Assignment of '*Alteromonas marinoglutinosa*' NCIMB 1770 to *Pseudoalteromonas mariniglutinosa* sp. nov., nom. rev., comb. nov.

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The taxonomic position of the marine bacterium '*Alteromonas marinoglutinosa*' NCIMB 1770 was investigated in a polyphasic study. Analysis of 16S rDNA sequence and DNA–DNA reassociation values confirmed the phylogenetic position of strain NCIMB 1770 within the genus *Pseudoalteromonas* as a separate species, distinct from all *Pseudoalteromonas* species with validly described names. On the basis of physiological and molecular properties, it is proposed that strain NCIMB 1770 is classified as *Pseudoalteromonas mariniglutinosa* sp. nov., nom. rev., comb. nov., with the type strain NCIMB 1770^T (= KMM 3635^T).

The genus *Alteromonas*, originally described by Baumann *et al.* (1972), was revised (Gauthier *et al.*, 1995) with the creation of two genera: *Alteromonas* (containing a single species, *Alteromonas macleodii*) and *Pseudoalteromonas* (including the 12 remaining species that previously belonged to the genus *Alteromonas*). In the following years, several novel species have been classified as members of the genus *Pseudoalteromonas*, which (at the time of writing) comprises 29 species with validly described names, including the recently described species *Pseudoalteromonas issachenkonii* (Ivanova *et al.*, 2002a), *Pseudoalteromonas ruthenica* (Ivanova *et al.*, 2002b), *Pseudoalteromonas maricaloris* and *Pseudoalteromonas flavipulchra* (Ivanova *et al.*, 2002c), *Pseudoalteromonas translucida* and *Pseudoalteromonas paragorgicola* (Ivanova *et al.*, 2002d), *Pseudoalteromonas agarivorans* (Romanenko *et al.*, 2003) and *Pseudoalteromonas phenolica* (Isnansetyo & Kamei, 2003).

Strain NCIMB 1770 was isolated from the diatom *Chaetoceros lauderi*, collected from sea water of the Marseille Gulf (Berland *et al.*, 1969). The strain was identified as '*Pseudomonas marinoglutinosa*' on the basis of its phenotypic features, which were originally described by

ZoBell & Allen (1935) for another marine agarolytic bacterium named '*Pseudomonas marinoglutinosa*'. DNA–rRNA hybridization experiments revealed that '*P. marinoglutinosa*' NCIMB 1770 should be assigned to the genus *Alteromonas* (De Vos *et al.*, 1989). Strain NCIMB 1770 was not included in the phylogenetic study that led to the description of the genus *Pseudoalteromonas* (Gauthier *et al.*, 1995), hence its taxonomic status was not specified. In the present study, strain NCIMB 1770 was subjected to biochemical and molecular analyses to clarify its taxonomic position. On the basis of phenotypic and molecular properties, *Pseudoalteromonas mariniglutinosa* sp. nov., nom. rev., comb. nov. is proposed.

Strain '*Alteromonas marinoglutinosa*' NCIMB 1770 was received from the National Collection of Industrial and Marine Bacteria, Aberdeen, UK. It was grown routinely on Marine 2216 Agar (MA), Marine Broth (MB) (both Difco) and nutrient sea-water-containing medium (SWM) at 28 °C. The composition of SWM was as follows (g l⁻¹): peptone, 5.0; yeast extract, 2.5; glucose, 1.0; K₂HPO₄, 0.2; MgSO₄, 0.05; agar, 15.0; sea water, 750 ml and distilled water, 250 ml. The bacterium was stored at –70 °C in liquid nutrient medium that was supplemented with 30 % (v/v) glycerol. Phenotypic properties, including Gram-staining, oxidase, catalase, gelatinase, caseinase, lipase, DNase, amylase and chitinase activities, were tested as described by Smibert & Krieg (1994) and Baumann *et al.* (1972), by

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using MA or SWM as basal media. Hydrolysis of κ -carrageenan was determined as described by Yaphe & Baxter (1955). Growth at 4–45 °C and pH 5.0–10.0 was examined on MA and in MB, respectively. Sea-water requirement and tolerance of 0–15 % NaCl were determined using nutrient medium prepared on the artificial sea-water base, supplemented with appropriate amounts of NaCl. The medium of Leifson (1963) was used to test acid production from sugars, with 1 % (w/v) of each test sugar. The agar-diffusion method was applied to test antibiotic sensitivity on MA plates, by using discs impregnated with antibiotics (per disc): ampicillin, 10 µg; benzylpenicillin, 10 U; gentamicin, 10 µg; kanamycin, 30 µg; erythromycin, 30 µg; carbenicillin, 25 µg; lincomycin, 15 µg; oleandomycin, 15 µg; polymyxin, 300 U; streptomycin, 30 µg; tetracycline, 30 µg; O/129, 150 µg; and neomycin, 15 µg. Phenotypic characteristics were additionally determined by using API 20 NE and API ZYM galleries (bioMérieux) and the Biolog GN identification system as recommended by the manufacturers, except that the culture was suspended in 3 % (w/v) NaCl solution. Motility was examined by the hanging-drop method. Cell morphology was examined by transmission electron microscopy of exponential-phase cells grown on MA. DNA G + C content was determined by the methods described by Marmur & Doty (1962) and Owen *et al.* (1969). DNA–DNA hybridization was performed by the initial reassociation rate kinetic method of De Ley *et al.* (1970). Cellular fatty acid and lipid compositions were identified as described by Svetashev *et al.* (1995) and Ivanova *et al.* (2000b). The 16S rRNA gene sequence was determined as described by Rainey *et al.* (1996). The 16S rDNA sequence of strain NCIMB 1770 was aligned manually with nucleotide sequences obtained from GenBank/EMBL and phylogenetic dendrograms were constructed by using different treeing algorithms [distance matrix (DeSoete, 1983; Felsenstein, 1993) and neighbour-joining (Felsenstein, 1993)].

16S rDNA similarity values between strain NCIMB 1770 and *Pseudoalteromonas prydzensis* ACAM 620^T (99.3 %) were high, while those with several type strains of the genus *Pseudoalteromonas* were lower by about 1 %, i.e. *Pseudoalteromonas atlantica* IAM 12376^T (98.2 %), *P. issachenkonii* KMM 3549^T (98.2 %), *Pseudoalteromonas espejiana* NCIMB 2127^T (98.2 %), *Pseudoalteromonas distincta* KMM 638^T (98.1 %), *Pseudoalteromonas elyakovii* KMM 162^T (98.1 %), *P. agarivorans* KMM 255^T (98.1 %), *Pseudoalteromonas carrageenovora* IAM 12662^T (98.1 %), *Pseudoalteromonas haloplanktis* ATCC 14393^T (98.0 %), *Pseudoalteromonas nigrificiens* NCIMB 8614^T (98.0 %), *Pseudoalteromonas undina* NCIMB 2128^T (98.0 %), *Pseudoalteromonas tetradonis* IAM 14160^T (98.0 %) and *P. antarctica* CECT 4664^T (97.7 %). Type strains of the other species of the genus are less closely related to strain NCIMB 1770. The phylogenetic position of strain NCIMB 1770, obtained by two different algorithms, agrees in the order of the major branches. The statistical significance of the majority of these branching points is confirmed by high bootstrap values. The resolution of lineages defined by highly related organisms is

Fig. 1. Neighbour-joining analysis of 16S rRNA gene sequences, showing the position of strain KMM 3635^T (=NCIMB 1770^T) among phylogenetically closely related *Pseudoalteromonas* species. Less related *Pseudoalteromonas* species served as the root (only one type strain is shown). Numbers at branching points refer to bootstrap analyses (percentage of 500 resamplings). Bar, 5 nucleotide substitutions per 100 nucleotides.

poor, and is not supported by high bootstrap values. Fig. 1 depicts a neighbour-joining dendrogram (Felsenstein, 1993).

Strain NCIMB 1770 was aerobic, Gram-negative, non-pigmented, motile by a single polar flagellum and capable of agar and carrageenan hydrolysis. The strain required Na⁺ ions for growth and grew in 1–9 % NaCl. The temperature range for growth was 5–37 °C; growth was not detected at 40 °C. On complex marine media, agarolytic, smooth, convex, slimy, non-pigmented colonies were formed. Two types of colonies, either whitish or translucent, were homogeneous with respect to metabolic properties and were considered to be variants.

Strain NCIMB 1770 was positive for oxidase, catalase, lipase, caseinase, DNase and gelatinase activities and starch, agar and carrageenan decomposition, when tests were performed by conventional methods. It should be noted that the API ZYM test revealed negative reactions for α -galactosidase and β -galactosidase, whereas agarolytic activity was detectable by routine methods. The main phenotypic features of strain NCIMB 1770 and some phylogenetically related species are displayed in Table 1.

Major fatty acids were C_{16:0} (24.0 %), C_{16:1} ω 7c (38.9 %) and C_{17:1} ω 8c (9.4 %). The following were present in smaller amounts (>1.5 %): C_{14:0} (1.7 %), C_{15:0} (3.9 %), iso-C_{16:0} (2.8 %), C_{17:0} (3.9 %) and C_{18:1} ω 7c (5.9 %). As traces (<1.0 %), the following fatty acids were present: C_{11:0}, C_{12:1}, iso-C_{13:0}, C_{13:0}, C_{13:1}, iso-C_{14:0}, C_{14:1} ω 7c, C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0}, C_{15:1} ω 6c, C_{11:0} 3-OH, C_{16:1} ω 5c, C_{12:0} 3-OH, iso-C_{17:0}, C_{17:1} ω 6c, iso-C_{18:0}, C_{18:0},

Table 1. Phenotypic characteristics of *P. mariniglutinosa* NCIMB 1770^T and related *Pseudoalteromonas* species

Taxa: 1, *P. mariniglutinosa*; 2, *P. atlantica*; 3, *P. carrageenovora*; 4, *P. elyakovii*; 5, *P. prydzensis*; 6, *P. issachenkonii*; 7, *P. agarivorans*; 8, *P. espejiana*; 9, *P. distincta*; 10, *P. haloplanktis*; 11, *P. nigrifaciens*; 12, *P. undina*; 13, *P. tetradonis*; 14, *P. antarctica*. Data were taken from Chan *et al.* (1978); Baumann *et al.* (1984); Akagawa-Matsushita *et al.* (1992); Bozal *et al.* (1997); Bowman (1998); Ivanova *et al.* (1996, 2000a, 2001, 2002a); Sawabe *et al.* (2000); Romanenko *et al.* (2003). +, Positive test reaction; –, negative test reaction; v, variable results between strains; W, weak reaction; ND, not determined. All strains were positive for the following tests: Na⁺ requirement for growth, growth at 10–30 °C, motility, oxidase and catalase activities and production of gelatinase. All strains were negative for growth at 40 °C, indole production and arginine dihydrolase activity.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Growth at/in:														
4 °C	–	–	–	v	+	+	–	–	+	–	v	v	+	+
37 °C	+	–	–	v	–	+	–	–	–	–	–	–	–	–
≥12% NaCl	–	–	–	–	+	+	–	–	–	–	–	–	–	v
Hydrolysis of:														
Starch	+	+	–	+	+	–	+	+	–	+	+	v	–	–
Agar	+	+	–	–	–	–	+	–	–	–	–	–	–	–
Carrageenan	+	v	+	–	–	–	+	–	–	–	–	–	–	–
Chitin	–	–	–	–	+	+	–	–	–	v	–	+	–	–
Utilization of:														
D-Glucose	+	+	+	+	+	ND	W	+	–	+	+	+	+	+
D-Fructose	–	+	+	+	–	+	–	v	–	v	v	–	–	–
L-Arabinose	+	–	–	ND	+	ND	–	–	–	v	v	–	–	–
Mannose	W	+	–	+	+	–	–	v	–	+	+	–	–	v
N-Acetylglucosamine	+	–	–	v	+	–	–	–	–	+	ND	+	ND	v
Sucrose	+	+	+	+	+	+	–	+	–	+	+	+	+	–
Glycerol	–	+	+	v	+	–	–	–	–	–	–	–	–	ND
DNA G+C content (mol%)	40.3	40.6–41.7	39.5	38.5–38.9	38.0–39.0	42.9–43.3	42.2–43.8	43.0–44.0	43.8	41.8–44.4	39.0–41.7	43.0–44.0	41.5	41.0–42.0

C_{18:1}ω11c, C_{18:1}ω9c, C_{19:1}, C_{12:0}, C_{15:1}ω8c and anteiso-C_{17:0}. Phospholipids included phosphatidylethanolamine (67.1%), phosphatidylglycerol (28.6%), diphosphatidylglycerol (DPG; 3.2%) and bis-phosphatidic acid (1.2%). The presence of smaller amounts of DPG in *P. nigrifaciens* and *P. agarivorans* (1.4 and 0.9–1.3%, respectively) has been reported previously by Frolova *et al.* (2000) and Romanenko *et al.* (2003).

16S rDNA sequence analysis (1483 nucleotides) revealed that strain NCIMB 1770 is a member of the genus *Pseudoalteromonas*. Except for *P. prydzensis*, with which 99.3% sequence similarity was shared, all other species had similarity values lower than 98.2%. Previous DNA–DNA reassociation studies on the phylogenetically closely related type strains depicted in Fig. 1 revealed that DNA relatedness values were significantly lower than 70% (Ivanova *et al.*, 2000a; Mikhailov *et al.*, 2002; Romanenko *et al.*, 2003). Thus, 97.5% 16S rRNA gene similarity, indicated to be the threshold value above which it has been advised to perform DNA–DNA reassociation studies to determine species status (Stackebrandt & Goebel, 1994), is clearly higher for members of the genus *Pseudoalteromonas* and should be set to 99.0%. A similar situation has recently been observed for the actinobacterial genus *Amycolatopsis* (Wink *et al.*, 2003). Although closely related by 99.3% 16S

rRNA gene similarity, strain NCIMB 1770 and its closest phylogenetic relative, *P. prydzensis* ACAM 620^T, share only 35% DNA–DNA similarity. A selection of nine type strains from the phylogenetically closely related cluster (Fig. 1) confirmed the low DNA–DNA similarity value obtained with strain NCIMB 1770, i.e. similarities with *P. elyakovii* KMM 162^T (45%), *P. carrageenovora* IAM 12662^T (32%), *P. undina* IAM 12922^T (42%), *P. distincta* KMM 638^T (42%), *P. espejiana* IAM 13640^T (16%), *P. haloplanktis* IAM 12915^T (15%), *P. tetradonis* IAM 14160^T (28%), *P. atlantica* IAM 12376^T (28%) and *P. agarivorans* KMM 255^T (16%) were well below 50% (*P. antarctica* CECT 4664^T is a patented strain and has not been released for this study). Such low DNA–DNA similarity data indicate that strain NCIMB 1770 does not belong to any previously described species (Wayne *et al.*, 1987). On the basis of the results obtained from the present study, strain '*Alteromonas marinoglutinosa*' NCIMB 1770 is proposed as the type strain of a novel species, *Pseudoalteromonas mariniglutinosa* sp. nov., nom. rev., comb. nov.

Strain NCIMB 1770^T shared some common properties with *P. atlantica*, *P. carrageenovora* and *P. agarivorans* in the hydrolysis of starch, agar and carrageenan, but differed from these species in growth at 37 °C and physiological properties. Growth at 37 °C is shared with *P. issachenkonii*

and some strains of *P. elyakovii*. Differences in metabolic properties, such as hydrolysis of starch, agar, carrageenan and chitin, as well as the utilization of glucose, L-arabinose, sucrose and N-acetylglucosamine and frequently the DNA G+C content, allow the discrimination of strain NCIMB 1770^T from other phylogenetically closely related species (Table 1).

Description of *Pseudoalteromonas mariniglutinosa* sp. nov., nom. rev., comb. nov.

Pseudoalteromonas mariniglutinosa (ma.ri.ni.glu.ti.no'sa. L. adj. *marinus* of the sea; L. adj. *glutinosa* glutinous, slimy; N.L. fem. adj. *mariniglutinosa* from the sea, forming glutinous, slimy colonies).

Gram-negative, strictly aerobic, rod-shaped cells, 0.6–0.8 µm in diameter and 1.7–2.0 µm in length, motile by a single, polar, unsheathed flagellum. Endospores are not formed. Oxidase- and catalase-positive. Na⁺ is essential for growth; growth is observed in 1–9% NaCl. Mesophilic and neutrophilic chemo-organotroph. Grows at 5–37 °C with optimal growth at 20–28 °C; no growth at 4 or 40 °C. On complex marine media, smooth, convex, slimy, non-pigmented, whitish or translucent colonies that are depressed into the agar are formed. In addition to the phenotypic characters indicated in Table 1, the type strain exhibits the following reactions: acid is produced from sucrose, maltose, L-arabinose and D-mannitol by using Leifson's method; acid is not produced from glucose, lactose, mannose, D-galactose, D-xylose, melibiose, rhamnose or glycerol. L-Malate, citrate, propionate and L-serine are utilized, whereas caprate and butyrate are not. DNase activity is positive. According to the API 20 NE panel, the following tests are positive: aesculin and gelatin hydrolysis, assimilation of glucose, arabinose, mannose (weakly), mannitol, N-acetylglucosamine, maltose, malate and citrate. The following tests are negative: nitrate reduction, indole production, arginine dihydrolase and urease activities, *p*-nitrophenyl-D-galactopyranoside (PNPG) test, gluconate, caprate, adipate and phenylacetate assimilation. In API ZYM analysis, strain NCIMB 1770^T exhibits positive reactions for alkaline phosphatase, esterase-lipase C8 (weakly), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase. Negative reactions are observed for esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase. As shown by Biolog GN MicroPlate tests, the type strain utilizes α-cyclodextran, dextran, N-acetyl-D-glucosamine, α-D-glucose, D-mannitol, D-mannose, methylpyruvate, mono-methylsuccinate, acetic acid, citric acid, DL-lactic acid, propionic acid, succinic acid, L-glutamic acid, L-proline (weak) and L-serine. Negative reactions are observed for utilization of the other organic compounds included in Biolog GN MicroPlate tests. Susceptible to streptomycin, polymyxin, gentamicin (weakly), kanamycin, carbenicillin and neomycin, but resistant to ampicillin, oleandomycin,

lincomycin, benzylpenicillin, O/129, oxacillin and tetracycline. Major fatty acids are C_{16:0}, C_{16:1ω7c} and C_{17:1ω8c}. Phospholipids include phosphatidylethanolamine and phosphatidylglycerol as main components and diphosphatidylglycerol and bis-phosphatidic acid as minor components. The DNA G+C content is 40.3 mol% (thermal denaturation method).

The type strain, NCIMB 1770^T (= KMM 3635^T), was isolated from the diatom *Chaetoceros lauderi*.

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