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Oceanimonas smirnovii sp. nov., a novel organism isolated from the Black Sea

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Abstract

A slightly creamy, melanogenic, Gram-negative, aerobic bacterium was isolated from seawater sample collected in the Karadag Natural Reserve of the Eastern Crimea, the Black Sea. The novel organism was chemoorganotrophic, had no obligate requirement in NaCl, tolerated to 12% NaCl, grew between 10 and 45 °C, was slightly alkaliphilic, and was not able to degrade starch, gelatin, agar, and Tween 80. 16S rRNA gene sequence-based analyses of the new organism revealed that *Oceanimonas doudoroffii* ATCC 27123^T, *Oceanimonas baumanii* ATCC 700832^T, and *Oceanisphaera litoralis* DSM 15406^T were the closest relatives (similarity around 97%–96%). The G+C content of the DNA of the strain 31-13^T was 55.5 mol%. Phosphatidylethanolamine (49.0%), phosphatidylglycerol (41.8%), and diphosphatidylglycerol (9.2%) were the predominant phospholipids. The major fatty acids were 16:0 (24.1%), 16:1ω7 (40.3%), and 18:1ω7 (29.2%). On the basis of the significant differences demonstrated in the phenotypic and chemotaxonomic characteristics, it is suggested that the bacterium be classified as a novel species; the name *Oceanimonas smirnovii* sp. nov. is proposed. The type strain is 31-13^T (UCM B-11076^T = LMG 22147^T = ATCC BAA-899^T).

Keywords: *Oceanimonas smirnovii*; The Black Sea; Seawater

Introduction

In 2001, Brown et al. proposed to create a genus *Oceanimonas* for accommodation of the new phenol degrading bacterium *Oceanimonas baumannii* and [*Pseudomonas*] *doudoroffii* [6,16]. The taxonomic status and

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phylogenetic relationships within 'Gammaproteobacteria' of the latter species for long time remained unclear because of its initial phenotypic misclassification as close relative of *Aeromonas hydrophila* and *Tolumonas auensis* [2–3,6,20].

In this study, we report on the characterization of new mesophilic bacterium of the genus *Oceanimonas* isolated from seawater sample collected in the Karadag Natural Reserve of the Eastern Crimea. This work was part of a taxonomic survey of free-living microbial populations of the Black Sea. During the course of this work 51 *Alteromonas*-like strains of different phenotypes were isolated. The majority of the strains had most of the *Pseudoalteromonas* phenotypic features, while a few had distinct phenotypes. Further phylogenetic analysis revealed that one of such strains, 31-13^T, showed close phylogenetic association with *Oceanimonas* spp, Pheno-, chemotaxonomic, genetic, and detailed phylogenetic analyses confirmed this conclusion and allowed to assign this organism to a new species, for which we propose the name *Oceanimonas smirnovii*.

Materials and methods

Isolation procedure, bacterial strain, and growth conditions

Water sample was collected in July 2000 from a depth of 1–3 m (salinity, 17‰, temperature, 16 °C) using a standard hydrological plastic bathometer in the Karadag Natural Reserve of the Eastern Crimea (a central part of the Black Sea coastal line) in July 2000. Samples were kept at 4 °C and processed within 4–8 h. A portion of seawater (0.1 ml) was plated onto marine agar 2216 (Difco) or medium B which contained 0.2% (w/v) Bacto peptone (Difco), 0.2% (w/v) casein hydrolysate (Merck), 0.2% (w/v) Bacto yeast extract (Difco), 0.1% (w/v) glucose, 0.02% (w/v) KH_2PO_4, 0.005% (w/v) $MgSO_4 \cdot 7H_2O$, 1.5% (w/v) Bacto agar (Difco), 50% (v/v) of natural seawater, and 50% (v/v) distilled water at pH 7.8. Plates were incubated aerobically at room temperature (ca. 22–25 °C) for 5–7–10 days. The isolation and purification of the bacterial strains has been described elsewhere [12]. Strains isolated were stored at −80 °C in marine 2216 broth (Difco) supplemented with 20% (v/v) of glycerol.

Physiological analysis

Unless otherwise indicated, the phenotypic properties used for characterization of *Alteromonas*-related species were tested following established procedures described previously and hence the results obtained are comparable with those published previously [2,12,13,21]. The range of pH for growth was studied with Marine agar (Difco) adjusted to various pH values: pH 6.0 (by adding NCl), pH 7.0 (addition of 1.0 M sodium phosphate buffer, pH 7.0), pH 8.0 (addition of 100 ml 1.0 M Na_2HPO_4), pH 9.0 (addition of 1 M $NaHCO_3$), pH 10.0 (addition of 1 M sodium sequicarbinate buffer, Na_2CO_3, pH 10). Starch, casein and gelatin hydrolysis was tested by the methods of Smibert and Krieg [21]. Degradation of macromolecules by the strains was tested with medium B. Chitin (1% w/v), elastin (0.1% w/v) and alginate (sodium salt) (0.1% w/v) hydrolysis was determined by development of clear zones around the colonies. Growth at different temperatures, NaCl concentrations or pH was measured at an optical density at 660 nm following 24-h incubation in the appropriate medium. Incubation temperatures ranged from 4 to 45 °C. NaCl concentrations used were 0% to 10%. Cultures were incubated on a rotary shaker at 160 rpm for 24–36 h at 28 °C. Assimilation of carbon compounds was determined as described elsewhere [12]. Production of brown, soluble pigment was studied as described previously [12]. Susceptibility to antibiotics was tested by the routine diffusion plate method, employing medium B agar and disks (Oxoid) impregnated with the following antibiotics: kanamycin (30 μg), ampicillin (10 μg), benzylpenicillin (10 μg), streptomycin (30 μg), gentamicin (10 μg), lincomycin (15 μg), neomycin (30 μg), polymyxin B (25 μg), tetracycline (30 μg). Agar plates were seeded with light lawn of bacteria and incubated at 28 °C for 24 h. A distinct inhibition zone indicated susceptibility to the antibiotic.

Cellular morphology was examined by phase contrast light microscopy of 24 h old cultures grown on agar plates. Electron micrographs of negatively stained cells were prepared using a Zeiss EM 10 CA electron microscope (80 kV). A drop of particle-free (autoclaved and ultracentrifuged), distilled water was placed on the bacterial growth of 24-h-culture. The sample (30 μl) of resulting bacterial suspension was applied to carbon- and formvar-coated 400-mesh copper grids, a drop of 1.25% uranyl acetate was added, and the bacteria were allowed to adhere for 1 min at room temperature. Superfluous liquid was gently removed by using a piece of filter paper.

Antibacterial activity was performed by the agar diffusion assay, described elsewhere [12]. Antimicrobial activities were tested against *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 885-653, phyto-pathogenic fungi *Penicillium chrysogenum* 001125Z (= UCMF-57627) and *Cohliobolus sativus* 000748Z (= UCMF-11224), and cyanobacteria *Synechocystis minuscula* (UCM A-14).