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NOTE

Pseudoalteromonas maricaloris sp. nov., isolated from an Australian sponge, and reclassification of [*Pseudoalteromonas aurantia*] NCIMB 2033 as *Pseudoalteromonas flavipulchra* sp. nov.

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A marine, Gram-negative, aerobic bacterium that produced cytotoxic, lemon-yellow, chromopeptide pigments that inhibited the development of sea urchin eggs has been isolated from the Australian sponge *Fascaplysinopsis reticulata* Hentschel. The cells of the organism were rod-shaped with a single polar flagellum and they required NaCl for growth (0·5–10%) with optimum growth at 1–3% NaCl. The temperature for growth was 10–37 °C, with optimum growth at 25–30 °C. Growth occurred at pH values from 6·0 to 10·0, with optimum growth at pH 6·0–8·0. Major phospholipids were phosphatidylethanolamine, phosphatidylglycerol and lyso-phosphatidylethanolamine. Of 26 fatty acids with 11–19 carbon atoms that were detected, 16:1ω7, 16:0, 17:1ω8 and 18:1ω7 were predominant. The DNA G+C content was 38·9 mol%. All of these phenotypic and chemotaxonomic characters place the organism in the genus *Pseudoalteromonas* (Gauthier *et al.*, 1995). These data are consistent with the phylogenetic analyses that confirmed that strain KMM 636^T is a member of the *Pseudoalteromonas* cluster in the γ-subclass of the *Proteobacteria*. DNA–DNA hybridization experiments revealed that the levels of relatedness between the DNA of the strain studied and DNAs of type strains of the species that clustered together (on the basis of 16S rDNA sequences) and [*Pseudoalteromonas aurantia*] NCIMB 2033 ranged from 19 to 35%, and that the DNA–DNA homology between [*P. aurantia*] NCIMB 2033 and other phylogenetically and/or phenotypically similar type strains ranged from 32 to 52%. According to the polyphasic evidence presented in this study, it is proposed that strain KMM 636^T (= LMG 19692^T = CIP 106859^T) be classified as *Pseudoalteromonas maricaloris* sp. nov. and [*P. aurantia*] NCIMB 2033 be reclassified as *Pseudoalteromonas flavipulchra* NCIMB 2033^T (= KMM 3630^T = LMG 20361^T) sp. nov.

Keywords: marine *Proteobacteria*, *Pseudoalteromonas maricaloris*, *Pseudoalteromonas flavipulchra*

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Abbreviation: NSW, natural seawater.

The GenBank accessions numbers for the 16S rDNA sequences of *Pseudoalteromonas maricaloris* KMM 636^T and *Pseudoalteromonas flavipulchra* NCIMB 2033^T are AF144036 and AF297958.

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The genus *Pseudoalteromonas*, as currently defined (Gauthier *et al.*, 1995), comprises heterotrophic, Gram-negative, aerobic, rod-shaped, polarly flagellated bacteria that are common inhabitants of marine environments (sea water, algae, marine invertebrates) (Baumann & Baumann, 1981; Baumann *et al.*, 1984; Gauthier & Breittmayer, 1992). Despite difficulties concerning the identification of environmental isolates, the list of species of the genus *Pseudoalteromonas* is constantly growing (Bozal *et al.*, 1997; Bowman, 1998, Sawabe *et al.*, 1998b; Holmström *et al.*, 1998).

Taxonomic investigations of marine heterotrophic bacteria from the Collection of Marine Micro-organisms (KMM) of the Pacific Institute of Bio-organic Chemistry revealed a diverse group of *Alteromonas*-like bacteria that represent a few novel taxa (Ivanova *et al.*, 2000a; Sawabe *et al.*, 2000) and recognized species with unusual features (Ivanova *et al.*, 1996, 1998). In this study, we describe a bacterium associated with the sponge *Fascaplysinopsis reticulata* Hentschel 1912 collected in the Coral Sea (Great Barrier Reef). The strain produced chromopeptide pigments with antibiotic and cytotoxic activities that inhibited the development of sea urchin eggs. The results of phenotypic, chemotaxonomic, genetic and phylogenetic analysis of the novel bacterium and some other yellow-pigmented strains of the genus have led us to the conclusion that strain KMM 636^T should be classified as *Pseudoalteromonas maricaloris* sp. nov. and that [*Pseudoalteromonas aurantia*] strain NCIMB 2033 should be reclassified as *Pseudoalteromonas flavipulchra* sp. nov.

The sponge *Fascaplysinopsis reticulata* Hentschel 1912 (family Irciniidae, order Dicetyoceratida) was collected from a depth of 10 m (salinity 32‰, temperature 20 °C) in the Coral Sea of the Pacific Ocean (Isle Lizzard; latitude 14° 30′ 2″ S, longitude 144° 56′ 9″ E) in December 1990. After initial isolation, a strain was purified on medium B [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, 0·002% (w/v) KH_2PO_4, 0·005% (w/v) $MgSO_4.7H_2O$, 50% (v/v) natural seawater (NSW)] at pH 7·5–7·8 as described elsewhere (Ivanova *et al.*, 1996). The bacterium was maintained on the same semi-solid medium B in tubes under mineral oil at 4 °C and stored at −80 °C in marine broth (Difco) supplemented with 30% (v/v) glycerol. The strain was streaked on agar plates from tubes every 6 months to control purity and viability.

Pseudoalteromonas citrea ATCC 29719^T, *P. aurantia* DSM 6057^T, [*P. aurantia*] NCIMB 2033, *Pseudoalteromonas luteoviolacea* ATCC 33492^T, *Pseudoalteromonas rubra* ATCC 29570^T, *Pseudoalteromonas peptidolytica* MBICC F1250A1^T, *Pseudoalteromonas piscicida* IAM 12932^T and *P. piscicida* NCMB 645 were obtained from the ATCC (Manassas, VA, USA), the National Collection of Industrial and Marine Bacteria (NCIMB, UK) and the Marine Biotechnology Institute (MBI, Japan). All reference strains were cultured routinely on plates of marine agar 2216 (Difco).

Unless indicated otherwise, phenotypic properties used for characterization of *Alteromonas*-like species were assessed by using standard procedures (Baumann *et al.*, 1972; Baumann & Baumann, 1981; Smibert & Krieg, 1994; Ivanova *et al.*, 1996). Temperature tolerance for growth was examined on medium B incubated at 4, 10, 37 and 42 °C. Utilization of various organic substrates [0·1% (w/v); listed in Table 1] as sole carbon sources was tested using BM broth medium (Baumann *et al.*, 1972). Bacteria were grown at 24–26 °C. The ability to oxidize organic substrates was investigated using BIOLOG-GN plates (BIOLOG) as described elsewhere (Ivanova *et al.*, 1998).

Yellow pigments were extracted with methanol from 5-d-old bacterial cells grown on plates of Tryptic soy agar (TSA, Oxoid) at 28 °C. Dried extract was separated by Sephadex LH-20 with chloroform/methanol (1:1) as eluant and isocratic reversed-phase HPLC (30% aqueous methanol) revealed a mixture of two pigments (A and B).

For bioassays of cytotoxic and antibacterial activities, eggs and sperm were taken from the gonads of the sea urchin *Strongylocentrotus intermedius* collected in Troitza Bay of the Sea of Japan (Russia). The cleavage rate of blastomers was determined in accordance with Biyiti *et al.* (1990). Eggs were rinsed, filtered and diluted with NSW to a concentration of 2000 eggs ml^{-1}. Sperm was collected 'dry' and the semen was diluted (1:50) shortly before use with NSW.

Cytotoxicity on Ehrlich cells was tested as described by Sasaki *et al.* (1985). Ascites tumour cells of Ehrlich carcinoma (tetraploid strain) were taken 7–8 d after tumour inoculation into stainless white mice of both sexes. The cell suspension was diluted 5:1 (v/v) with saline, centrifuged at 3000 ***g*** for 10 min and the cells were then resuspended in culture medium 199 containing penicillin and streptomycin (250 U ml^{-1}) to a final concentration of 6–8 million cells ml^{-1}.

Antibacterial activity was assessed by the agar diffusion assay, based on the method described by Barry (1980). Cultures (0·1 ml) of test strains were spread on TSA plates in which circular wells (diameter 10 mm) had been cut. Samples (0·1 ml) were added to the wells and areas of inhibited bacterial growth were measured after incubation for 48 h at 28 °C. Zones of inhibited growth of the indicator strains surrounding the wells were observed. Mean diameters were measured and 10 mm was subtracted (representing the diameter of the well). Antibacterial activities were tested against *Staphylococcus aureus* CIP 103594^T, *Escherichia coli* ATCC 15034, *Proteus vulgaris* IFO 3851, *Enterococcus faecium* CIP 104105, *Bacillus subtilis* ATCC 6051^T and *Candida albicans* KMM 455. Test strains were obtained from the collection of bacterial strains of the Pasteur Institute (CIP), France, the ATCC and the

Table 1. Phenotypic features of *P. maricaloris* KMM 636^T, *P. flavipulchra* NCIMB 2033^T and other yellow-pigmented species of the genus *Pseudoalteromonas*

Strains: 1, *P. maricaloris* KMM 636^T; 2, *P. flavipulchra* NCIMB 2033^T; 3, *P. piscicida* IAM 12932^T; 4, *P. citrea* ATCC 29719^T; 5, *P. aurantia* DSM 6057^T; 6, *P. peptidolytica* MBICC F1250A1^T. All strains studied exhibit polar flagella, require sodium ions for growth, are positive for oxidase and catalase, are negative for denitrification, arginine dihydrolase activity and production of agarase and chitinase, do not produce lipase, produce amylase and gelatinase, grow at 10, 28 and 30 °C and 1, 3, 6 and 10 % NaCl, utilize D-glucose, D-mannose and fructose, are susceptible to kanamycin, erythromycin, gentamicin and oleandomycin and are not susceptible to benzylpenicillin, oxacillin, lincomycin or O/129. Data were taken from this study and from Gauthier & Breittmayer (1992), Hansen *et al.* (1965), Ivanova *et al.* (1998) and Venkateswaran & Dohmoto (2000). ND, No data available.

Characteristic	1	2	3	4	5	6
Requirement for organic growth factors	–	–	ND	+	+	–
Growth at:						
4 °C	–	–	–	–	+	–
37 °C	+	+	+	–	–	+
Growth at 10 % NaCl	+	+	+	+	–	+
Production of chitinase	–	+	–	–	–	ND
Utilization of:						
Maltose	+	+	–	–	–	+
D-Galactose	+	–	+	–	–	–
D-Arabinose	+	–	–	–	–	
Sucrose	+	+	–	–	–	–
Melibiose	+	–	–	–	–	ND
Mannitol	+	–	–	–	–	–
Sorbitol	+	–	–	–	–	–
Citrate	+	+	+	–	–	–
Glycerol	+	–	–	–	–	–
L-Arginine	+	–	ND	–	+	ND
Susceptibility to:						
Ampicillin (10 μg)	–	–	–	–	+	ND
Streptomycin (10 μg)	+	–	+	–	–	ND
Tetracycline (30 μg)	–	–	–	–	–	ND
Vancomycin (30 μg)	–	ND	+	+	–	ND

culture collection of the Institute for Fermentation (IFO), Osaka, Japan.

A heterotrophic marine bacterium associated with the sponge *Fascaplysinopsis reticulata* dwelling in the Coral Sea at a depth of 10–40 m had all the phenotypic characteristics of the genus *Pseudoalteromonas*. Cells of the novel isolate were Gram-negative, strictly aerobic, oxidase-positive, yellow-pigmented rods, 0·7–0·9 μm in diameter and 1·0–1·2 μm long with a single polar flagellum. Colonies were uniformly round, 2–3 mm in diameter, circular, regular, convex, translucent, smooth and lemon-yellow after incubation for 48 h on marine agar. No diffusible pigment was produced in the medium. The bacterium did not form endospores, did not accumulate poly-β-hydroxybutyrate as an intracellular reserve product and did not have an arginine dihydrolase, was oxidase- and catalase-positive and required the addition of 0·5–10 % NaCl or seawater for growth, with optimum growth at 1–3 % NaCl. The temperature range for growth was 10–37 °C, with optimum growth at 28–30 °C. No growth was detected at 42 °C. The pH range for growth was 6·0–10·0, with optimum growth at pH 7·5–8·0. Gelatin and starch were decomposed. Agar-agar and chitin were not hydrolysed. The range of substrates oxidized (according to BIOLOG) is presented in Table 1.

Two pigments extracted from wet cells and designated A and B had UV-VIS maxima at 400 nm and double $[M+H]^+$ signals at 844/846 and 858/860 (HPLC/electrospray ionization mass spectrometry), respectively. The mass differences, $\Delta m = 2$, of the molecular ions and their intensities pointed to monobrominated compounds. Pigments A and B were different from those of other pigmented pseudoalteromonads, *P. rubra* (Gauthier, 1976), *P. luteoviolacea* (Gauthier & Flatau, 1976; Gauthier, 1982), *Pseudoalteromonas denitrificans* (Enger *et al.*, 1987), *Pseudoalteromonas bacteriolytica* (Sawabe *et al.*, 1998b) and *Pseudoalteromonas tunicata* (Holmström *et al.*, 1998), and the water-soluble yellow pigments of *P. piscicida*, *P. citrea* and *P. aurantia* (Gauthier, 1977; Gauthier & Breittmayer, 1979). Based on these and other results obtained (Kuznetsova *et al.*, 1995), it may be concluded that pigments A and B represent a novel brominated chromopeptide. Both pigments of strain KMM 636^T showed antibacterial activity against *S.*

Table 2. Polar lipid and cellular fatty acid composition of *P. maricaloris* KMM 636^T; *P. flavipulchra* NCIMB 2033^T and some other pigmented *Pseudoalteromonas* species

Values are percentages of the total content. Strains: 1, *P. maricaloris* KMM 636^T; 2, *P. flavipulchra* NCIMB 2033^T; 3, *P. piscicida*; 4, *P. citrea* ATCC 29719^T; 5, *P. aurantia* DSM 6057^T; 6, *P. rubra* ATCC 29570^T; 7, *P. luteoviolacea* NCIMB 1893^T; 8, *P. peptidolytica* MBICC F1250A1^T. ND, No data available. Data for *P. piscicida* are means for strains IAM 12932^T and NCIMB 645. Data were taken from this study and from Ivanova *et al.* (2000b) and Venkateswaran & Dohmoto (2000).

Component	1	2	3	4	5	6	7	8
Polar lipids								
Phosphatidylethanolamine	49·2	63·5	77·1	77·5	66·3	64·0	67·8	ND
Phosphatidylglycerol	21·8	14·3	17·7	17·5	20·1	26·1	19·8	ND
Bisphosphatidic acid	1·3	2·2	1·5	1·7	2·9	2·0	4·2	ND
Lyso-phosphatidylethanolamine	22·1	16·3	1·0	2·0	5·6	5·0	5·1	ND
Phosphatidic acid	2·5	3·7	1·0	1·4	3·6	2·9	3·1	ND
PL-a*	0·8		1·2		1·5			ND
PL-b*	2·3							ND
Fatty acids								
11:0-3OH	0·16	0·22	0·2	0·18	0	0·2	0·13	ND
12:0	0	0	0·6	0	1·0	0	0	1·85
12:0-3OH	0·21	0·16	0·3	1·09	0·6	0·22	0·17	6·14
12:1	0·81	0·53	0·9	0·6	5·2	0·54	0·47	ND
i13:0	0	0	0	0	0	0	0·26	ND
13:0	0·34	0·16	0·4	0·31	0	0·51	0·8	ND
13:1	0·53	0·5	0·7	0·15	0	0·48	1·01	0·14
i14:0	0·1	0·1	0·05	0·2	0	0·19	0·44	ND
14:0	2·4	0·99	3·8	3·1	0·4	1·6	3·94	3·11
14:1ω7	0·62	0·24	1·0	0·99	1·5	0·46	0·83	ND
a15:0	0·25	0·16	0·25	0·54	1·0	0·19	0·31	ND
15:0	5·54	2·68	1·7	2·98	1·5	3·78	2·62	ND
15:1ω8	2·8	2·35	1·7	3·8	0·9	1·68	1·37	0·35
15:1ω6	0·38	0·22	0·1	0·19	0·1	0·19	0·16	ND
i16:0	0·95	1·96	0·7	1·12	0·2	1·71	1·14	0·12
16:0	16·3	17·58	27·4	21·24	24·1	16·5	17·13	17·46
16:1ω7	35·89	29·56	40·2	41·2	44·4	34·44	33·71	ND
16:1ω5	0·28	0·17	0·19	0·52	0·1	0·16	0·16	ND
i17:0	0·1	0·22	0·2	0·37	0·1	0·26	0·11	ND
a17:0	0·14	0·16	0	0·31	0·2	0	0	ND
17:0	4·04	6·72	2·15	1·57	1·9	3·58	3·25	ND
17:1ω8	15·98	18·95	5·5	6·44	2·9	14·23	9·4	0·19
17:1ω6	1·37	1·31	0·4	0·55	0·2	0·59	1·14	ND
i18:0	0·12	0·35	0	0·12	0	0·97	0·18	ND
18:0	0·44	0·65	1·4	0·52	1·1	0·61	0·84	0·93
18:1ω11	0·19	0·12	0	0	0	0·15	0·12	ND
18:1ω9	0·38	0·37	0·3	0·24	0	0·48	0·37	0·39
18:1ω7	6·76	6·85	7·6	7·09	11·3	11·19	16·03	3·03
19:1	0·41	0·72	0·16	0·13	0·1	0·84	0·54	ND

* Unidentified phospholipids.

aureus CIP 103594^T, *Enterococcus faecium* CIP 104105, *B. subtilis* ATCC 6051^T and *C. albicans* KMM 455 and cytotoxicity on Ehrlich cells and sea urchin eggs. Notably, the pigments of KMM 636^T differ from pigments isolated from the sponge *F. reticulata*, red fascaplysins (Gribble & Pelcman, 1992) and yellow aplysinopsin (Kazlauskas *et al.*, 1977). The ecological role and functions of bacterial cytotoxins and antibiotics have been discussed intensively though not fully elucidated (Ballester *et al.*, 1977; Roszak & Colwell, 1987; Anderson *et al.*, 1974; Lemos *et al.*, 1985; Gil-Turnes *et al.*, 1989). The likely symbiotrophic relationship between the bacterial population and the sponge might cause the bacterial production of cytotoxins as a chemical defence essential in aquatic environments.

Table 3. DNA relatedness among tested strains

Organism	G + C content (mol %)	Hybridization with DNA from: (%)	
		P. maricaloris KMM 636^T	*P. flavipulchra* NCIMB 2033^T
P. maricaloris KMM 636^T	38·9	100	
P. flavipulchra NCIMB 2033^T	41·7	21	100
P. piscicida IAM 12932^T	42·7	22	32
P. piscicida NCIMB 645	43·1	19	
P. peptidolytica MBICC F1250A1^T	37·9	27	45
P. aurantia DSM 6057^T	44·1	22	52
P. citrea ATCC 29719^T	42·1	24	48
P. antarctica CECT 4664^T	42·3	19	
P. atlantica IAM 12927^T	42·1	18	
P. elyakovii KMM 162^T	40·1	32	
P. haloplanktis IAM 12915^T	40·2	31	
P. luteoviolacea NCIMB 1893^T	42·0	21	
P. rubra ATCC 29570^T	39·0	31	
P. tunicata CCUG 26757^T	42·3	29	

For phospholipid analysis, the bacteria were harvested at late-exponential phase. Lipids were extracted by the method of Bligh & Dyer (1959). Phospholipids were separated by TLC on a silica gel plate (Serva) with solvents A [chloroform/methanol/acetone/acetic acid; 65:30:6:10 by vol.] and B [chloroform/methanol/acetone/acetic acid/benzene/water; 70:30:5:4:10:1 by vol.]. Phospholipids were visualized on the TLC plate by heating at 180 °C after spraying with 10% H_2SO_4 in methanol. The following specific reagents were used: for phospholipids (Bligh & Dyer, 1959), for amino-containing lipids (2% ninhydrin in acetone), Dragendorff's reagent for choline lipids and anthrone spray (0·5% anthrone in benzene and 5% H_2SO_4 in water) for glycolipids. Spots identified on the TLC plate were scraped off and extracted. Quantities of phospholipid were determined by the method of Bartlett (1959). The overall polar lipid pattern of KMM 636^T was similar to the patterns determined for both strains of *P. piscicida*, DSM 6057^T and NCIMB 645, *P. citrea* ATCC 29719^T, *P. aurantia* IAM 12932^T, [*P. aurantia*] NCIMB 2033, *P. rubra* ATCC 29570^T and *P. luteoviolacea* NCIMB 1893^T (Table 2). Major phospholipids (PL) were phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and lyso-phosphatidylethanolamine (LPE). The amount of the latter PL was notably higher and the amount of PE was relatively lower than those of the other tested species. Two unidentified PL were present in minor amounts.

Analysis of fatty acid methyl ethers (FAME) was performed by GLC as described previously by Svetashev *et al.* (1995). Of 26 fatty acids detected with 11–19 carbon atoms, 16:1ω7, 16:0, 17:1ω8 and 18:1ω7 were predominant (about 80% of the total), while 14:0, 15:0, 15:1ω8 and 17:0 were present in minor quantities (Table 2). FAME analysis of the type strains of the yellow-pigmented strains *P. citrea* ATCC 29719^T, *P. aurantia* IAM 12932^T, [*P. aurantia*] NCIMB 2033, two strains of *P. piscicida*, DSM 6057^T and NCIMB 645, and some other pigmented members of the genus, *P. rubra* ATCC 29570^T and *P. luteoviolacea* NCIMB 1893^T, showed similar patterns that are consistent with FAME profiles for the remaining *Pseudoalteromonas* species (Svetashev *et al.*, 1995; Bozal *et al.*, 1997; Holmström *et al.*, 1998). However, it is interesting to note that, when compared with other pigmented species, the FAME pattern of strain KMM 636^T was more similar to those of [*P. aurantia*] NCIMB 2033, *P. rubra* ATCC 29570^T and *P. luteoviolacea* NCIMB 1893^T. Also interesting in this regard are the amounts of 17:1ω8, which is more than twice as abundant, and 16:0, which is significantly lower, for strain KMM 636^T, [*P. aurantia*] NCIMB 2033, *P. rubra* ATCC 29570^T and *P. luteoviolacea* NCIMB 1893^T than for the two strains of *P. piscicida* studied, *P. citrea* ATCC 29719^T and *P. aurantia* IAM 12932^T.

DNA was isolated following the method of Marmur (1961) and the G+C content of the DNA was determined using the thermal denaturation method of Marmur & Doty (1962). DNA–DNA hybridization was performed spectrophotometrically and initial renaturation rates were recorded in 2 × SSC (1 × SSC is 0·15 M NaCl, 15 mM sodium citrate, pH 7·0) at optimal temperature [$T = 0{\cdot}51$ mol%(G+C) + 47·0] as described by De Ley *et al.* (1970). The G+C content of the DNA of strain KMM 636^T was 38·9 ± 0·4 mol% (thermal denaturation method). DNA–DNA hybridization results showed that the levels of genetic similarity of KMM 636^T to the type strains of the genus and some other yellow-pigmented strains ranged from 19 to 32% (Table 3). In addition, DNA–DNA hybridization data revealed that DNA from strain [*P. aurantia*] NCIMB 2033 showed 32–52% genetic relatedness to DNA from the type strains of *P. piscicida*, *P. peptidolytica*, *P. citrea* and *P. aurantia*. Based on generally accepted criteria for the definition of a species

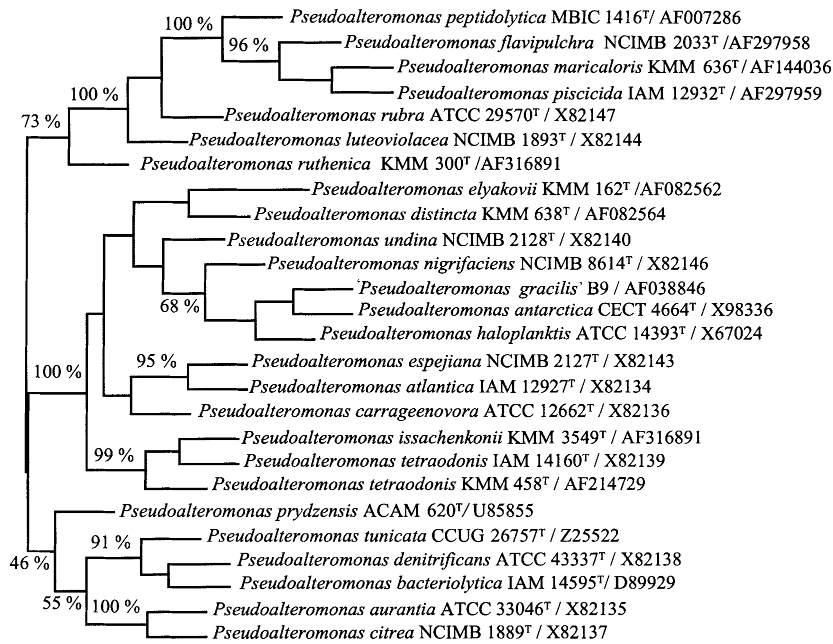

Fig. 1. Phylogenetic position of *Pseudoalteromonas maricaloris* KMM 636^{T} and *Pseudoalteromonas flavipulchra* NCIMB 2033^{T} within the genus *Pseudoalteromonas*. Unrooted tree obtained using a bioNJ algorithm, Kimura's two-parameter correction for distance calculations and 500 replications in a bootstrap analysis. Bootstrap percentages are indicated only for those branches that were also found in maximum-likelihood ($P < 0{\cdot}01$) and parsimony (most parsimonious tree) analyses.

(Wayne *et al.*, 1987), strain KMM 636^{T} and [*P. aurantia*] NCIMB 2033 can be assigned to two separate species.

The bacterium designated strain 201 (= NCIMB 2033 = ATCC 33042) was isolated originally by Gauthier & Breittmayer (1979) from seawater off Nice, France, as one of six orange-pigmented bacteria isolated either from water of the same area or from the surface of the seaweed *Ulva lactuca*. All six strains were assigned to *P. aurantia* (formerly *Alteromonas aurantia*), though the authors noted that two strains, NCIMB 2033 and ATCC 33043 (no longer available), had some phenotypic features of *P. piscicida* [formerly *Pseudomonas piscicida* (Bein) Buck *et al.* 1963]. During the course of this study, we re-investigated the phenotypic and chemotaxonomic characteristics of [*P. aurantia*] NCIMB 2033 and found a number of features that are useful for distinguishing this strain from other related species (Tables 1 and 2). In contrast to *P. piscicida*, [*P. aurantia*] NCIMB 2033 produced chitinase, utilized maltose and sucrose and was not susceptible to streptomycin. Strain KMM 636^{T} can be distinguished easily by utilization of glycerol, maltose, sucrose, D-arabinose, melibiose, mannitol and sorbitol, fatty acid and phospholipid composition and the production of chromopeptide pigments.

In order to clarify further the phylogenetic relationship between strain KMM 636^{T} and [*P. aurantia*] NCIMB 2033 and other species of the genus, we undertook a phylogenetic analysis. For DNA amplification and sequencing, bacterial DNAs were prepared using the Promega Wizard genomic DNA extraction kit according to the instruction manual. DNA templates (100 ng) were used in a PCR to amplify the small-subunit rRNA genes as described previously by Sawabe *et al.* (1998a, b). PCR conditions were as follows: initial denaturation step at 94 °C for 180 s, an annealing step at 55 °C for 60 s and an extension step at 72 °C for 90 s. The thermal profile consisted of 30 cycles. The amplification primers used in this study gave a 1·5 kb PCR product and corresponded to positions 25–1521 of the *Escherichia coli* sequence. The PCR products were purified using the Promega Wizard PCR preps DNA purification kit and sequenced directly by using a *Taq* FS dye terminator sequencing kit (ABI) and the protocol recommended by the manufacturer. DNA sequencing was performed with an Applied Biosystems model 373A automated sequencer. Nine primers were used for sequencing (Sawabe *et al.*, 1998a).

The 16S rDNA sequences were aligned automatically and then manually by reference to a database of 20000 already-aligned bacterial 16S rDNA sequences. Phylogenetic trees were constructed according to three different methods (bioNJ, maximum-likelihood and maximum-parsimony). For the neighbour-joining (NJ) analysis, a distance matrix was calculated according to Kimura's two-parameter correction. Bootstraps were done using 500 replications, bioNJ and Kimura's two-parameter correction. BioNJ was done according to Gascuel (1997) and maximum-likelihood and maximum-parsimony data were from PHYLIP (Phylogeny Inference Package, version 3.573c; distributed by J. Felsenstein, Department of Genetics, University of Washington, Seattle, WA, USA). Phylogenetic trees were drawn using NJPLOT (Perrière & Gouy, 1996) and CLARIS DRAW software for Apple Macintosh.

Domains used to construct phylogenetic trees were regions of the small-subunit rDNA sequences that were available for all sequences and excluding positions likely to show homoplasy. For Fig. 1, we used almost the entire 16S rDNA sequences of species of the genus *Pseudoalteromonas*, corresponding to positions

45–1351 of the KMM 636^T and [*P. aurantia*] NCIMB 2033 sequences. The topology shown is that of the bootstrap analysis. See Ivanova *et al.* (2002) for further consideration of the treeing analysis.

16S rDNA gene sequence analysis revealed that the bacteria studied are members of the γ-subclass of the *Proteobacteria*. These data indicate clearly that strains KMM 636^T and [*P. aurantia*] NCIMB 2033 form a robust clade with *P. piscicida* and that this clade is included in the genus *Pseudoalteromonas*. Phylogenetic analysis of 16S rDNA sequences alone does not allow it to be ascertained that these three strains indeed belong to different species, as phylogenetic analyses of 16S rDNA sequences always group them in a single clade (Fig. 1). A definitive allocation of these strains to three different species is shown by data from DNA–DNA hybridization experiments.

Based on the data of this study, we propose that strain KMM 636^T be placed in a novel species as *Pseudoalteromonas maricaloris* sp. nov. and that [*P. aurantia*] NCIMB 2033 be reclassified as the type strain of *Pseudoalteromonas flavipulchra* sp. nov.

Description of *Pseudoalteromonas maricaloris* sp. nov.

Pseudoalteromonas maricaloris (ma′ri.ca.lo.ris. L. n. *mare* the sea; L. n. *calor* warmth, heat; N.L. gen. n. *maricaloris* from the warm sea).

Rod-shaped cells, single, about 0·7–0·9 μm in diameter. Gram-negative. Motile, with a single polar flagellum. Strictly aerobic. Chemorganotroph with respiratory metabolism. Does not form endospores. Produces two yellow chromopeptide pigments with cytotoxic activity. Does not accumulate poly-β-hydroxybutyrate as an intracellular reserve product and has an arginine dihydrolase system. Oxidase- and catalase-positive. Requires Na^+ ions or seawater for growth. Growth occurs in media with 0·5–10 % NaCl. Temperature for growth ranges from 10 to 37 °C, with optimum growth at 25–35 °C. No growth is detected at 42 °C. The pH for growth ranges from 6·0 to 10·0, with optimum growth at pH 7·5–8·0. Decomposes gelatin and starch. Agar-agar and chitin are not hydrolysed. Positive for utilization of dextrin, glycogen, *N*-acetyl D-galactosamine, D-fructose, α-D-glucose, maltose, D-mannose, sucrose, D-trehalose, β-hydroxybutyric acid, propionic acid, alaninamide, L-alanine, L-asparagine, glycyl L-aspartic acid, glycyl L-glutamic acid, L-proline, inosine, uridine, glucose 1-phosphate and glucose 6-phosphate (according to BIOLOG). Major phospholipids are phosphatidylethanolamine, phosphatidylglycerol and lyso-phosphatidylethanolamine. The main cellular fatty acids are 16:1ω7, 16:0, 17:1ω8 and 18:1ω7 (about 80 % of the total).

Isolated from the sponge *Fascaplysinopsis reticulata*, collected from the Coral Sea. The G+C content of the DNA is 38·9 ± 0·4 mol %. The type strain is KMM 636^T (= LMG 19692^T = CIP 106859^T).

Description of *Pseudoalteromonas flavipulchra* sp. nov.

Pseudoalteromonas flavipulchra (fla′vi.pul.chra. L. adj. *flavus* golden-yellow; L. adj. *pulcher* beautiful; N.L. fem. adj. *flavipulchra* beautifully golden-coloured).

Rod-shaped cells, single, about 0·5–1·5 μm in diameter. Gram-negative. Motile, with a single polar flagellum. Strictly aerobic. Chemorganotroph with respiratory metabolism. Does not form endospores. Produces a non-carotenoid orange pigment. Does not accumulate poly-β-hydroxybutyrate as an intracellular reserve product and has an arginine dihydrolase system. Oxidase- and catalase-positive. Growth occurs in media with 0·5–10 % NaCl. Temperature for growth ranges from 10 to 44 °C, with optimum growth at 25–35 °C. The pH for growth ranges from 5·0 to 12·0, with optimum growth at pH 7·5–8·0. Decomposes gelatin and starch. Agar-agar and chitin are not hydrolysed. Utilizes the following as sole sources of carbon: glucose, mannose, trehalose, glucosamine, *N*-acetyl D-glucosamine, maltose, sucrose, glycogen, leucine, isoleucine, citrulline, succinate, fumarate, malate, pyruvate, citrate, arginine and L-tyrosine. Major phospholipids are phosphatidylethanolamine, phosphatidylglycerol and lyso-phosphatidylethanolamine. The main cellular fatty acids are 16:1ω7, 16:0, 17:1ω8 and 18:1ω7 (about 80 % of the total).

Isolated from surface seawater off Nice, France. The G+C content of the DNA is 41·7 ± 0·4 mol %. The type strain is NCIMB 2033^T (= KMM 3630^T = LMG 20361^T).

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