Catenovulum algicola sp. nov., an agar-hydrolysing, halophilic gammaproteobacterium isolated from kelp seedlings

Rui-Ying Chen
Shandong University
Yang-Yang Sun
Shandong University
Ya Gong
gongya@sdu.edu.cn
Shandong University
Zong-Jun Du
Shandong University

Research Article

Keywords: Catenovulum algicola, draft genome sequencing, nitrate reduction, sulfate reduction

Posted Date: March 19th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4063278/v1

Additional Declarations: No competing interests reported.

Abstract

A pale white, Gram-stain-negative, strictly aerobic, halophilic, agar-hydrolysing and rod-shaped bacterium, designated strain 2E275^T, was isolated from kelp seedlings at Weihai, China. The cells are motile by means of a single polar flagellum. The isolate had an absolute requirement for agar and formed a depression when grown on Marine Agar 2216 plates for 3 days. Strain 2E275^T growth occurred at 4–42°C (optimum, 37°C), with 0–12% (w/v) NaCl (optimum, 3%), and at pH 5.5–10.5 (optimum, pH 6.5-7.0). The isolate could not be cultured in MB, but when 0.2% agar was added, they grew well. According to the phylogenetic analysis of the 16S rRNA gene sequence, strain 2E275^T showed the highest sequence similarity of 96.0% with *Catenovulum agarivorans* YM01^T, followed by *Catenovulum sediminis* D2^T (94.1%) and *Catenovulum maritimum* Q1^T (93.7%). Strain 2E275^T could remarkably hydrolyse agar, cellulose, starch, sodium alginate, casein and Tweens 20, 60, 80, but not DNA and Tween 40. The major polar lipids of strains 2E275^T were phosphatidylglycerol, phosphatidylethanolamine and phosphoaminolipid. The major cellular fatty acids were summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), summed feature 8 ($C_{18:1}\omega7c$) and $C_{16:0}$. Ubiquinone 8 (Q-8) was found to be the major respiratory quinone. The DNA G+C content was 38.2%. On the basis of the phenotypic, genetic and physiological characteristics, strain 2E275^T should be classified as a novel species of the genus *Catenovulum*, for which the name *Catenovulum algicola* sp. nov. is proposed. The type strain is 2E275^T (MCCC 1H00528^T = KCTC 92643^T).

Introduction

The genus *Catenovulum* was proposed as a novel member of the family *Alteromonadaceae* by Yan *et al.* (2011). At the time of writing, three species have been validly published in this genus (https://lpsn.dsmz.de/genus/catenovulum, Parte *et al.* 2020). The type species *Catenovulum agarivorans* was isolated from seawater, *Catenovulum maritimum* from the marine alga *Porphyra yezoensis* Ueda (AST58-103), and *Catenovulum sediminis* from coastal sediment (Yan *et al.* 2011; Li *et al.* 2015; Shi *et al.* 2017). These species are Gram-stain-negative, agar-hydrolysing, rod-shaped cells with flagella.

In this study, a member of one more putative new species belonging to the genus *Catenovulum* was isolated from kelp seedlings in Weihai and its taxonomic properties were characterized using a polyphasic approach.

Materials and methods

Bacterial isolation and cultivation

Strain 2E275^T was isolated from kelp seedlings collected from a kelp nursery pond of Weihai, China, in August 2021. For the bacterial isolation, sterile cotton swabs were used to wipe the kelp seedlings

sample and immersed in the sterile seawater. The immersion sample was oscillated in a shaker at 150 rpm for one hour. The collected sample was serially diluted to 10^{-5} with sterilized seawater, and 0.1 mL aliquots of each dilution were spread on modified MA (Becton Dickinson). The modified MA medium contained (g L^{-1}; pH 7.6): peptone (0.5), yeast extract (0.1), sodium pyruvate (0.3), agar (20), sodium alginate (2), carboxymethylcellulose sodium (2), carrageenan (2). After incubation at 20°C for 10 days, a pale white colony was selected from the plate and re-streaked to obtain a pure isolate, 2E275^T. This strain was preserved at -80°C in sterile seawater supplemented with 20% (*v/v*) glycerol, with the lab accession number of SDUM 120004. Strain 2E275^T was cultivated on MA at 37°C for physiological, biochemical, and chemical analyses. MB (Becton Dickinson) supplemented with 0.2% agar was used as liquid medium. Strain *C. agarivorans* YM01^T (the type species of the genus, obtained from the laboratory of Professor Xiao-Hua Zhang) and *C. sediminis* D2^T were used as the closely related strains for comparative purposes and cultivated on modified MA at 28°C.

16S rRNA gene sequence analyses

The 16S rRNA gene was amplified from the genomic DNA by PCR using bacterial primers 27F and 1492R (Lane 1991). The purified PCR product was ligated into the T-vector pMD19 (Takara) and recombinant plasmids were reproduced in *Escherichia coli* DH5*α* cells. Sequencing was performed by Tsingke Biotech Co., Ltd., China. The determined 16S rRNA gene sequence was submitted to GenBank and similar sequences were obtained from the EzBioCloud Database (http://www.ezbiocloud.net/, Yoon *et al.* 2017). The 16S rRNA gene sequences of the related species were aligned using ClustalX (Thompson *et al.* 1997), and phylogenetic trees were reconstructed using the neighbour-joining (NJ) (Saitou and Nei 1987) and maximum-likelihood (ML) (Fitch 1971) methods in the MEGA version X (Kumar et al. 2018). Kimura's two-parameter model was used to estimate the evolutionary distances for the neighbour-joining tree. Bootstrap analyses (1000 replications) were carried out to evaluate the tree topologies (Felsenstein 1985).

Genome Analyses

Genomic DNA of strain 2E275^T was gained by using a Bacterial Genomic DNA Mini kit (Takara) according to the manufacturer's recommendations. The draft genome sequence of strain 2E275^T was sequenced at Novogene Bioinformatics Technology Co., Ltd using the Illumina PE150 platform with massively parallel sequencing Illumina technology. The draft genome assembly was obtained from high-quality reads using SOAP denovo version 2.04 software (Li *et al.* 2008; Li *et al.* 2010). The DNA G + C content was calculated according to the draft genome. The genes of strain 2E275^T were identified by NCBI (National Center for Biotechnology Information) Prokaryotic Genome Annotation Pipeline server online (Angiuoli *et al.* 2008). The genomes of *C. agarivorans* and *C. sediminis* were obtained from NCBI. The average nucleotide identity (ANI) values between the genomes were calculated by using the ChunLab's online ANI Calculator (www.ezbiocloud.net/tools/ani, Lee *et al.* 2016). The Genome-to-Genome Distance Calculator GGDC3.0 (http://ggdc.dsmz.de, Meier-Kolthoff 2022) was used to calculate the digital DNA-DNA hybridization (dDDH) values. Protein-coding regions were identified with the Rapid

Annotations using Subsystems Technology (RAST) server (https://rast.nmpdr.org/rast.cgi, Aziz *et al.* 2008). The presence of gene clusters encoding secondary metabolites of strain 2E275^T was predicted by using the antiSMASH 6.0 database (Blin *et al.* 2021). KEGG database (https://www.genome.jp/kegg/, Kanehisa *et al.* 2016) was used to annotate the genome and analyse the genes involved in metabolic pathways. Carbohydrate-active enzymes of strain 2E275^T was annotated using the dbCAN2 meta server (Zhang *et al.* 2018).

Physiology

The morphological, physiological and biochemical characteristics of strain 2E275^T were investigated after cultivation on MA at 37°C for 3 days. The Gram reaction was determined using the bioMérieux Gram-stain kit according to the manufacturer's instructions. Cell morphology and size were examined with light microscopy (E600; Nikon), scanning electron microscopy (Nova NanoSEM 450; FEI), and transmission electron microscopy (JEM-1200EX; JEOL). Motility was determined using the hang-drop method (Bernardet *et al.* 2002) and gliding motility was observed on MA solidified with 0.3% agar according to the methods of Bowman *et al.* (2000). Oxidase activity was tested using a bioMérieux oxidase reagent kit according to the manufacturer's instructions, and catalase activity was examined by the production of bubbles in a 3.0% (*v/v*) H_2O_2 solution. Growth at 4, 10, 20, 25, 28, 30, 33, 37, 40, 42 and 45°C was measured for 3 days on MA to determine the optimal temperature and temperature range for growth. The pH range for growth was determined on MA by the addition of appropriate buffer solution [MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5) and CAPSO (pH 9.0, 9.5, 10.0 and 10.5)]. Salt resistance was tested at 37°C by using modified MA, prepared with artificial seawater (0.32% $MgSO_4$, 0.23% $MgCl_2$, 0.12% $CaCl_2$, 0.07% KCl and 0.02% $NaHCO_3$, all *w/v*), and supplemented with different NaCl concentrations (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 10, 12 and 13%, *w/v*). Colony growth for temperature, salt tolerance and pH were all recorded every 6 hours. Reduction of nitrate was determined as described by Cowan and Steel (1974). Growth under anaerobic (N_2: H_2: CO_2 at 80:10:10 by vol) conditions were determined by incubation on modified MA with or without 0.1% (*w/v*) KNO_3 for 2 weeks. Hydrolysis of Tweens (20, 60, 40 and 80), DNA, alginate, agar, starch and cellulose were investigated according to previously described methods (Wang *et al.* 2015).

Susceptibility to antibiotics were investigated on MA plates at 37°C for 3 days (0.5 McFarland standard) using the disc diffusion method according to guidelines of the Clinical and Laboratory Standards Institute (2012). Enzyme activities and other physiological and biochemical characteristics were determined with the API 20E and API ZYM kits (bioMérieux). The ability to oxidize various carbon compounds was determined with Biolog GEN III MicroPlates. Acid production from carbohydrates was determined using the API 50CHB fermentation kit (bioMérieux). All the tests were performed according to the manufacturer's instructions.

Chemotaxonomy

For the cellular fatty acid analysis, cells were harvested after growing on MA under the optimum growth conditions for 48 h. 40 mg of cell material was used to extract fatty acid methyl esters. Cellular fatty

acids were determined using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.1, Athalye *et al.* 1985) equipped with an Agilent model 6890N gas chromatograph. For the respiratory quinones and polar lipids, cells were collected and freeze-dried after incubation on MA at 37°C for 48 h. Respiratory isoprenoid quinones were extracted from 300 mg freeze-dried cells and analysed as described by Tindall *et al.* (2007). A silica-gel TLC plate (Merck Kieselgel 60 F254) was used to separate the quinone, and the content of each quinone was subsequently analyzed via HPLC (Hiraishi *et al.* 1996). Polar lipids were extracted from 50 mg of freeze-dried cell material and separated via two-dimensional silica gel TLC, using a chloroform/methanol system according to the procedures described by Komagata and Suzuki (1988). Total lipid material was detected using molybdatophosphoric acid and the functional groups were determined using spray reagents specific for each one (Minnikin *et al.* 1984).

Results and Discussion

16S rRNA gene sequence analyses

A near-complete 16S rRNA gene sequence (1532 bp) of strain 2E275^T was obtained, and the comparison results revealed that strain 2E275^T shared the highest 16S rRNA gene sequence similarity with *C. agarivorans* YM01^T (96.0%). In the neighbor-joining phylogenetic tree constructed using these 16S rRNA gene sequences, strain 2E275^T clustered with species of the genus *Catenovulum* (Fig. 1). This topology was also supported by the maximum-likelihood trees (Fig. S1). Based on the sequence similarity and phylogenetic position in the trees, it can be inferred that strain 2E275^T may represent a novel species within the genus *Catenovulum*.

Genome Analyses

The draft genome sequence of strain 2E275^T consisted of 4,565,251 bp in length, producing 156 contigs which length was between 301 bp and 299,961 bp, and the N50 value was 79,060. The sequencing depth of coverage was 150×. 3901 genes, with 3788 protein-coding genes, 3 rRNAs and 64 tRNAs, were identified. The DNA G + C content was 38.2%, which was different from that of *C. agarivorans* YM01^T (44.8 %) and *C. sediminis* D2^T (40.4 mol%). One 16S rRNA gene sequence (1525 bp) was extracted from the genome of strain 2E275^T, showing 100% similarity with that from traditional Sanger sequencing. The ANI value between genomes of strain 2E275^T and *C. agarivorans* YM01^T, *C. sediminis* D2^T were 73.5% and 72.2% respectively, and the dDDH value were 22.9% and 21.2% respectively (Table S1). Both of them were lower than the species differentiation thresholds of 95% and 70%, respectively (Thompson *et al.* 2013; Meier-Kolthoff *et al.* 2013). Furthermore, we calculated the ANI values between *Catenovulum* species and *Algibacillus agarilyticus* which are in the same node and found that the values were both less than 75. It was also useful for distinguishing strain *Algibacillus agarilyticus* from the genus *Catenovulum*.

Based on secondary metabolite analysis predicted by antiSMASH, strain 2E275^T has 5 putative biosynthetic gene clusters (BGCs), including ectoine cluster, NRPS-like cluster, RiPP-like biosynthetic

cluster, one arylpolyene region and one hserlactone region, which suggested strain 2E275^T might synthesize these active compounds. The similarity between ectoine and the known cluster was 83%. We also found that strain 2E275^T shares four secondary metabolites with its most similar strain *C. agarivorans* YM01^T. Furthermore, 2,187 entries (56.3%) were annotated and assigned to functional categories by KEGG. The genes *narB*, *narD*, *narG*, *narH* and *narL* have been predicted, and the annotation results showed that strain 2E275^T had dissimilatory nitrate reduction metabolism pathway. During the dissimilatory nitrate reduction, nitrate was reduced to nitrite under the function of genes *narG*, *narH* and *narL*, and then to ammonia under the action of genes *narB* and *narD*. In addition, strain 2E275^T contained the complete pathway of assimilatory sulfate reduction. During this reaction, sulfate is reduced to H_2S.

For the polysaccharides utilization, the genome of strain 2E275^T has been analyzed by the CAZy database. Strain 2E275^T contains 117 carbohydrate-active enzymes, glycoside hydrolases (GHs) are the largest number of enzymes. In the GH family, there are one *α*-agarase (EC 3.2.1.158) and four *β*-agarases (EC 3.2.1.81). *α*-agarase belongs to GH96 family and *β*- agarases belong to GH50 family. According to report, they can hydrolyze the *α*-1,3 glycosidic bond and *β*-1,4 glycosidic bond between two agarbiose respectively (Michel *et al.* 2006). This discovery could explain why strain 2E275^T can degrade agar significantly (Fig. 2).

Physiology

Cells of strain 2E275^T were Gram-stain-negative, strictly aerobic, rod-shaped and motile by means of a single polar flagellum. Strain 2E275^T was approximately 0.4-1.0 μm width and 1.0–3.0 μm length (Fig. S2). Strain 2E275^T could grow at 4–42°C (optimum, 37°C), at pH 5.5–10.5 (optimum, pH 6.5-7.0) and with 0–12% (*w/v*) NaCl (optimum, 3%). Strain 2E275^T hydrolyze Tweens (20, 60 and 80), starch, agar, alginate, and cellulose, but not DNA and Tween 40. Strain 2E275^T was positive for catalase and oxidase. Nitrate is reduced to ammonia. Strains 2E275^T was resistant to cefotaxime sodium (30 μg), vancomycin (30 μg), gentamycin (10 μg), ampicillin (10 μg), chloramphenicol (30 μg), ceftriaxone (30 μg), ofloxacin (5 μg), norfloxacin (10 μg), tobramycin (10 μg), erythromycin (15 μg), rifampin (5 μg), penicillin (10 μg), clarithromycin (15 μg), kanamycin (30 μg), but susceptible to streptomycin (10 μg), neomycin (30 μg), tetracycline (30 μg), lincomycin (2 μg). Strains 2E275^T could be distinguished from the closely related strains by a few physiological and biochemical characteristics, including the results of API and Biolog tests (Table 1). The complete morphological, physiological and biochemical characteristics of strains 2E275^T were provided in the species description and Table 1.

Chemotaxonomy

Fatty acid analyses revealed that the major fatty acids of strain 2E275^T were summed feature 3 (42.1%, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{16:0}$ (26.1%) and summed feature 8 (18.4%, $C_{18:1}\omega7c$). Although *C. agarivorans* YM01^T and *C. sediminis* D2^T share the same major cellular fatty acids summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), summed feature 8 ($C_{18:1}\omega7c$) and $C_{16:0}$ with strain 2E275^T, the proportion of

the major cellular fatty acids differentiate strain 2E275^T from the others, as shown in Table 2. Ubiquinone-8 was detected as the predominant respiratory quinone of strain 2E275^T, which is consistent with other *Catenovulum* strains. The polar lipids of strains 2E275^T were phosphatidylglycerol (PG), phosphatidylethanolamine (PE), one phosphoaminolipid (PN), two unidentified phospholipids (PL) and three unknown lipids (Fig. S3).

Description of Catenovulum algicola sp. nov.

Catenovulum algicola (*al.gi'co.la. L. fem. n. alga, seaweed, alga; L. masc./fem. n. suff. -cola, inhabitant, dweller; N.L. masc./fem. n. algicola, alga-dweller*).

Cells are Gram-stain-negative, motile by means of a single polar flagellum, strictly aerobic. Cells are straight to slightly curved rods, 0.4–1.0 μm in width and 1.0–3.0 μm in length. Colonies on MA are pale white colored, smooth, circular and 1.0–1.5 mm in diameter after 2 days incubation at 37°C. Growth occurs at 4–42°C, pH 5.5–10.5 and in the presence of 0–12% (*w/v*) NaCl. Optimal growth is observed at 37°C, pH 6.5-7.0 and in the presence of 3% (*w/v*) NaCl. Nitrate is reduced to nitrite. The genomic DNA G + C content of the type strain is 38.2%. Agar, starch, alginate, Tweens (20, 60 and 80), cellulose are hydrolysed, but DNA and Tween 40 are not. Cells are positive for catalase, oxidase activity, but negative for utilization of citrate, indole and H_2S production. Voges–Proskauer reaction are positive, glucose can be fermented. Alkaline phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidases, acid phosphatases, *α*-glucosidase, *α*-galactosidase and naphthol-AS-BI-phosphohydrolase are present, but lipase (C14), valine arylamidases, cystine aminopeptidase, trypsin, chymotrypsin, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, urease, gelatinase, tryptophan deaminase, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, N-acetyl-*β*-glucosaminidase, *α*-mannosidase and *β*-fucosidase are absent. Acid can be produced from l-arabinose, d-xylose, methyl *β*-d-Xylopyranoside, d-xylose, d-glucose, d-fructose, d-mannose, l-Rhamnose, inositol, amygdalin, d-cellobiose, d-maltose, d-lactose, d-melibiose, d-sucrose, inulin, d-raffinose, d-turanose, d-melezitose, salicin, glycogen, starch, d-gentiobiose, d-lyxose and potassium 5-ketogluconate. According to Biolog GEN III MicroPlates assays, strain 2E275^T can utilize the following substrates as carbon and energy sources: Dextrin, d-Maltose, d-Cellobiose, Gentiobiose, Sucrose, d-Turanose, Stachyose, d-Raffinose, *α*- d-Lactose, d-Melibiose, *β*-Methyl- d-Glucoside, d-Salicin, *α*-d-Glucose, d-Mannose, d-Fructose, d-Galactose, 3-Methyl Glucose, d-Fucose, l-Fucose, l-Rhamnose, d-Fructose-6-PO_4, l-Histidine, Pectin, d-Galacturonic, l-Galactonic, d-Gluconic Acid, d-Glucuronic Acid, Glucuronamide, *α*-Keto-Glutaric Acid, Acetoacetic Acid. The predominant isoprenoid quinone is ubiquinone-8. The polar lipids are PE and PG as major components, two unidentified phospholipids (PL), aminophospholipid (PN) and three unidentified lipids (L) as moderate to minor components. The major cellular fatty acids are summed feature 3 ($C_{16:1}$ω7c and/or $C_{16:1}$ω6c), summed feature 8 ($C_{18:1}$ω7c) and $C_{16:0}$.

The type strain, 2E275^T (= MCCC 1H00528^T = KCTC 92643^T), was isolated from kelp seedlings collected from a kelp nursery pond of Weihai, China.

Abbreviations

MA, Marine Agar 2216; MB, Marine Broth 2216; MCCC, Marine Culture Collection of China; KCTC, Korea Collection for Type Culture; MEGA, Molecular Evolutionary Genetics Analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; CAPSO, 3-cyclohexylamino-2-hydroxypropanesulfonic acid sodium salt; HEPES, N-(2hydroxyethyl) piperazine-N'-2-ethanesulfonic acid; PIPES, Piperazine-1,4-bisethanesulfonic acid; MES, 2-(N-Morpholino) ethanesulfonic acid; HPLC, high-performance liquid chromatography; TLC, Thin Layer Chromatography.

The GenBank accession number for the 16S rRNA gene sequences of 2E275 is OP493183. The Whole Genome Shotgun projects of 2E275 has been deposited at DDBJ/ENA/GenBank under the accession numbers JAOPFU000000000.

Declarations

Funding information

This work was supported by Science & Technology Fundamental Resources Investigation Program (Grant No. 2022FY101100, 2019FY100700) and the National Natural Science Foundation of China (32070002).

Data availability

The sequences of 16S rRNA gene and genome of strain 2E275^{T} have been deposited in GenBank/EMBL/DDBJ with the accession numbers OP493183 and JAOPFU000000000, respectively.

Acknowledgements

Scanning electron microscopy of this work was supported by Physical-Chemical Materials Analytical & Testing Center of Shandong University at Weihai.

Conflicts of interests and ethical statements

The authors declare that they have no conflict of interest.

Author contributions

RYC: investigation, performed research, data analyses, writing—original draft, writing—review and editing, project administration. YYS: investigation and writing—review and editing. YG: project administration and writing—review and editing. ZJD: funding acquisition. All authors read and approved the final manuscript.

References

1. **Yan S, Yu M, Wang Y, Shen C, Zhang XH** (2011) Catenovulum agarivorans gen. nov., sp. nov., a peritrichously flagellated, chain-forming, agar-hydrolysing gammaproteobacterium from seawater. *Int J Syst Evol Microbiol* 61:2866-2873. http://doi.org/10.1099/ijs.0.027565-0
2. **Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M** (2020). List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. International journal of systematic and evolutionary microbiology, 70(11), 5607–5612. https://doi.org/10.1099/ijsem.0.004332
3. **Yan S, Yu M, Wang Y, Shen C, Zhang XH** (2011) Catenovulum agarivorans gen. nov., sp. nov., a peritrichously flagellated, chain-forming, agar-hydrolysing gammaproteobacterium from seawater. Int J Syst Evol Microbiol 61(Pt 12):2866-2873. doi:10.1099/ijs.0.027565-0
4. **Li DQ, Zhou YX, Liu T, Chen GJ, Du ZJ** (2015) Catenovulum maritimus sp. nov., a novel agarolytic gammaproteobacterium isolated from the marine alga Porphyra yezoensis Ueda (AST58-103), and emended description of the genus Catenovulum. *Antonie Van Leeuwenhoek* 108:427-434. http://doi.org/10.1007/s10482-015-0495-2
5. **Shi MJ, Du WJ, Wang C, Chen GJ, Du ZJ** (2017) Catenovulum sediminis sp. nov., isolated from coastal sediment. *Int J Syst Evol Microbiol* 67:3894-3898. http://doi.org/10.1099/ijsem.0.002219
6. **Lane DJ** (1991) 16S/23S rRNA Sequencing. Nucleic Acid Techniques in Bacterial Systematics. In: Stackebrandt E, Goodfellow M (editors). *Nucleic acid techniques in bacterial systematics*. New York: Wiley, pp. 115-175.
7. **Yoon SH, Ha SM, Kwon S, Lim J, Kim Y et al** (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 67:1613-1617. http://doi.org/10.1099/ijsem.0.001755
8. **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 analysis tools. *Nucleic Acids Res* 25:4876-4882. http://doi.org/10.1093/nar/25.24.4876
9. **Saitou N, Nei M** (1987) The Neighbor-Joining Method - a New Method for Reconstructing Phylogenetic Trees. *Mol Biol Evol* 4:406-425.
10. **Fitch WM** (1971) Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 20:406-416.
11. **Kumar S, Stecher G, Li M, Knyaz C, Tamura K** (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35:1547-1549.
12. **Felsenstein J** (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Soc Study Evol* 39:783-791. http://doi.org/j.1558-5646.1985.tb00420.x
13. **Li RQ, Li YR, Kristiansen K, Wang J** (2008) SOAP: short oligonucleotide alignment program. *Bioinformatics* 24:713-714. http://doi.org/10.1093/bioinformatics/btn025
14. **Li RQ, Zhu HM, Ruan J, Qian WB, Fang XD et al** (2010) De novo assembly of human genomes with massively parallel short read sequencing. *Genome Res* 20:265-272. http://doi.org/10.1101/gr.097261.109

15. **Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D et al** (2008) Toward an online repository of Standard Operating Procedures (SOPs) for (meta)genomic annotation. *OMICS* 12:137-141. http://doi.org/10.1089/omi.2008.0017
16. **Lee I, Ouk Kim Y, Park SC, Chun J** (2016) OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 66:1100-1103. http://doi.org/10.1099/ijsem.0.000760
17. **Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Goker M** (2022) TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Res* 50:D801-D807. http://doi.org/10.1093/nar/gkab902
18. **Aziz RK, Bartels D, Best A, DeJongh M, Disz T et al** (2008) The RAST Server: Rapid annotations using subsystems technology. *BMC Genomics* 9:75. http://doi.org/10.1186/1471-2164-9-75
19. **Blin K, Shaw S, Kloosterman AM, Powers ZC, Weezel GP et al** (2021) antiSMASH 6.0: improving cluster detection and comparison capabilities. *Nucleic Acids Research* 49:W29–W35. http://doi.org/10.1093/nar/gkab335
20. **Kanehisa M, Sato Y, Morishima K** (2016) BlastKOALA and GhostKOALA: KEGG Tools for Functional Characterization of Genome and Metagenome Sequences. *J Mol Biol* 428:726-731. http://doi.org/10.1016/j.jmb.2015.11.006
21. **Zhang H, Yohe T, Huang L, Entwistle S, Wu P et al** (2018) dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. *Nucleic Acids Res* 46:W95-W101. http://doi.org/10.1093/nar/gky418
22. **Thompson CC, Chimetto L, Edwards RA, Swings J, Stackebrandt E et al** (2013) Microbial genomic taxonomy. *BMC Genomics* 14:913. http://doi.org/10.1186/1471-2164-14-913
23. **Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M** (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:60. http://doi.org/10.1186/1471-2105-14-60
24. **Michel G, Nyval-Collen P, Barbeyron T, Czjzek M, Helbert W** (2006) Bioconversion of red seaweed galactans: a focus on bacterial agarases and carrageenases. *Appl Microbiol Biotechnol* 71:23-33. http://doi.org/10.1007/s00253-006-0377-7
25. **Bernardet JF, Nakagawa Y, Holmes B, Subcommittee On The Taxonomy Of F, Cytophaga-Like Bacteria Of The International Committee On Systematics Of P** (2002) Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family. *Int J Syst Evol Microbiol* 52:1049-1070. http://doi.org/10.1099/00207713-52-3-1049
26. **Bowman JP** (2000) Description of Cellulophaga algicola sp nov., isolated from the surfaces of Antarctic algae, and reclassification of Cytophaga uliginosa (ZoBell and Upham 1944) Reichenbach 1989 as Cellulophaga uliginosa comb. nov. *Int J Syst Evol Micr* 50:1861-1868. http://doi.org/Doi 10.1099/00207713-50-5-1861
27. **Cowan ST, Steel KJ** (1974) *Bacterial characters and characterization*, 2nd ed. Cambridge: Cambridge University Press.

28. **Wang ZJ, Liu QQ, Zhao LH, Du ZJ, Chen GJ** (2015) Bradymonas sediminis gen. nov., sp. nov., isolated from coastal sediment, and description of Bradymonadaceae fam. nov. and Bradymonadales ord. nov. *Int J Syst Evol Microbiol* 65:1542-1549. http://doi.org/10.1099/ijs.0.000135
29. **CLSI** (2012) *Performance standards for antimicrobial susceptibility testing, Twenty-Second Informational Supplement CLSI document M100-S22*. Clinical and Laboratory Standards Institute.
30. **Athalye M, Noble WC, Minnikin DE** (1985) Analysis of cellular fatty acids by gas chromatography as a tool in the identification of medically important coryneform bacteria. *J Appl Bacteriol* 58:507-512. http://doi.org/10.1111/j.1365-2672.1985.tb01491.x
31. **Tindall BJ, Sikorski J, Smibert RM, Krieg NR** (2007) *Phenotypic characterization and the principles of comparative systematics*, 3rd ed. Washington DC: ASM.
32. **Hiraishi A, Ueda Y, Ishihara J, Mori T** (1996) Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* 42:457-469. http://doi.org/10.2323/jgam.42.457
33. **Komagata K, Suzuki KI** (1988) 4 Lipid and Cell-Wall Analysis in Bacterial Systematics. *Methods Microbiol* 10:161–207.
34. **Minnikin DE, Odonnell AG, Goodfellow M, Alderson G, Athalye M et al** (1984) An Integrated Procedure for the Extraction of Bacterial Isoprenoid Quinones and Polar Lipids. *J Microbiol Meth* 2:233-241. http://doi.org/10.1016/0167-7012(84)90018-6

Tables

Table 1

Differential characteristics between strain 2E275^T and type strains of related species of the genus *Catenovulum* Strains: 1. 2E275^T; 2. *C. agarivorans* YM01^T; 3. *C. sediminis* D2^T. All data were from this study, except for the labeled data. All three strains are Gram negative, strictly aerobic, positive for oxidase and have flagellum. The predominant menaquinone detected of all is Q-8. All strains are positive for the following characteristics: hydrolysis of starch and agar, enzyme activity of oxidase, Alkaline phosphatase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase, assimilation of glucuronamide. All strains are negative for the following characteristics: enzyme activity of lipase (C14), trypsin, chymotrypsin, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, *α*-mannosidase and *β*-fucosidase. +, positive; -, negative; Data from: a, Yan *et al.* [1]; b, Shi *et al.* [2].

Characteristic	**1**	**2**	**3**
Ranges (optima) for growth			
Temperature (°C)	4–42 (37)	10–42 (28)[a]	10–42 (37)[b]
NaCl (%, w/v)	0–12.0 (3.0)	0.5–3.0[a]	0.5–8[b]
pH	5.5–10.5 (6.5-7.0)	7.5–10.0 (8.0–9.0)[a]	6.0–9.0 (7.5–8.0)[b]
Catalase reaction	+	+[a]	-[b]
Nitrate reduction	+	+[a]	-[b]
Hydrolysis of			
Cellulose	+	+[a]	-[b]
Tween 80	+	+[a]	-[b]
Enzyme activity			
esterase (C4)	+	+	-
esterase lipase (C8)	+	+	-
valine arylamidase	-	-	+
Acid phosphatase	+	+	+
α-glucosidase	+	-	-
α-Galactosidase	+	+	-
Acid production from			
d-xylose	+	-	+
d-turanose	+	-	+
d-melibiose	+	+	-
d-sucrose	+	+	-

Characteristic	1	2	3
Ranges (optima) for growth			
Amygdalin	+	+	-
d-glucose	+	+	-
potassium 5-ketogluconate	+	-	+
d-ribose	-	-	+
l-sorbose	-	-	+
aesculin	-	-	+
d-tagatose	-	+	+
inulin	+	-	-
d-raffinose	+	-	-
d-melezitose	+	-	-
salicin	+	-	-
glycogen	+	-	-
starch	+	-	-
d-gentiobiose	+	-	-
d-lyxose	+	-	-
d-cellobiose	+	-	-
d-maltose	+	-	-
d-lactose	+	-	-
l-arabinose	+	-	-
methyl *β*-d-Xylopyranoside	+	-	-
d-fructose	+	-	-
l-rhamnose	+	-	-
inositol	+	-	-
DNA G + C content (%)	38.2	44.8[a]	40.4 mol[b]

Table 2
Cellular fatty acid contents (%) of strains 2E275^T and related species Strains: 1, 2E275^T; 2, *C. agarivorans* YM01^T; 3, *C. sediminis* D2^T. All data were obtained from this study. Fatty acids representing < 1.0% in all strains tested are not shown. TR < 1.0%; -, not detected.

Fatty acid	**1**	**2**	**3**
$C_{12:0}$	1.7	1.5	1.2
$C_{16:0}$	26.1	28.7	26.5
$C_{18:0}$	TR	1.2	1.6
$C_{10:0}$ 3-OH	6.0	5.6	6.3
Summed Feature*			
2	1.0	TR	TR
3	42.1	43.1	44.8
8	14.4	15.8	12.6

*Summed Features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 2 contained iso-$C_{16:1}$ and/or $C_{14:0}$ 3-OH; summed feature 3 contained $C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c; summed feature 8 contained $C_{18:1}$ ω7c.

Figures

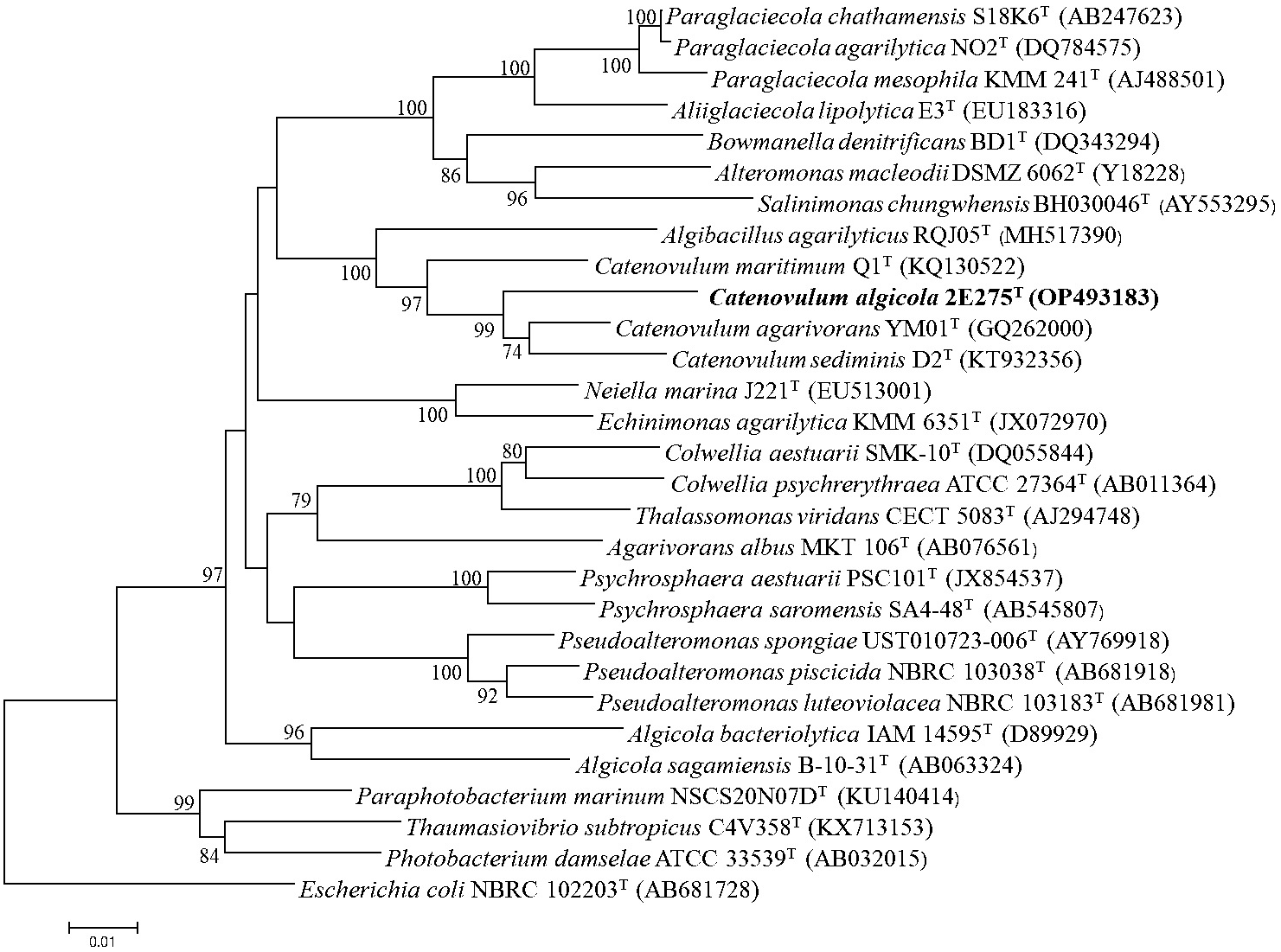

Figure 1

Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain 2E275^T and other related species

Only bootstrap values (expressed as percentages of 1,000 replications) of >70% are shown at branching points. *Escherichia coli* NBRC 102203^T (AB681728) is used as an out group. Scale bar 0.01 substitutions per nucleotide position. The two numbers at the node in the figure legend represent bootstrap values of neighbor-joining and maximum-likelihood algorithms respectively.

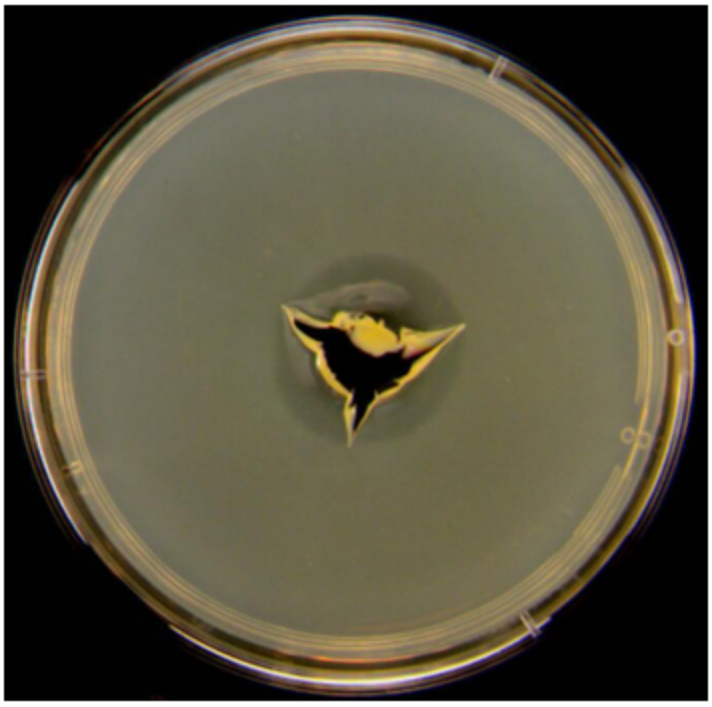

Figure 2

Agar utilization by strain 2E275 after 3days of growth on MA

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementary..docx