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Pseudoalteromonas tunicata sp. nov., a bacterium that produces antifouling agents

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A dark-green-pigmented marine bacterium, previously designated D2, which produces components that are inhibitory to common marine fouling organisms has been characterized and assessed for taxonomic assignment. Based on direct double-stranded sequencing of the 16S rRNA gene, D2^T was found to show the highest similarity (93%) to members of the genus *Pseudoalteromonas*. The G+C content of D2^T is 42 mol%, and it is a facultatively anaerobic rod and oxidase-positive. D2^T is motile by a sheathed polar flagellum, exhibited non-fermentative metabolism and required sodium ions for growth. The strain was not capable of using citrate, fructose, sucrose, sorbitol and glycerol but it utilizes mannose and maltose and hydrolyses gelatin. The molecular evidence, together with phenotypic characteristics, showed that this bacterium which produces an antifouling agent constitutes a new species of the genus *Pseudoalteromonas*. The name *Pseudoalteromonas tunicata* is proposed for this bacterium, and the type strain is D2^T (= CCUG 26757^T).

Keywords: *Pseudoalteromonas tunicata*, pigment, antifouling bacterium, marine, 16S rRNA sequence

INTRODUCTION

Heterotrophic bacteria which are Gram-negative and motile through the use of flagella are commonly isolated from marine environments. These culturable bacteria can be divided into two groups based on their capacity to ferment carbohydrates. The fermentative strains belong to the genera *Vibrio*, *Listonella*, *Photobacterium*. *Colwellia* and *Aeromonas* and the non-fermentative strains belong to *Alteromonas*, *Pseudomonas*, *Alcaligenes*, *Halomonas*, *Deleya*, *Marinomonas*, *Shewanella* and *Flavobacterium* (Kita-Tsukamoto *et al.*, 1993). The two new genera *Listonella* and *Colwellia* have been suggested for species formerly considered to be members of the genus *Vibrio* (Deming *et al.*, 1988; MacDonell & Colwell, 1985) while most members of the genus *Alteromonas* have been reclassified within the new genus *Pseudoalteromonas* (Gauthier *et al.*, 1995).

Gauthier *et al.* (1995) made a comparison of the phylogenetic relationships among 17 isolates of the genera *Alteromonas*, *Shewanella* and *Moritella*. The results suggested that the genus *Alteromonas* should be separated into two genera. The new genus was designated *Pseudoalteromonas*. Originally the genus *Alteromonas* contained four species and was created for marine bacteria that are *Pseudomonas*-like but which have a lower G+C content (Baumann *et al.*, 1972). The new genus, *Pseudoalteromonas*, was recommended to contain 11 species previously belonging to species of *Alteromonas* and one species that previously was placed in the genus *Pseudomonas*, *Pseudoalteromonas piscicida* comb. nov. (Gauthier *et al.*, 1995). The genus *Alteromonas* is thereby restricted to a single species, *Alteromonas macleodii*.

The strain in this study, a pigmented Gram-negative marine bacterium, designated D2^T (T = type strain), was isolated from an adult tunicate, *Ciona intestinalis*. The bacterium was shown to produce extracellular components that kill the larvae of *Balanus amphitrite* and *Ciona intestinalis* (Holmström *et al.*, 1992). In addition, recent studies have demonstrated that the isolate produces at least four different extracellular compounds with various degrees of specific inhibitory activity against a variety of organisms including bacteria, algae, diatoms and fungi (Egan, 1995; Holmström *et al.*, 1992, 1996, 1997; Holmström &

The GenBank accession number for the 16S rDNA sequence of strain D2^T is Z25522.

Kjelleberg, 1994; James *et al.*, 1996). In this study, we carried out a phenotypic and genetic characterization of the isolate for taxonomic classification. The non-fermentative nature of this strain precludes its inclusion in the genus *Vibrio*, and the G+C content of the genome is too low for the inclusion in the genera *Pseudomonas* and *Marinomonas*. Isolate D2^T revealed 93·4% 16S rDNA sequence similarity to the next closest related bacterial strain (*Pseudoalteromonas citrea*) in current DNA sequence databases (GenBank, EMBL and RDP). This relationship is well within the range of % similarities of the genus *Pseudoalteromonas* (> 91%). We therefore propose isolate D2^T as a new species of the genus *Pseudoalteromonas*, *P. tunicata* on the basis of its physiological, biochemical and genetic features.

METHODS

Bacterial strain and isolation. Strain D2^T was isolated from an adult tunicate *Ciona intestinalis* which was collected from a depth of 10 m in Gullmarsfjorden on the western coast of Sweden. A platinum loop was used to obtain samples from the surface of tunicates and loopfuls of the samples were diluted in sterile nine salt solution (NSS, which consists of, per litre, 17·6 g NaCl, 1·47 g Na_2S0_4, 0·08 g $NaHCO_3$, 0·25 g KCl, 0·04 g KBr, 1·87 g $MgCl_2.6\ H_2O$, 0·41 g $CaCl_2.2\ H_2O$, 0·008 g $SrCl.6\ H_20$, 0·008 g H_3BO_3, pH 7). Aliquots of the diluted samples were spread on VNSS agar plates (VNSS agar consists of per litre NSS, 1·0 g peptone, 0·5 g yeast extract, 0·5 g glucose, 0·01 g $FeSO_4.7\ H_2O$, 0·01 g Na_2HPO_4 and 15 g agar) and incubated for 3–5 d at 20 °C. Isolates were maintained on VNSS agar plates at 4 °C. Bacteria were also stored at −70 °C in 30% (v/v) glycerol. Isolate D2^T (= CCUG 26757^T) has been deposited in the Culture Collection of the University of Göteborg. All media used in this study were sterilized at 121 °C for 20 min, unless otherwise stated.

Phenotypic characterization of strain D2^T. The bacterium was routinely grown on the complex marine medium, VNSS, and incubated at 20 °C to provide inocula for the biochemical tests.

Oxidative or fermentative utilization of glucose was determined by the method of Hugh & Leifson (1953). Catalase activity was determined by the methods of Skerman (1967) and oxidase activity was tested by the Kovaks method (Kovaks, 1956).

The salinity tests were performed using the medium VNSS with NaCl concentrations ranging from 0 to 10% (w/v). We also tested for growth on Tryptone soy broth (TSB) (Oxoid) and Luria broth (LB 20) supplemented with 2% salt (w/v) (Östling *et al.*, 1991). A marine minimal medium (MMM) (Östling *et al.*, 1991) was used during tests of growth of D2^T on different substrates as sole carbon and energy sources at the concentration of 4 g l^{-1}.

Swarming (Givskov *et al.*, 1996) was tested on four different media, VNSS, TSB, LB 20 and AB (Clark & Maaloe, 1967). The last medium was supplemented with 0·4% glucose, 0·5% Casamino acids, and all four media were supplemented with 0·6% Bacto agar.

The susceptibility of strain D2^T to the antibiotics (Bauer *et al.*, 1966) erythromycin, rifampicin, gentamicin, tetracycline, ampicillin, neomycin, kanamycin and nalidixic acid was tested at 100 μg ml^{-1} and its sensitivity to the vibriostatic agent 0/129 was tested at a concentration of 150 μg ml^{-1}. Disks (6·0 mm in diameter) impregnated with antibiotics were placed on VNSS agar plates which had been surface-inoculated with D2^T suspension.

Some of the biochemical properties of the isolate were determined using the API 20E system (Analytab Products) and exponential-phase bacterial cells which were washed three times in minimal medium (MMM) before being inoculated into the test cupules.

The motility of the cells was determined with a 100× oil-immersion objective.

Negative staining and electron microscopy. Cell morphology and size and the presence and type of flagellum were examined by using cells from an 18 h bacterial growth culture. The cells were suspended in a fixative (2·5% glutaraldehyde in 0·1 M sodium cacodylate buffer), fixed for 30 min, and washed three times in sterile Milli-Q water. A drop of the cell suspension was mixed with one drop of sodium phosphotungstate stain (2% aqueous) for 10 s using a Formvar grid (300-square copper grid). The grid was thereafter blotted with filter paper and dried for 10 min before examination using a Hitachi H7000 electron microscope operated at 75 kV.

Fatty acid analysis. D2^T was grown on VNSS medium at 25 °C for 24 h. The cells were harvested, washed, and freeze-dried. The supernatant was collected and filtered through a 0·22 μm filter and thereafter freeze-dried. A modified Bligh–Dyer lipid extraction (Bligh & Dyer, 1959; White *et al.*, 1979) was performed on the extracts followed by silicic acid chromatography to fractionate the lipids. Finally mild alkaline methanolysis was performed (White *et al.*, 1979). The samples were then separated, quantified and identified by capillary gas-liquid chromatography/electron impact mass spectrometry (Guckert *et al.*, 1985). The results were based on three replicates.

Genome analysis. The G+C content was determined by using a thermal denaturation procedure (Johnson, 1994). The DNA samples used for the analysis were extracted and purified as described by Wilson (1992) and the results were based on four replicates. *Pseudoalteromonas haloplanktis* ACAM 547 was used as a reference strain (G+C = 43·0 mol%).

16S rDNA amplification and sequencing. Bacterial cells for the DNA extraction were grown in a complex marine medium VNSS and harvested (1·5 ml bacterial suspension) in late-exponential phase of growth. The DNA extraction, PCR-mediated amplification of the 16S rDNA and purification of PCR products were carried out as described previously by Hallbeck *et al.* (1993). Both strands were sequenced with the Sequenase version 2.0 DNA sequencing kit (USB). The PCR product was sequenced directly, using the primers as described by Hallbeck *et al.* (1993).

Nucleotide sequence accession numbers. EMBL/GenBank/RDP accession numbers (in parentheses) for small-subunit rDNA sequences of strains other than D2^T used in this study are as follows: *Alteromonas macleodii* IAM 12920^T (X82145), *Marinomonas vaga* ATCC 27119^T (X67025), *Moritella marinus* NCIMB 1144^T (X82142), *Pseudoalteromonas antarctica* CECT4664^T (X98336), *Pseudoalteromonas atlantica* IAM 12927^T (X82134), *Pseudoalteromonas aurantia* ATCC 33046^T (X82135), *Pseudoalteromonas carrageenovora* IAM 12662^T (X82136), *Pseudoalteromonas citrea* NCIMB 1889^T (X82137), *Pseudoalteromonas denitrificans* ATCC 43337^T (X82138), *Pseudoalteromonas espejiana* NCIMB

2127^T (X82143), *Pseudoalteromonas haloplanktis* subsp. *haloplanktis* ATCC 14393^T (X67024), *Pseudoalteromonas luteoviolacea* NCIMB 1893^T (X82144), *Pseudoalteromonas nigrifaciens* NCIMB 8614^T (X82135), *Pseudoalteromonas piscicida* ATCC 15057^T (X82215), *Pseudoalteromonas rubra* ATCC 29570^T (X82147), *Pseudoalteromonas haloplanktis* subsp. *tetraodonis* IAM 14160^T (X82139), *Pseudoalteromonas undina* NCIMB 2128^T (X82140), *Pseudoalteromonas* sp. IC006 (U85856), *Pseudoalteromonas* sp. IC013 (U85859), *Pseudoalteromonas* sp. MB6-05 (U85860), *Pseudoalteromonas* sp. MB6-03 (U85857), *Pseudoalteromonas* sp. MB8-11 (U85855), *Pseudoalteromonas* sp. SW08 (U85861), *Pseudoalteromonas* sp. SW29 (U85862), *Pseudoalteromonas* sp. S9 (U80834), *Pseudoalteromonas* sp. Y (AF030381), *Pseudoalteromonas* sp. ANG.RO2 (AF022407), *Pseudoalteromonas peptidysin* F12-50-A1^T (AF007286), *Shewanella benthica* ATCC 43992^T (X82131), *Shewanella hanedai* CIP 103207^T (X82132), *Shewanella putrefaciens* ATCC 8071^T (X82133), *Aeromonas allosaccharophila* CECT 4199^T (S39232), *Photobacterium* sp. SS9 (U91586), *Vibrio fischeri* ATCC 7744^T (X74702) and *Escherichia coli* (J01859). Culture collection designations are: ATCC, American Type Culture Collection, Rockville, MD, USA; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK; IAM, Institute of Applied Microbiology, Tokyo, Japan; CIP, Collection of Institute Pasteur, Paris, France.

Phylogenetic analysis. DNA sequences were aligned using the programs PILEUP, GCG (Wisconsin package, 1994) and the multiple sequence alignment tool from CLUSTAL W (Thompson *et al.*, 1994). Manual confirmation of the sequence alignment was performed and checked against both primary and secondary structure considerations of the 16S rRNA molecule. The aligned sequences were applied to genetic distance and maximum parsimony methods for phylogenetic inference. For all multiple sequence alignments and phylogenetic inference programs the input order of taxa was randomized. Genetic distances were calculated using the formula of Jukes & Cantor (1969), where $D = -3/4 \ln(1 - 4/3d)$ and d is the sequence dissimilarity. Phylogenetic inference protocols, DNADIST, NEIGHBOR, DNAPARS, CONSENSE and SEQBOOT were supplied by the PHYLIP package (version 3.57c) (Felsenstein, 1989). All sequence manipulation and phylogeny programs were made available through the Australian National Genome Information Service (ANGIS, Sydney, Australia).

RESULTS

Phenotypic characterization of the marine isolate

Strain D2^T grew at 4 °C but not at 37 °C, with optimal growth at approximately 28 °C. Growth at the lower temperatures of 4 and 10 °C was very slow compared with that at 28 °C. The water temperature surrounding the tunicate specimen, at the time of the isolation, was approximately 10 °C. D2^T tolerated a pH range of 5·5–9 (optimum pH 7–8) while the low-molecular-mass component, which is active against invertebrate larvae, tolerated a pH range of 2–11 without losing its inhibitory activity (Holmström, 1993). D2^T required Na^+ at a concentration of 0·3% (w/v) NaCl, and optimal salinity for growth of the strain was found to be 1–2% (w/v) NaCl concentration.

The isolate exhibited gelatinase activity while β-galactosidase, arginine dihydrolase, lysine-ornithine decarboxylase and urease activities were not detected. Did not show any H_2S or NO_2 production. Growth on different carbon and energy sources showed that the bacterium utilizes trehalose, glucose, maltose and Tween 20 after 24 h incubation and mannose and proline after 48 h. We have not been able to demonstrate growth on additional carbohydrates or amino acids. It did not ferment sugar in the Hugh–Leifson test and the cells were sensitive to erythromycin, rifampicin, gentamicin, tetracycline, ampicillin, neomycin, kanamycin and nalidixic acid at concentrations of 100 μg ml^{-1}. The isolate is sensitive to the vibriostatic agent 0/129 at a concentration of 150 μg and is oxidase and catalase-positive. Strain D2^T cells are 2·0–3·4 μm in length, motile and rod-shaped, and possess a single sheathed polar flagellum. Swarming of the bacteria was not detected (Table 1).

Table 1. Phenotypic characteristics of *Pseudoalteromonas tunicata*

−, Negative; +, positive.

Characteristic	*P. tunicata*
Swarming	−
Growth at 4 °C	+
Growth at 37 °C	−
Optimal NaCl concentration (%)	1–2
Optimal pH	7–8
Hugh–Leifson test	−
Production of:	
β-Galactosidase activity	−
Arginine dihydrolase activity*	−
Lysine decarboxylase activity*	−
Ornithine decarboxylase activity*	−
Hydrolysis of:	
Urease	−
Gelatin	+
Utilization of:	
Glucose, maltose, trehalose	+
Mannose	+†
Galactose, fructose, xylose, sucrose, lactose, raffinose, melibiose, rhamnose, arabinose, glycerol, mannitol, sorbitol, inositol, dulcitol, melizitose, xylitol, erythritol, glycine, citrate	−
DL-Threonine	−
L-Asparagine	−
L-Proline	+†
L-Histidine	−
Tween 20	+
Oxidase	+
Catalase	+
Sheathed polar flagellum	+
G+C content (mol%)	42·2±0·7

* Tests using the API 20E system.

† Growth after 48 h.

Table 2. Fatty acid profiles of cell and supernatant extracts of *P. tunicata*

Results are expressed as mean mol% (±SD).

Fatty acid	Extract	
	Cell	Supernatant
12:0	0·03±0·01	0·00
13:0	0·06±0·02	0·00
14:1a	0·44±0·01	0·00
14:1b	0·03±0·00	0·00
14:0	2·10±0·02	0·00
15:1a	0·45±0·02	0·00
15:1b	0·08±0·01	0·00
15:0	0·90±0·01	0·00
16:0i	0·13±0·01	0·00
16:1ω9*c*	0·58±0·04	0·00
16:1ω7*c*	47·47±0·76	23·47±0·53
16:1ω7*t*	6·69±0·06	0·00
16:1ω5*c*	0·35±0·13	19·51±6·13
16:0	18·31±0·38	46·99±7·53
17:0i	0·01±0·00	0·00
17:0a	3·54±0·03	0·00
17:1a	0·20±0·00	0·00
17:1b	0·23±0·01	0·00
17:0	1·30±0·07	2·84±4·02
18 unknown	0·04±0·00	0·00
18:1ω9*c*	0·36±0·01	0·00
18:1ω7*c*	14·46±0·29	3·15±4·45
18:1ω7*t*	0·29±0·01	0·00
18:1ω5*c*	0·08±0·03	0·00
18:0	1·50±0·09	4·04±5·72
18:1br	0·02±0·01	0·00
19:1	0·17±0·01	0·00
20:1ω9*c*	0·16±0·02	0·00
20:0	0·04±0·01	0·00

The colony and cellular morphology on different growth media showed that young colonies grow in punctiform with a diameter around 2 mm on TSB and LB 20 agar plates or 1 to 2 mm on VNSS plates. The colonies were smooth and convex with regular edges. However, the occurrence of pigmentation varied. After 24 h on VNSS medium, the colonies turned dark green, while on LB 20 and TSB the colonies remained white. Streaking white colonies from plates on VNSS plates gave dark green colonies after 24 h incubation and vice versa.

Fatty acid analysis

The unsaturated fatty acid 16:1ω7*c* was shown to be the predominant fatty acid of D2^T cells (mean level, 47·47±0·76%), whereas the fatty acids 16:0, 18:1ω7*c* and 16:1ω7*t* were the second, third and fourth most common fatty acids (relative percentages, 18·31±0·38, 14·46±0·29 and 6·69±0·06%), respectively. These four major components accounted for almost 90% of the total cellular fatty acids. The balance of the profile of D2^T cells consisted of saturated and unsaturated fatty acids and a trace of terminally branched fatty acids. There was one unknown 18 carbon fatty acid, however, this constituted less than 1% of the profile. The supernatant also contained normal and monoenoic fatty acids but the biomass of the supernatant was four orders of magnitude less than that of the cells themselves. The saturated fatty acid 16:0 was the major fatty acid (46·99±7·53) in the supernatant while 16:1ω7*c* and 16:1ω5*c* were the second and third most common fatty acids (23·47±0·53 and 19·51±6·13%), respectively (Table 2).

DNA base composition

The G+C content of the DNA of *Pseudoalteromonas tunicata* was 42·2±0·7 mol%. The accepted range for members of the genus *Pseudoalteromonas* is 38–50 mol%.

16S rDNA sequence and phylogenetic characterization

The sequencing strategy used in the current investigation generated 1370 bases of the 16S rRNA gene between positions 34 and 1404 corresponding to the numbering of nucleotides in the *E. coli* 16S rRNA molecule (Brosius *et al.*, 1981). The resulting sequence was aligned to currently available 16S rRNA gene sequences which showed significant identity from the EMBL, GenBank and RDP databases. The derived multiple sequence alignment, including both conserved and variable domains of the 16S rRNA primary structure, was used as the basis to generate pairwise similarities and genetic distances between D2^T and related bacteria. Several phylogenetic trees were constructed using different methods to analyse the sequence data, including genetic distance matrices and maximum parsimony. The input order of sequences into the various phylogenetic programs and the selection of outgroups were also varied during this study. Statistical evaluation of the derived genetic divergences was performed by bootsrap resampling of the sequence data.

The tree topology shown in Fig. 1 was identical to other statistical representations of the sequence data, including parsimony and consensus trees. Based on phylogenetic characterization, the strain D2^T belongs to the gamma-3 subclass of *Proteobacteria* and was most closely branched to the monophyletic cluster containing members of the genus *Pseudoalteromonas*. However, in relation to the *Pseudoalteromonas* clade, this strain comprised a distinct ancestral lineage, as seen in both parsimony (data not shown) and genetic distance trees (Fig. 1). The isolate shared 93% 16S rDNA sequence similarity with *P. citrea* and *P. aurantia*. Within this genus, D2^T had a range of sequence identities between 91·3–93·4%, for the

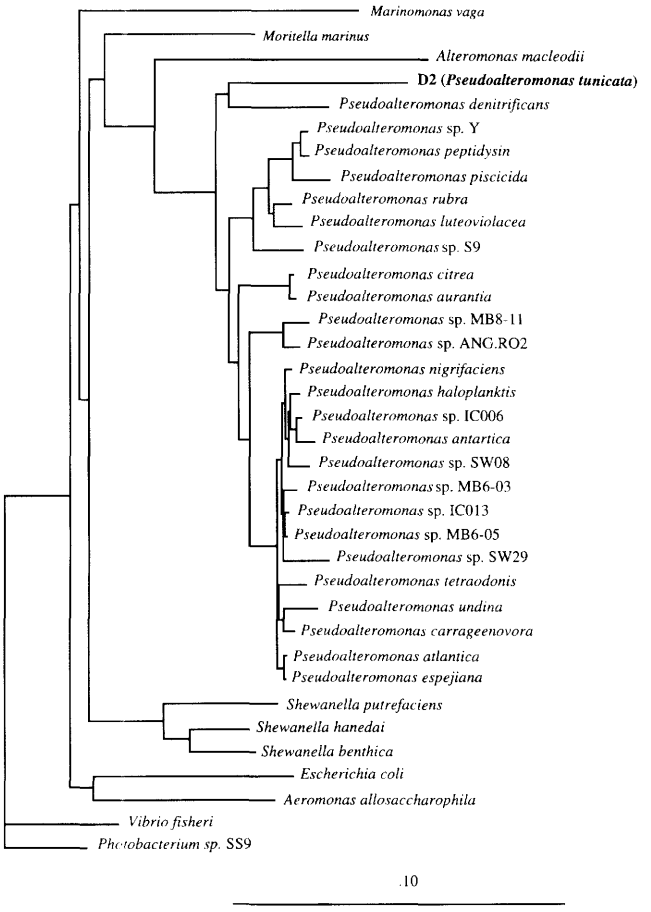

Fig. 1. Phylogenetic affiliations between D2^T (*P. tunicata*), strains of the genus *Pseudoalteromonas*, and other closely related bacteria as indicated by near complete 16S rRNA gene sequence alignment. An alignment of 1370 characters was used to calculate genetic distances according to the method of Jukes & Cantor (1969). The phenogram was reconstructed from the pairwise distance matrix using the neighbour-joining method of Saitou & Nei (Satuito *et al.*, 1996). The scale represents 1 base substitution per 10 nucleotide positions. Strain designations, source culture collections, and sequence database accession numbers are listed in the text.

Pseudoalteromonas strains included in this analysis. The next highest sequence similarities to this genus was 88%, for members of *Shewanella* and *Moritella*, and 87 and 85% for the genera *Alteromonas* and *Marinomonas*, respectively.

DISCUSSION

The strain that we investigated was isolated from an adult tunicate collected in Gullmarsfjorden on the western coast of Sweden. The isolate is a pigmented, motile and Gram-negative rod, with a G+C content of 42·2 ± 0·7 mol %. Strain D2^T is oxidase-positive, can hydrolyse gelatin and requires Na^+ ion for growth with an optimal growth at 1–2% Na^+ ion concentration. D2^T can grow at 4 °C but not at 37 °C and is susceptible to all antibiotics tested. Strain D2^T possesses a sheathed flagellum which may disallow inclusion in the genus *Pseudoalteromonas*. However, earlier studies have suggested that similar bacteria with sheathed flagella may be admitted to the genus *Pseudoalteromonas* (Novic & Tyler, 1985). Enger *et al.* (1987) also classified an isolate, *P. denitrificans*, with a sheathed flagellum to the genus *Pseudoalteromonas*. A further distinctive difference to the generic description of the genus *Pseudoalteromonas* is that D2^T is a facultative anaerobic bacterium, however, similar results have also been reported for *P. denitrificans* (Enger *et al.*, 1987) and *Pseudoalteromonas* sp. S9 (Techkarnjanaruk *et al.*, 1997; Wrangstadh *et al.*, 1986). We therefore do not regard the presence of a flagellar sheath and the ability to grow anaerobically as decisive for exclusion of our strain from the genus *Pseudoalteromonas*.

The genus *Pseudoalteromonas* classification is somewhat unsatisfactory (Akagawa-Matsushita *et al.*, 1993) given that the present members of this taxonomic group are related by 16S rDNA sequence identities as low as 91%. The D2^T isolate showed the highest similarities with *P. citrea* (93·4%) and *P. aurantia* (93·3%). These two species also have many characteristics in common with *P. tunicata*. When comparing 23 taxonomic parameters (Table 3) the proposed type strain of *P. tunicata* (D2^T) differed by only six characteristics from the profiles of *P. citrea* and *P. aurantia*. All three species are pigmented, can hydrolyse gelatin, and can utilize mannose. None of the species can utilize sucrose, citrate, glycerol and sorbitol. In Table 3, the comparisons of characteristics between *P. tunicata* and other *Pseudoalteromonas* strains show that most strains differ by between nine and six characteristics except *P. haloplanktis* subsp. *haloplanktis* (12 characteristics) and *Alteromonas macleodii* (13 characteristics). The latter species also showed less than 90% similarities with D2^T 16S rDNA sequence. With respect to evolutionary distances among strains assigned to genus *Pseudoalteromonas* the closest phylogenetic relative to *P. tunicata* is *P. denitrificans*. *P. denitrificans* has been classified to have a sheathed flagellum and can grow anaerobically. These strains, like D2^T cells, can show varied expression of pigment depending on the growth medium that is employed (Enger *et al.*, 1987). This is a characteristic which is also expressed by *Shewanella hanedai* (Baumann *et al.*, 1984) and *P. nigrifaciens* (Ivanova *et al.*, 1996).

P. tunicata express at least one diffusable dark pigment when grown on the complex marine medium VNSS. The identity of the pigment(s) are not yet known but it was observed that the pigment was produced and released into the supernatant at the same time as the antilarval and antibacterial components. However, it has clearly been shown that the dark pigment produced by the bacterium is not a fouling-inhibitory component (Holmström *et al.*, 1992). However, it is possible that the active components may be produced by the same pathway or by a branch of the pathway leading to the pigment production. The genus *Pseudoalteromonas*

Table 3. Differential characteristics of *Pseudoalteromonas* species and *Alteromonas macleodii*

Data from references 4 and 12. +, Positive; −, negative; d, 11–89% of the strains are positive; ND, not determined.

Characteristic	*P. tunicata*	*P. haloplanktis* subsp. *haloplanktis*	*P. denitrificans*	*P. macleodii*	*P. undina*	*P. rubra*	*P. citrea*	*P. aurantia*
Growth at 4 °C	+	−	+	−	d	−	−	+
Growth at 35 °C	+	d	−	+	−	+	d	−
Pigmentation	+	−	+	−	−	+	+	+
Hydrolysis of gelatin	+	+	+	+	+	+	+	+
Utilization of:								
Mannose	+	+	ND	−	−	+	+	+
Sucrose	−	+	−	+	+	−	−	
Maltose	+	+	+	+	+	−	−	d
Sorbitol	−	−	−	−	−	−	−	−
Fructose	−	d	−	+	−	−	+	+
Citrate	−	+	−	−	−	−	−	−
Glycerol	−	−	−	+	−	−	−	−
Galactose	−	d	−	+	−	−	−	−
Lactose	−	−	−	+	−	−	−	−
Melibiose	−	−	ND	+	−	ND	−	−
Mannitol	−	d	−	d	−	−	−	−
Xylose	−	−	ND	d	−	−	−	−
Trehalose	+	d	ND	+	+	+	+	+
Inositol	−	−	ND	−	−	−	−	−
Rhamnose	−	−	ND	−	−	−	−	−
Glycine	−	d	ND	d	+	ND	ND	+
L-Proline	+	d	+	−	d	ND	−	−
L-Histidine	−	d	ND	−	−	ND	−	d
Oxidase	+	+	+	+	+	+	+	+
G+C content (mol%)	42–43	41–45	37	43–44	43–44	46–48	41–45	38–43
No. of characteristics differing from *P. tunicata*		12	9	13	7	6	6	6

contains both pigmented and non-pigmented strains. Many of the strains within this genus produce metabolites active against other organisms. Five pigmented species belonging to *Pseudoalteromonas* have been found to produce inhibitory components. Four of these species, *P. rubra*, *P. luteoviolacea*, *P. citrea* and *P. aurantia* have been shown to produce high-molecular-mass components which are autotoxic and active against both Gram-positive and Gram-negative bacteria (Baumann *et al.*, 1984). This is a characteristic which *P. tunicata* also possesses (James *et al.*, 1996). The fifth species, *P. piscida*, has been suggested to cause fish death (Bein, 1954). Furthermore, the non-pigmented *P. haloplanktis* subsp. *tetraodonis* (Akagawa-Matsushita *et al.*, 1993; Simidu *et al.*, 1990) has been found to produce tetrodotoxin (Simidu *et al.*, 1990). *P. denitrificans* can also produce autotoxic substances that kill cells and inhibit growth in dense bacterial cultures (Enger *et al.*, 1987). Specific biological activity has furthermore been demonstrated for other *Pseudoalteromonas* strains. *Pseudoalteromonas* sp. S9 and *Pseudoalteromonas colwelliana* have been shown to contribute to larval settlement (Szewzyk *et al.*, 1991; Weiner *et al.*, 1988).

P. tunicata cells are dominated by monoenoic fatty acids (> 70%) which is typical of Gram-negative bacteria. The fatty acids 16:1ω7*c* and 16:0 were the dominant fatty acids in both the cell and the supernatant extracts. These two fatty acids were also the dominant molecules when D2^T was grown in Tryptone soy broth complemented with 2% (w/v) NaCl (data not shown). This result is in agreement with the findings by Bozal *et al.* (1997) who performed a fatty acid analysis on *Pseudoalteromonas antarctica* species. They showed that the two fatty acids,16:1ω7*c* and 16:0, were the dominant components. Svetashev *et al.* (1995) determined cellular fatty acid composition of seven type strains of *Pseudoalteromonas* species. They also showed that the fatty acids 16:1ω7*c* and 16:0 were the main components. In addition, only low amounts of branched-chain and hydroxy fatty acids were detected in the seven type strains (Svetashev *et al.*, 1995). These results are in agreement with the data on the fatty acid composition of *P. tunicata* cells.

Description of *Pseudoalteromonas tunicata*

Pseudoalteromonas tunicata (tu.ni.ca'ta L. fem. n. *tunicata* shirt-like classical piece of Roman underwear, tunic; L. fem. adj. *tunicata* clothed with a tunic).

Facultative anaerobic Gram-negative rod shaped cells that are 2·0–3·4 μm in length. Motile by means of a polar sheathed flagellum. Growth on VNSS medium results in dark-green-pigmented colonies while growth on TSB medium results in white colonies. Does not ferment sugar in the Hugh–Leifson test. Sodium ions are required for growth with the optimal NaCl concentration of 1–2%. Cannot grow at pH 5 or 11; optimal pH range for growth is pH 7–8. Slow growth occurs at 4 °C and no growth is detectable at 37 °C. Oxidase- and catalase-positive. Utilizes trehalose, glu-

cose, maltose and Tween 20 at a concentration of 4 g l^{-1} but can not grow on galactose, fructose, xylose, sucrose, lactose, raffinose, melibiose, glycerol, mannitol, rhamnose, sorbitol, inositol, dulcitol, melexitose, xylitol, erythritol, glycine, threonine, arabinose, L-aspargine and L-histidine. Positive for hydrolysis of gelatin. Sensitive to erythromycin, rifampicin, gentamicin, tetracycline, ampicillin, neomycin, kanamycin and nalidixic acid at a concentration of 100 μg ml^{-1} and is sensitive to the vibriostatic agent 0/129 at a concentration of 150 μg ml^{-1}. The G+C content of the DNA is 42·2±0·7 mol%. The main cellular fatty acids are 16:1ω7*c*, 16:0, 18:1ω7*c* and 16:1ω7*t*, respectively. Strain D2^T is negative for H_2S and NO_2 production and no β-galactosidase, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase activity has been detected. Isolated from an adult tunicate, *Ciona intestinalis*, collected from water off the western coast of Sweden. The type strain has been deposited in the Culture Collection of University of Göteborg as strain CCUG 26757^T.

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