Paracoccus seriniphilus sp. nov., an L-serine-dehydratase-producing coccus isolated from the marine bryozoan Bugula plumosa

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A novel marine Gram-negative, non-motile, non-spore-forming, aerobic bacterium, associated with the bryozoan Bugula plumosa, was isolated in a screening programme for strains containing enzymes able to convert the amino acid L-serine. Strain MBT-A4 T produced L-serine dehydratase and was able to grow on L-serine as the sole carbon and nitrogen source. The nearest phylogenetic neighbour was Paracoccus marcusii, as determined by 16S rDNA sequence analysis (97.6 % similarity). The DNA–DNA reassociation value obtained for Paracoccus marcusii DSM11574 T and MBT-A4 T was 32.6 %. The major ubiquinone was Q-10. Based on genotypic, chemotaxonomic and physiological characteristics, a new species of the genus Paracoccus is proposed, Paracoccus seriniphilus sp. nov., the type strain being strain MBT-A4 T (= DSM 14827 T = CIP 107400 T ).

The development of molecular methods has led to the recognition of high marine microbial diversity, which can be used as a resource for the detection of novel marine natural products. Marine invertebrates and their cultivatable bacterial associates have become a focal point for marine natural products research. Bacteria which are symbiotic or able to colonize surfaces in marine waters have been shown to produce secondary metabolites (Holmström & Kjelleberg, 1999; Davidson et al., 2001). Also, the formation of biofilms has important implications for biochemical reactions and ecological functions in a specific ecological niche (Dagostino et al., 1991; Sternberg et al., 1999). Here, we report on the isolation and characterization of a novel bacterial strain, MBT-A4 T , isolated from colonies of the North Sea bryozoan Bugula plumosa.

Isolation and morphology of strain MBT-A4 T

Bryozoan samples were taken in August 1999 by divers from the south pier of the entrance harbour of the island Helgoland. Sampling depth was 7 m and samples were placed directly into polyethylene bags containing seawater from the local site. The temperature of the seawater was 17.5 °C. Samples of Bugula plumosa were washed three times for 30 min in sterile artificial sea water (Sea Salts; Sigma). For extraction of bacteria from the surface of a Bugula plumosa colony, samples (1 g) were placed into a sterile plastic bag (Seward Medical) containing 9 ml artificial seawater, and treated in a Stomacher Lab blender (Seward Medical) twice for 120 s at the highest speed. Aliquots of the resultant bacterial suspension were used as the inoculum for an enzyme screening. The L-serine basal medium used for screening experiments consisted of L-serine, 3 g l⁻¹; Sea Salts, 34.3 g l⁻¹; 1 ml l⁻¹ trace element solution containing (l⁻¹): 0.1 g ZnSO₄·7H₂O, 0.03 g MnCl₂·4H₂O, 0.3 g H₂BO₃, 0.2 g CoCl₂·2H₂O, 0.01 g CuCl₂·2H₂O, 0.02 g NiCl₂·6H₂O, 0.03 g Na₂MoO₄·2H₂O; 1 ml l⁻¹ vitamin solution containing (l⁻¹): 60 mg m-inositol, 30 mg calcium pantothenate, 60 mg thiamine.HCl (B₁); 150 μg pyridoxol.HCl (B₆), 30 μg biotin. Experiments were performed in microplates (TechnoPlastic). L-Serine minimal medium (200 μl) was inoculated with 4 μl bacterial suspension extracted from Bugula plumosa. The plate was incubated at 37°C using an Eppendorf thermal shaker. After 6 days, growth was observed in some wells, and strains able to use L-serine as their sole energy source were subcultured on L-serine basal agar medium to obtain pure cultures.

Cells of strain MBT-A4 T were Gram-negative, non-spore-forming, aerobic, non-motile and catalase- and oxidase-positive. They were able to grow on marine agar (Difco), forming smooth, circular, colourless to creamy white colonies which were 1–2 mm in diameter. Strain MBT-A4 T was capable of aerobic growth on L-serine as the sole carbon and nitrogen source, and produced an L-serine dehydratase (EC 4.2.1.13). This enzyme catalyses the irreversible non-oxidative deamination of L-serine to equimolar amounts
of pyruvate and ammonia. The name dehydratase originates from the first partial reaction, which is a dehydration. This \( \beta \)-elimination is followed by the tautomerization of the aminoacrylate and hydrolysis of the resulting imine.

**Microscopy**

Cells were observed by using an Olympus BH-2 microscope equipped with phase-contrast optics. Furthermore, bacteria grown on liquid broth (yeast extract/peptone medium, see Physiological characteristics) were adsorbed onto a sterile filter after reaching the mid-exponential growth phase, and fixed with 3 % glutaraldehyde in cacodylate buffer (0.1 M, pH 7.2) for 1 h at room temperature (Robinson et al., 1984). The samples were washed, dehydrated with a graded series of ethanol, critical-point-dried and sputter-coated with a gold film; samples were then examined in a Digital Scanning Microscope DSM 940 (Zeiss). Cells were cocci and short rods, 0.5–1 x 0.7–1 \( \mu m \) in size (Fig. 1) and grew as single cells or as pairs to short chains. Aggregation was observed in cultures grown in minimal medium.

**Phylogenetic analyses**

Genomic DNA was extracted from the bacteria and purified as described previously (Pukall et al., 1998). The primer pair 27f (5’-GAGTTTGATCCTGGCTCAG-3’) and 1385r (5’-CGGTGTGTRCAAGGCCC-3’) was used for amplification of the 16S rRNA gene (Lane, 1991). PCR amplification of 16S rDNA was done as described previously (Pukall et al., 1999). Analysis of the 16S rDNA sequence obtained from isolate MBT-A4\(^T\) followed the method described by Rainey et al. (1996), using a Taq DyeDeoxy Terminator Cycle Sequencing kit and a model 373A automated DNA sequencer (both Applied Biosystems). The sequence was manually aligned and compared with published sequences from the DSMZ 16S rDNA database, which consists of more than 6000 sequence entries, including those from the Ribosomal Database Project (Maidak et al., 2001) and EMBL. Similarity values were transformed into phylogenetic distances that compensate for multiple substitutions at any given site in the sequence (Jukes & Cantor, 1969). The algorithm of DeSoete (1983) and the neighbour-joining method contained in the PHYLIP package (Felsenstein, 1993) were used to construct phylogenetic dendrograms. All analyses were done on a SUN SparcII workstation. 16S rDNA sequence analysis indicated that strain MBT-A4\(^T\) was affiliated to the genus *Paracoccus* within the Rhodobacter group of the \( \alpha \)-subclass of the Proteobacteria. The highest similarity values were 97.6, 97.4 and 97.1\% with sequences of *Paracoccus marcusii*, *Paracoccus carotinifaciens* and *Paracoccus alcaliphilus*, respectively, while similarities with type strains of other *Paracoccus* species ranged from 91.9 to 96.8\%. Dendrograms of 16S rDNA relationships obtained with the two different treeing algorithms were almost identical. The position of strain MBT-A4\(^T\) relative to its phylogenetic neighbours is shown by neighbour-joining analysis (Fig. 2).

**DNA G+C content and DNA–DNA hybridization**

Isolation of DNA (Cashion et al., 1977) and determination of the DNA G+C content by HPLC (Mesbah et al., 1989)
followed described procedures. DNA–DNA similarity studies were performed by using the renaturation method (Escarà & Hutton, 1980; Hüb et al., 1983). Hybridization conditions were as follows: 2 × SSC, 10 % DMSO, 69 °C. Similarity values were calculated according to the method of Jahnke (1992). Determination of DNA relatedness between strain MBT-A4ᵀ and its closest phylogenetic relative, *Paracoccus marcusii* DSM 11574ᵀ, revealed a low DNA–DNA similarity of 32-6 %. The G + C content of the DNA was 63·3 mol%.

**Chemotaxonomic analyses**

Fatty acids were determined in cells grown in TSB medium. Fatty acid methyl esters were obtained from freeze-dried biomass (approx. 10 mg) by saponification, methylation and extraction using the modifications (Kuykendall et al., 1988) of the method of Miller (1982). The fatty acid methyl ester mixtures were separated using the 5898A Microbial Identification System (Microbial ID), which consisted of a model 3392 gas chromatograph fitted a with 5 % phenyl–methyl silicone capillary column (0·2 mm × 25 m), a flame-ionization detector, a model 7673A automatic sampler and a model Kayak XA computer (all from Hewlett Packard). Peaks were integrated automatically, and fatty acid composition was determined by the identification system. The gas-chromatographic parameters were as follows: carrier gas, ultrahigh-purity hydrogen; column head pressure, 60 kPa; injection volume, 2 μl; column split ratio, 100:1; septum purge, 5 ml min⁻¹; column temperature, 170–270 °C at 5 °C min⁻¹; injection port temperature, 250 °C; and detector temperature, 300 °C. Isoprenoid quinones were extracted from strain MBT-A4ᵀ grown in marine broth (Difco), as described by Collins (1985). Analysis was performed by HPLC (Groth et al., 1996) and the ubiquinone type was determined by using a QP2000 mass spectrometer fitted with a direct sample inlet device DI20 (Shimadzu) as given by Collins (1994).

The major fatty acid for strain MBT-A4ᵀ, detected by the Microbial ID system, was C₁₈:₁t07c (83-46 %), whereas other fatty acids were detected in smaller amounts as follows: C₁₀:₀ 3-OH (7-92 %), C₁₈:₀ (2-51 %) and 11-methyl-C₁₈:₁t07c (1-48 %). This profile is characteristic of members of the α-subclass of the Proteobacteria, including members of the genus *Paracoccus* (Kelly et al., 2000). The amounts of the major fatty acid C₁₈:₁ detected in *Paracoccus marcusii* DSM 11574ᵀ and *Paracoccus carotinifaciens*IFO 16211ᵀ were 79-4 and 82·0 %, respectively (Harker et al., 1998; Tsubokura et al., 1999). The major isoprenoid quinone in strain MBT-A4ᵀ was ubiquinone Q-10.

**Physiological characteristics**

Routinely, a complex medium containing 5 g peptone l⁻¹, 5 g yeast extract l⁻¹ and 34·3 g Sea Salts l⁻¹ was used for bacterial cultivation and analysis of physiological characteristics. To induce L-serine dehydratase activity, 1 g filter-sterilized L-serine l⁻¹ was added after autoclaving. The temperature range for growth was determined by incubating the inoculated medium (pH 7-0) at 18, 20, 25, 28, 35 and 40 °C. Growth was assessed at pH 4–11. Furthermore, the NaCl requirement was tested using 1–9 % NaCl: for this, NaCl content was adjusted by reduction of the amount of sea salts added to the medium (pH 7·0), or by addition of extra NaCl. Results were recorded after 16 h incubation.

The optimal temperature for growth was 30 °C. Growth was very poor below 20 °C and above 37 °C. Growth was observed in 1–9 % NaCl, the optimum being 3 %. Strain MBT-A4ᵀ was able to grow at pH 5–10 with an optimum range of pH 6·5–8·0. Aggregates were formed at NaCl concentrations below 2 % and at low concentrations of nutrients in minimal medium (< 20 %).

The ability to utilize a variety of substrates as carbon sources was tested using the GN2 Microplates of the MicroLog system (Biolog). Bacteria were suspended in Sea Salts solution (20 g l⁻¹), and 150 μl of this suspension was added to each well. After 24 h incubation at 30 °C, reduction of the tetrazolium dye was determined by using a Microlog plate reader. Tests for the production of arginine dihydrolase, urease, β-glucosidase, protease, β-galactosidase, cytochrome oxidase, fermentation of glucose, indole production from tryptophan and the reduction of nitrites to nitrates were performed using the API 20 NE system (bioMérieux) according to the manufacturer’s instructions, with the exception that cells were resuspended in Sea Salts solution (34 g l⁻¹). Characteristics that differentiate strain MBT-A4ᵀ from other representatives of the genus *Paracoccus* are given in Table 1.

**Table 1.** Comparison of selected characteristics of *Paracoccus seriniphilus* sp. nov. with those of its nearest phylogenetic neighbours within the genus *Paracoccus*

<table>
<thead>
<tr>
<th>Character</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>Motility</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Pigmentation</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>Urease</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>β-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>NR</td>
<td>NR</td>
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<td>Growth on:</td>
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<tr>
<td>6 % NaCl</td>
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<td>–</td>
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<tr>
<td>Formate</td>
<td>+</td>
<td>+</td>
<td>NR</td>
<td>–</td>
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<td>Sucrose</td>
<td>–</td>
<td>+</td>
<td>NR</td>
<td>–</td>
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<td>Glycerol</td>
<td>+</td>
<td>+</td>
<td>NR</td>
<td>W</td>
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<td>Mannitol</td>
<td>+</td>
<td>+</td>
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<td>Inositol</td>
<td>+</td>
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<td>L-Serine</td>
<td>+</td>
<td>–</td>
<td>NR</td>
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</table>
Other properties are indicated in the species description. Strain MBT-A4\(^\text{T}\) can be differentiated from its nearest phylogenetic neighbours by pigmentation, ability to reduce nitrate, lack of \(\beta\)-glucosidase and growth on media supplemented with different carbon sources. Strain MBT-A4\(^\text{T}\) also differs from \(P.\) marcusii (Harker et al., 1998) by its inability to utilize gentiobiose, arabinose, maltose, cellobiose, lactose, trehalose, furanose or sucrose, and by its ability to utilize L-serine as a carbon and energy source.

16S rDNA sequence analysis and chemotaxonomic properties indicate membership of the genus \(Paracoccus\), and the profile of metabolic properties and low DNA–DNA reassociation with its nearest phylogenetic neighbour indicate that strain MBT-A4\(^\text{T}\) represents a new species of the genus \(Paracoccus\), for which the name \(Paracoccus\) seriniphilus sp. nov. is proposed.

**Description of \(Paracoccus\) seriniphilus sp. nov.**

\(Paracoccus\) seriniphilus (se.ri.ni’phi.lus. Gr. adj. philos loving; N.L. masc. adj. serinus loving).

Gram-negative, aerobic, non-motile, non-spore-forming cocci to rods, 0.5–1 x 0.7–1 \(\mu\)m in size. Colonies on marine agar are circular and colourless to creamy white. Growth occurs at 18–37°C with an optimum of 30°C. Grows at pH 6.5–8. NaCl is required for growth (1–9 %, optimum 3 %). Nitrate is reduced. Glucose is not fermented. No indole is produced from tryptophan. Cytochrome oxidase and \(\beta\)-galactosidase activities are present. Arginine dihydrolase, urease and \(\beta\)-glucosidase activities are absent. Gelatin hydrolysis is not detected. Grows on L-serine as sole carbon and nitrogen source. Cells in lag phase of growth tend to form aggregates. Utilizes Tween 40, Tween 80, \(N\)-acetyl-\(D\)-galactosamine, adonitol, \(D\)-arabitol, \(L\)-erythritol, \(D\)-fructose, \(D\)-galactose, \(\alpha\)-D-glucose, \(m\)-inositol, \(D\)-mannitol, \(D\)-mellibiose, \(D\)-sorbitol, xylitol, methyl pyruvate, acetic acid, \(cis\)-aconitic acid, citric acid, formic acid, \(D\)-galactonic acid lactone, \(D\)-gluconic acid, \(D\)-glucuronic acid, \(\alpha\)-hydroxybutyric acid, \(\beta\)-hydroxybutyric acid, \(\alpha\)-hydroxyphenylacetic acid, \(D\)-lactic acid, propionic acid, \(D\)-alanine, \(L\)-alanine, \(L\)-asparagine, \(L\)-aspartic acid, \(L\)-glutamic acid, \(L\)-histidine, \(L\)-leucine, \(L\)-ornithine, \(L\)-proline, \(L\)-pyroglutamic acid, \(L\)-serine, \(D\)-carnitine, \(\gamma\)-aminobutyric acid, 2,3-butanediol and glycerol. The following substrates are not utilized: \(\alpha\)-cyclodextrin, dextrin, glycogen, \(N\)-acetyl-\(D\)-galactosamine, \(L\)-arabinose, cellobiose, \(L\)-fucose, gentiobiose, \(\alpha\)-D-glucose, lactulose, maltose, \(D\)-mannose, \(\beta\)-methyl-\(D\)-glucose, \(D\)-psicose, \(D\)-raffinose, \(L\)-rhamnose, sucrose, \(D\)-trehalose, furanose, \(L\)-monomethyl succinate, \(D\)-galacturonic acid, \(D\)-glucosaminic acid, \(\gamma\)-hydroxybutyric acid, itaconic acid, \(\alpha\)-ketobutyric acid, \(\alpha\)-ketoglutaric acid, \(\alpha\)-ketovaleric acid, malonic acid, quinic acid, \(D\)-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinic amide, glucuronamide, alaninamide, \(L\)-alaninolglycine, glycyl-\(L\)-aspartic acid, glycyl-\(L\)-glutamic acid, hydroxy-\(L\)-proline, \(L\)-phenylalanine, \(L\)-serine, \(L\)-threonine, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, DL-\(\alpha\)-glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate. Ubiquinone Q-10 is the major isoprenoid quinone. In TSB medium the main fatty acid is \(C_{18:1}\)\(\gamma\)7c (\(cis\)-11-octadecenoic acid; \(cis\)-vaccenic acid). The DNA G+C content is 63.3 mol%.

Isolated from the North Sea bryozoan Bugula plumosa. Type strain is MBT-A4\(^\text{T}\) (= DSM 14827\(^\text{T}\) = CIP 107400\(^\text{T}\)).

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**References**


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