/acs.jnatprod.9b01159 (2020).
69. Danevcic, T., Borić, M. & Stopar, D. Microbial ecophysiology of *Vibrio ruber*. *Food Technol. Biotechnol.* **52**(2), 198–203. https://hrcak.srce.hr/122344 (2014).

70. Pirttijärvi, T. S. M., Andersson, M. A. & Salkinoja-Salonen, M. S. Properties of *Bacillus cereus* and other bacilli contaminating biomaterial-based industrial processes. *Int. J. Food Microbiol.* **60**(2–3), 231–239. https://doi.org/10.1016/S0168-1605(00)00313-5 (2000).
71. Schoch, C. L. et al. NCBI Taxonomy: A comprehensive update on curation, resources and tools. Database (Oxford) https://doi.org/10.1093/database/baaa062 (2020) (**PubMed: 32761142 PMC: PMC7408187**).
72. Setiyono, E. et al. An Indonesian marine bacterium, *Pseudoalteromonas rubra*, produces antimicrobial prodiginine pigments. *ACS Omega* **5**(9), 4626–4635. https://doi.org/10.1021/acsomega.9b04322 (2020).
73. Srimathi, R., Priya, R., Nirmala, M. & Malarvizhi, A. Isolation, identification, optimization of prodigiosin pigment produced by *Serratia marcescens* and its applications. *Int. J. Latest Eng. Manag. Res.* **2**(9), 11–21 (2017).

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