Microbulbifer variabilis sp. nov. and *Microbulbifer epialgicus* sp. nov., isolated from Pacific marine algae, possess a rod–coccus cell cycle in association with the growth phase

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Phylogenetic and taxonomic characterization was performed for 14 strains of bacteria that produce anticancer antibiotics (pelagiomycins) (represented by strain Ni-2088^T) and one strain that produces UV-absorbing substances (strain F-104^T), isolated from marine algae and seagrass collected from coastal areas of tropical Pacific islands and a subtropical island of Japan. All 15 isolates were Gram-negative, strictly aerobic, non-motile and non-spore-forming. Sequence analysis of the 16S rRNA gene showed that the isolates occupied positions in the phylogenetic radiation of the genus *Microbulbifer*, with similarities of 93.6–97.6%. The cells possessed a clearly discernible rod–coccus cell cycle in association with the growth phase; cells were rods during the growth phase and all converted to coccoid–ovoid cells when proliferation ceased. The coccoid–ovoid cells were optically denser than the rod cells and were viable for extended periods. They were considered to constitute a resting form. The type strains of described species of *Microbulbifer* were also found to possess identical rod–coccus cell cycles. The G + C content of the DNA was 48.1–49.7 mol%. The major respiratory quinone system was ubiquinone-8. The major fatty acids were C_{18:1}ω7c and C_{16:0}, and the hydroxy acids comprised C_{10:0} 3-OH, C_{12:0} 3-OH and iso-C_{11:0} 3-OH. The polar lipids comprised phosphatidylethanolamine, phosphatidylglycerol and phosphatidylserine. The group of 14 pelagiomycin-producing strains and strain F-104^T each constituted a single genomic species. Based on phylogenetic affiliation, phenotypic characteristics and genomic distinctness, the isolates represent two novel species in the genus *Microbulbifer*, for which the names *Microbulbifer variabilis* sp. nov. (type strain Ni-2088^T = MBIC01082^T = ATCC 700307^T) and *Microbulbifer epialgicus* sp. nov. (type strain F-104^T = MBIC03330^T = DSM 18651^T) are proposed.

Abbreviation: UVAS, UV-absorbing substance.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains Ni-2088^T, F-104^T and C-38 are AB167354, AB266054 and AB266055, respectively, that for the *gyrB* gene sequence of strain Ni-2088^T is AB276367 and those for the *ftsZ* gene sequences of strains Ni-2088^T, F-104^T, *M. hydrolyticus* ATCC 700072^T, *M. salipaludis* JCM 11542^T, *M. elongatus* DSM 6810^T and *M. maritimus* JCM 12187^T are AB371302, AB371303, AB371308, AB371307, AB371306 and AB371305, respectively.

Details of strain isolation, a neighbour-joining tree based on concatenated *gyrB* and *ftsZ* sequences, micrographs of cells of the novel strains and reference strains, fatty acid profiles and a dendrogram based on fatty acid data, 2D TLC of polar lipids of representative novel strains and reference strains and detailed DNA–DNA hybridization results are available as supplementary material with the online version of this paper.

The genus *Microbulbifer* was described by González *et al.* (1997) for a biopolymer-decomposing marine gammaproteobacterium, *Microbulbifer hydrolyticus*. Since then, five species of the genus have been isolated from various marine sources (Yoon *et al.*, 2003a, 2004, 2007; Miyazaki *et al.*, 2008) and one species, the agar-degrading *Pseudomonas elongata* (Humm, 1946), has been transferred to the genus (Yoon *et al.*, 2003b).

In the course of screening for marine bacteria that produce bioactive substances, we isolated 14 strains that produce anticancer phenazine antibiotics (pelagiomycins; Imamura *et al.*, 1997) and one strain that produces UV-absorbing substances (UVAS; Nagao *et al.*, 2000) from marine algae

and seagrass collected from tropical Pacific islands and the subtropical Okinawa Prefecture of Japan. Phylogenetically, the isolates belonged to the genus *Microbulbifer* and were found to possess a clearly discernible cell cycle of rod-shaped vegetative cells and resting coccoid cells in association with the growth phase. In this study, we report that this cell cycle is also present in all described *Microbulbifer* species. Based on phylogenetic affiliation, phenotypic characteristics and genetic distinctness, these bacteria represent two novel species of the genus *Microbulbifer*.

The sources of the isolates were marine macroalgae (green, red and brown), seagrass and a cyanobacterium collected from coastal areas of the tropical Pacific islands of Palau in 1991, 1994 and 1995 and Yap in 1994, as well as from the island of Ishigaki Jima in Okinawa Prefecture, a subtropical area of Japan, in 1996 (for detailed sources and sites of isolation, see Supplementary Table S1, available in IJSEM Online). Isolation was conducted immediately after collecting the algal samples at the sampling sites. The surface of an alga or seagrass sample was rubbed with a small piece of sterile gauze, which was then soaked in sterilized seawater and shaken vigorously. Aliquots (100 µl) of each undiluted sample and of a sample diluted 10-fold with artificial seawater (ASW) (Tropic Marin; Aquariene-technik or Marine Art SF; Osaka-Yakken) were spread onto agar isolation plates. The isolation media used were marine agar 2216 (MA; Difco), 1/10-strength MA (1/10 MA) [3.74 g marine broth 2216 (MB) powder (Difco), 750 ml ASW, 250 ml distilled water and 15 g agar, pH 7.6] and MA amended with 10 p.p.m. polymyxin B and 2 p.p.m. penicillin G. The 14 strains of pelagiomycin-producing bacteria and the single UVAS-producing strain were isolated and subjected to phylogenetic and taxonomic characterization. *Microbulbifer hydrolyticus* ATCC 700072^T, *Microbulbifer salipaludis* JCM 11542^T, *Microbulbifer elongatus* ATCC 10144^T, *Microbulbifer maritimus* JCM 12187^T, *Microbulbifer celer* KCTC 12973^T, *Microbulbifer agarilyticus* JCM 14708^T and *Microbulbifer thermotolerans* JCM 14709^T were used as reference strains.

PCR amplification of the 16S rRNA gene was performed using bacterial universal primers 8F (5'-AGAGTTTGAT-CCTGGCTCAG-3') and B9 (5'-TACGGCTACCTTGTTA-CGACTT-3'; Hanzawa *et al.*, 1995). PCR products were purified in a QIAquick PCR purification column (Qiagen) and then sequenced with an ABI Big Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems) and an ABI 3700 DNA sequencer (Applied Biosystems). The 16S rRNA gene sequences of the isolates were compared with other sequences in the public databases (GenBank/DBJ/EMBL) using BLAST (Altschul *et al.*, 1997). Sequences were aligned with CLUSTAL W (Thompson *et al.*, 1994) and edited with the BioEdit program (Hall, 1999). A phylogenetic tree was constructed by analysing the 16S rRNA gene sequences corresponding to positions 64–1411 (*Escherichia coli* numbering system) using the software MEGA version 3.0 (Kumar *et al.*, 2004) and the neighbour-joining method

(Saitou & Nei, 1987). The *gyrB* gene fragment covering positions 274–1525 of the *E. coli gyrB* gene was amplified using deoxyinosine-containing primers UP1Ei (5'-GAAG-TCATCACCGTTCTGCAYGSIGGIGGIAARTTYRA-3') and UP2ri (5'-AGCAGGGTACGGATGTGCGAGCCRTCIACR-TCIGCRTICIGTCAT-3'). Sequencing was performed using primers UP1s (5'-GAAGTCATCACCGTTCTGCA-3') and UP2rs (5'-AGCAGGGTACGGATGTGCGAGCC-3'), as described by Yamamoto & Harayama (1995). The fragment covering positions 141–1110 of the *E. coli ftsZ* gene was amplified and sequenced using forward primer 5'-AAYACNGAYGCNCARGCNYT-3' and reverse primer 5'-AANGCNGGDATRTCNNARRTA-3'. Sequencing and sequence alignment of the *gyrB* and *ftsZ* genes were performed as described for the 16S rRNA gene. A phylogenetic tree based on the concatenated dataset of *gyrB* and *ftsZ* gene sequences was constructed in the same way. The reliability of cluster formation was assessed by a bootstrap analysis based on 1000 resamplings of the dataset (Felsenstein, 1985). Accession numbers of the reference strains used in the phylogenetic analysis are given in Fig. 1 and Supplementary Fig. S1.

Almost-complete 16S rRNA gene sequences were determined for strains Ni-2088^T, C-38 (a pelagiomycin-producing strain) and F-104^T. A comparison against 16S rRNA gene sequences available in DNA databases indicated that the isolates belonged to the class *Gammaproteobacteria* (Fig. 1). The degree of sequence similarity between strains Ni-2088^T and C-38 was 99.9%, and the respective values for strain F-104^T to strains Ni-2088^T and C-38 were 99.3 and 99.2%. Among genera with validly published names, the sequences most closely associated with these strains were those of the genus *Microbulbifer*, which comprises seven species: *M. hydrolyticus* (the type species; González *et al.*, 1997), *M. salipaludis* (Yoon *et al.*, 2003a), *M. elongatus* (formally *Pseudomonas elongata*; Humm, 1946; Yoon *et al.*, 2003b), *M. maritimus* (Yoon *et al.*, 2004), *M. celer* (Yoon *et al.*, 2007), *M. agarilyticus* (Miyazaki *et al.*, 2008) and *M. thermotolerans* (Miyazaki *et al.*, 2008). The respective degrees of sequence similarity of strains Ni-2088^T and F-104^T to the type strain of *M. hydrolyticus* were 94.9 and 94.5%. The isolates formed a phylogenetic cluster with the type strains of *M. maritimus* and *M. thermotolerans* with a bootstrap value of 100%, while the remaining *Microbulbifer* species formed a separate cluster, also with a bootstrap value of 100% (Fig. 1). Other aerobic marine genera in the class *Gammaproteobacteria*, including the next closest relative *Saccharophagus*, were more distantly related (sequence similarity <91%). Phylogenetic analysis of the concatenated sequences of the *gyrB* and *ftsZ* genes gave two separate clusters composed of the novel isolates and three *Microbulbifer* species, with respective bootstrap support of 100 and 98%. *M. maritimus* formed part of the cluster formed by the *Microbulbifer* species, but with low bootstrap support (Supplementary Fig. S1). Analysis of *gyrB* gene sequences gave a phylogenetic tree with topology similar

Fig. 1. Phylogenetic affiliation of *Microbulifer variabilis* sp. nov. strains Ni-2088^T and C-38 and *Microbulifer epialgicus* sp. nov. F-104^T based on 16S rRNA gene sequences. The tree, which was constructed using the neighbour-joining method, is based on a comparison of approximately 1350 nucleotides. Bootstrap values, expressed as percentages of 1000 replications, are provided at branch points. Bar, 0.02 substitutions per nucleotide position.

to that given by the analysis of the concatenated *gyrB* and *ftsZ* sequences (not shown).

Cultural characteristics were observed for colonies grown on MA and 1/10 MA plates. The morphology of rod and coccoid cells was observed mainly for cultures grown on 1/10 MA (1.5 % agar or agarose; Agarose S, Wako Chemicals) at 20 and 24 °C using a slide culture technique. An agar medium film (about 0.25 mm thick) was prepared by pouring a small amount of molten 1/10 MA onto a sterile glass slide. The agar medium film was spot-inoculated, and the slides were placed on 1/10 MA in a Petri dish, which was then sealed with tape to prevent evaporation of water during incubation. After growth, a small drop of 75 % ASW was added to the microcolony, which was covered with a coverslip. The peripheral portion of the growth was observed by phase-contrast light microscopy. Cells were released from the microcolony into the 75 % ASW, adhered to the surface of the agar medium film following release or remained at the periphery. Coccoid cells were observed by scanning electron microscopy as well as by optical microscopy. They were then taken from the microcolony, which had formed on 1/10 MA for 4 days at 24 °C, and fixed with 2.5 % glutaraldehyde (pre-fixation) and 2 % osmium tetroxide (post-fixation). Following dehydration in an ethanol series, the samples were dried to the critical point in CO₂ and coated with platinum–palladium. Prepared specimens were viewed using an S-2500 scanning electron microscope (Hitachi). Fibrous structures on the rod cells were observed with an H-7000 transmission electron microscope (Hitachi) for cells grown in MA and negatively stained with uranyl acetate.

Conventional taxonomic features were determined according to the procedures described by Cowan and revised by Barrow & Feltham (1993), except that all media used were supplemented with 3 % (w/v) NaCl or prepared with 75 % ASW. Incubation was at 30 °C unless otherwise noted. The production of acid from glucose and growth under aerobic

and anaerobic conditions was examined in BBL phenol red broth base (Difco) prepared with 75 % ASW and with 1 % (w/v) glucose added. The use of ammoniacal nitrogen and growth factor requirements were evaluated by observing growth in marine salts basal medium (BM) described by Baumann & Baumann (1981) supplemented with 0.2 % glucose. The use of carbohydrates, related compounds, organic acids and amino acids was determined using BM supplemented with the following vitamins (mg l⁻¹): riboflavin (2), pyridoxine hydrochloride (4), pyridoxal hydrochloride (4), *p*-aminobenzoic acid (2), calcium pantothenate (2), niacin (2), folic acid (0.8), nicotinic acid (0.8), thiamine hydrochloride (2) and biotin (0.01). Each carbon compound was added at a concentration of 0.2 % (w/v). Tests for urease, β -galactosidase (PNPG), indole production, arginine dihydrolase and aesculin hydrolysis were conducted using the API 20NE system (bioMérieux). In addition, API ZYM tests were conducted to examine enzyme activities. Both test kits were used as described in the manufacturer's manual, except that cells for inoculation were suspended in 75 % ASW. Incubation time in the API ZYM tests was extended to 24 h, which gave reliable results. MB was used to study growth responses to temperature and pH. For the pH experiment, MB was supplemented with Good's buffers (MES, Tris, MOPS, HEPES, EPPS, CHES and CAPS; Dojin) at a concentration of 0.1 M each. To determine the requirements for Na⁺, Mg²⁺ and Ca²⁺, a peptone-yeast extract-glucose medium was used as a base containing 5 g Bacto peptone (Difco), 1 g Bacto yeast extract (Difco), 10 g glucose, 0.1 g ferric citrate, 5.9 g MgCl₂ · 7H₂O and 1.8 g CaCl₂ · 2H₂O in 1 l distilled water at pH 7.6. Growth, if any, was observed with or without either 2 % (w/v) NaCl or equimolar KCl to examine Na⁺ requirement and with 2 % (w/v) NaCl added with MgCl₂, CaCl₂ or both omitted to examine requirements for Ca²⁺ and/or Mg²⁺. The dependence of growth initiation on NaCl concentration was investigated with the basal medium supplemented with 0–10 % NaCl (w/v) at 0.5 % intervals.

The characteristics of colonies on MA are given in the species descriptions. Colonies of strains Ni-2088^T and F-104^T grown on 1/10 MA plates at 24 °C were very thin and spread across the surface of the medium, sometimes

reaching several centimetres in diameter after 3 months. All the isolates were Gram-negative, non-motile and non-spore-forming. The peripheral portion of a microcolony on 1/10 MA in glass slide culture showed terraced rows of rod

Fig. 2. Phase-contrast micrographs of cells of *M. variabilis* sp. nov. Ni-2088^T and *M. epialgicus* sp. nov. F-104^T. The cells were grown on an agar film of 1/10 MA on a glass slide in a moist chamber at 24 °C, unless otherwise stated, and were prepared for observation using a drop of 75 % (v/v) ASW and a coverslip over the periphery of the microcolony. Bars, 2 µm. (a) Peripheral portion of a microcolony of strain F-104^T incubated for 2 days at 24 °C, showing terracing of six or seven rows of rod cells. (b, c) Straight rod cells of strains Ni-2088^T (b) and F-104^T (c); the cells, grown for 43 h, were released into 75 % ASW. (d, e) Curved and bent rod cells of strains Ni-2088^T (d) and F-104^T (e); the cells, grown at 20 °C for 2 days and at 24 °C for 24 h, respectively, were released into 75 % ASW and adhered to the surface of an agar medium film. (f, g) Micrographs showing the process of conversion of rod cells to coccoid cells of strain Ni-2088^T through successive division and probable simultaneous fragmentation. The cells were grown for 2 days at 20 °C (f) and 3 days at 24 °C (g). (h, i) Coccoid cells of strains Ni-2088^T (h) and F-104^T (i) from a microcolony incubated for 7 days. (j) Ovoid cells of strain Ni-2088^T from a microcolony incubated for 7 days. (k–n) Micrographs showing reversion of coccoid cells to rod cells of strain Ni-2088^T. Coccoid cells were incubated for 7, 9, 12 and 24 h, respectively. (o) Spherical bodies of strain Ni-2088^T. The growth was incubated for 2 days, covered with a coverslip after adding a drop of 75 % ASW and incubated for a further 24 h.

cells, with approximately 6–12 µm between each row (Fig. 2a). Each row of rod cells seemed to constitute the edge of a 'sheet of rod cells' formed by the arrangement of cells in a single horizontal plane. The cells were rod-shaped at the periphery of growing colonies and were coccoid–ovoid in the inner portion of colonies incubated for 2 days or more on 1/10 MA and 2–3 days or more on MA at 24 °C. The rod cells were very flexible and elastic; cells released from the periphery of growth into 75 % ASW were straight and slender (Fig. 2b, c), whereas those that were released and adhered to the agar medium surface were curved and bent to various degrees (Fig. 2d, e). Nuclear staining with a fluorescent dye (4',6-diamidino-2-phenylindole) revealed that the rod cells were multinucleate (not shown). The rod cells of both Ni-2088^T and F-104^T possessed peritrichous appendages of a fine fibrous nature on the cell surface (Fig. 3). Because all strains were isolated from the surfaces of marine algae and seagrass, the peritrichous fibrous structures on the cell surface could play a role in attachment of the cells to the surface of marine algae. Romanenko *et al.* (2003) hypothesized that external fibril-like structures on cells of *Oceanisphaera*

litoralis might be an adaptation to enable attachment to the surfaces of marine sand sediment. The coccoid–ovoid cells were optically much denser than the rod cells and had coarse surfaces, which might play a role in attachment to fresh materials when drifting in seawater (Fig. 4).

All the isolates were aerobic, catalase- and oxidase-positive, mesophilic, neutrophilic and slightly halophilic. Strains Ni-2088^T and F-104^T both required Na⁺, which could not be replaced by K⁺. The isolates grew in peptone-yeast extract-glucose medium containing 2.0 % NaCl in the presence of either Mg²⁺ or Ca²⁺, but they could not grow in the absence of both. The isolates grew in BM with added glucose, were capable of using ammoniacal nitrogen as a

Fig. 3. Transmission electron micrograph of a negatively stained rod cell of *M. variabilis* sp. nov. Ni-2088^T possessing peritrichous fibrous appendages on the cell surface. The cell was taken from a culture grown on MA at 30 °C for 24 h. Bar, 1 µm.

Fig. 4. Scanning electron micrograph of coccoid cells of *M. epialgicus* sp. nov. F-104^T. The cells were taken from a culture grown on 1/10 MA at 24 °C for 4 days. Bar, 2.5 µm.

nitrogen source and did not require growth factors. The range of compounds used as sole carbon sources was limited. Assimilation profiles of 36 compounds tested were almost identical for all the isolates, with differences between the pelagiomycin-producing strains and UVAS-producing strain F-104^T in acetate and L-arginine assimilation. Additional and more detailed taxonomic features are given in Table 1 and in the species descriptions.

The cells exhibited a clearly discernible cycle of rod and coccoid–ovoid cells in association with the growth phase. The conversion of rods to coccoid cells and the reversion of coccoid cells to rods was followed by the slide culture technique on 1/10 MA at 24 °C. The ability of coccoid cells to survive for extended periods was examined for cells taken from the inner to central portion of colonies on a 1/10 MA plate, which had been sealed with tape to prevent drying and maintained at room temperature (23–27 °C) for up to 14 months for the novel isolates and 4 months for *Microbulbifer* reference strains. Viability of the isolates was examined by spreading a loopful of cells on a 1/10 MA plate and incubating at 25 °C for 5–7 days. For the *Microbulbifer* reference strains, cells were suspended in 2 ml 1/10 MB and treated at 50 or 55 °C, depending on the strain, to kill rod cells that might not have converted to coccoid cells and could still be viable. To examine the heat resistance of rod cells, 100 µl of a young culture in 1/10 MB was added to 2 ml semi-solid 1/10 MA (0.2 % agar, w/v) in a test tube that had been pre-equilibrated to the test temperature. The cell suspensions were held at test temperatures of 45 to 65 °C at intervals of 5 °C. The heat resistance of coccoid cells was examined as for the rods, except that a loopful of cells was taken from a culture on a 1/10 MA plate grown and kept at 23–27 °C for 2–4 weeks followed by heat treatment at 45–80 °C for 10 min. For the *Microbulbifer* reference strains, cells were suspended in 1/10 MB instead of a semi-solid agar medium and, after heat treatment, 50 µl of each cell suspension was spread on 1/10 MA. The inoculated media were incubated at 25 °C for 5–7 days to monitor the occurrence of colonies in the semi-solid agar medium or on agar medium plates.

The long rod cells observed during the growth phase converted to coccoid–ovoid cells when proliferation ceased. Just inside the periphery of a microcolony on 1/10 MA in slide culture incubated for 2–3 days, almost all the cells were of a coccoid–ovoid form. The conversion of rod cells to coccoid–ovoid cells occurred through a process of successive division and a probable simultaneous fragmentation (Fig. 2f, g), with subsequent rounding of each unit to form coccoid–ovoid cells (Fig. 2h–j). The coccoid cells were arranged in orderly chains, with a faint track of rod cells between the adjacent cells. The coccoid cells in the chain probably adhered to the adjacent cells. The chains of coccoid cells also probably adhered to adjacent chains on a single horizontal plane, thus forming a ‘sheet of coccoid cells’ (Fig. 2h–j and Supplementary Figs S3c–g and S4). Some sheets of coccoid cells remained at the periphery of the microcolony and others were released

from it and floated in the 75 % ASW. Supplementary Fig. S2(a, b) shows two sheets of coccoid cells floating in 75 % ASW with different depths of focus under a microscope with their peripheries at right angles. Nearly all the rod cells had converted to coccoid–ovoid cells. The coccoid cells reverted to a rod form when transferred to fresh medium. The outgrowth of rods from the coccoid cells occurred after incubation for 6–7 h (Fig. 2k), and they grew vigorously to become long and curved/bent rod cells within 24 h (Fig. 2l–n), thus completing a rod–coccus cell cycle. Coccoid cells were similarly formed on 75 % ASW agar containing 0.05 % (w/v) glucose and 0.05 % (w/v) NH₄Cl but no other organic nutrient. Dramatic conversion from rod to coccoid cells was observed when cells grown in MB at 13 °C for 7 days with shaking were transferred to 30 °C: the rod cells, which had been very long to filamentous (up to 50 µm long), converted into coccoid cells within 24 h. The type strains of all seven described species of *Microbulbifer* also exhibited a rod–coccus cell cycle in association with the growth phase, which was identical to that of the isolates. Micrographs of coccoid–ovoid cells of the *Microbulbifer* reference strains are presented in Supplementary Fig. S3(c–g). The outgrowth of rods from coccoid cells of *M. hydrolyticus* ATCC 700072^T is shown in Supplementary Fig. S3(h).

The presence of a rod–coccus cell cycle as observed here has not been described previously for *Microbulbifer* species (Humm, 1946; González *et al.*, 1997; Yoon *et al.*, 2003a, 2004, 2007; Miyazaki *et al.*, 2008). While the presence of coccoid cells has been described in *M. elongatus* (Humm, 1946; Palleroni, 1984; Yoon *et al.*, 2003b), *M. agarilyticus* (Miyazaki *et al.*, 2008) and *M. thermotolerans* (Miyazaki *et al.*, 2008), features of the coccoid cells have not. The processes of formation and germination of coccoid–ovoid cells in the present isolates apparently differ from those in various Gram-negative bacteria, including *Azotobacter* cysts (Sadoff, 1975), *Azospirillum* cysts (Sadasivan & Neyra, 1985, 1987), *Myxococcus* myxospores (Dworkin, 1996; Reichenbach & Dworkin, 1992), *Azotobacter*-type cysts, exospores and lipid cysts of methanotrophs (Whittenbury *et al.*, 1970), *Sporocytophaga* microcysts (Reichenbach, 1992), *Rhodocista* cysts (Berleman & Bauer, 2004; Kawasaki *et al.*, 1992), *Bdellovibrio* bdellocysts (Hoeniger *et al.*, 1972; Tudor & Conti, 1977) and *Ramlibacter* cysts (Heulin *et al.*, 2003). The resting coccoid cells of the novel isolates could not be regarded as cysts, since shedding of the cyst coat was not observed upon germination (Fig. 2k, l).

The coccoid cells of the isolates formed on 1/10 MA plates were viable after being kept at room temperature (23–27 °C) for at least 14 months. In these old plate cultures, some coccoid cells remained optically dense, but the majority became optically faint as ‘ghost cells’ (Supplementary Fig. S4). For most of the 1/10 MA plate cultures examined, coccoid cells treated at 55 °C (Ni-2088^T) or 50 °C (F-104^T) prior to spreading on a 1/10 MA plate (for procedure, see the description of the heat resistance of rod cells) were not able to form colonies

Table 1. Differential phenotypic characteristics of strains Ni-2088^T and F-104^T and the seven described *Microbulbifer* species

Taxa: 1, *M. variabilis* sp. nov. Ni-2088^T; 2, *M. epialgicus* sp. nov. F-104^T; 3, *M. hydrolyticus* (unless indicated, data from González *et al.*, 1997); 4, *M. salipaludis* (Yoon *et al.*, 2003a); 5, *M. elongatus* (Humm, 1946); 6, *M. maritimus* (Yoon *et al.*, 2004); 7, *M. celer* (Yoon *et al.*, 2007); 8, *M. agarilyticus* (Miyazaki *et al.*, 2008); 9, *M. thermotolerans* (Miyazaki *et al.*, 2008). +, Positive; −, negative; w, weakly positive; ND, no data.

Characteristic	1	2	3	4	5	6	7	8	9
Pigmentation	Cream (young colonies), yellowish green (diffusible)	Yellowish brown	Cream	Greyish yellow	Yellowish brown (diffusible)	Yellowish brown	Greyish yellow	Cream	Brown
Morphology	Rods-cocci	Rods-cocci	Rods-cocci*	Rods-cocci*	Rods-cocci*	Rods-cocci*	Rods-cocci*	Rods-cocci*	Rods-cocci*
Resting coccoid cells	+	+	+	+	+	+	+	+	+
Motility	−	−	−	−	+	−	+	−	+
Nitrate reduction	+	+	−	+	−	+	−	+	+
Temperature for growth (°C)									
Optimum	30	30	37	37	25–30, 34 ^{a†}	37	ND	31–35	43–49
Maximum	42	40	41	45	38 ^a	48	48	35–38	52–54
Growth at 10 % NaCl	−	−	−	+	− ^a	+	+	−	−
Aesculin hydrolysis	+	−	+	− ^a	+	−	+	−	+
Agarase	−	−	−	w	+	−	−	+	+
Cellulase	−	−	+	+	+	ND	ND	ND	ND
Chitinase	−	−	+	−	+	−	ND	+	+
Lecithinase	+	+	−	−	−	−	ND	ND	ND
Utilization of:									
Glucose	+	+	+	ND	+	+	−	+	+
Acetate	+	−	+	ND	+	+	+	ND	ND
Malate	−	−	+	ND	+	+	−	ND	ND
Succinate	−	−	+	ND	+	+	−	ND	ND
Glutamate	+	+	+	ND	+	−	−	ND	ND
API ZYM tests‡									
Trypsin	+	+	−	−	−	−	−	−	−
α-Mannosidase	−	+	−	−	−	−	−	−	−
α-Glucosidase	+	+	+	w*	w*	w*	−	−	−
N-Acetyl-β-glucosaminidase	−	−	−	+	−	−	−	−	+
Ca ²⁺ or Mg ²⁺ requirement	+	+	−	−	−	−	ND	ND	ND
Major fatty acid(s) (approx. >15 %)	C _{18:1} ω7c, C _{16:0}	C _{18:1} ω7c, C _{16:0}	iso-C _{15:0} *	C _{18:1} ω7c, iso-C _{15:0} , iso-C _{17:1} ω9c*	C _{18:1} ω7c, iso-C _{15:0} *	C _{18:1} ω7c, iso-C _{15:0} , iso-C _{17:1} ω9c*	iso-C _{15:0} *	C _{18:1} ω7c, iso-C _{15:0} *	C _{18:1} ω7c, iso-C _{15:0} , iso-C _{17:1} ω9c*
Major hydroxy fatty acid(s)	C _{10:0} 3-OH, C _{12:0} 3-OH, iso-C _{11:0} 3-OH	C _{10:0} 3-OH, C _{12:0} 3-OH, iso-C _{11:0} 3-OH	iso-C _{11:0} 3-OH*	iso-C _{11:0} 3-OH*	iso-C _{11:0} 3-OH*	iso-C _{11:0} 3-OH*	iso-C _{11:0} 3-OH*	iso-C _{11:0} 3-OH*	iso-C _{11:0} 3-OH*
Major polar lipids§	PE, PG, PS, AL	PE, PG, PS, AL	PE, PG*	PE, PG*	PE, PG*	PE, PG*	ND	ND	ND
DNA G+C content (mol%)	48.7	49.0	57.7	59.0	58.9*	59.9	57.7	55.2–55.3	56.0–56.2
16S rRNA gene sequence similarity to strain Ni-2088 ^T (%)	100	99.3	94.9	94.0	95.1	97.6	94.5	95.1	96.5

*Determined in this study for the type strain.

†Data from: a, Miyazaki *et al.* (2008); b, Yoon *et al.* (2003a).

‡Incubation time was extended to 24 h in this study.

§AL, Unidentified aminolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine.

although, on a few plates, coccoid cells withstood heat treatment, showing that the heat resistance of coccoid cells was lost with age. In contrast to coccoid cells, the very few rod cells that had remained unconverted in a 1- to 2-week plate culture became even more scarce after being kept for at least 8 months. The coccoid cells of the four *Microbulbifer* reference strains that were tested were viable after at least 4 months. Based on the observations that almost all rod cells converted to coccoid cells and that only coccoid cells survived for extended periods, it is considered that the coccoid–ovoid cells are a resting form of these organisms, probably playing a survival role in the life cycle.

While the conversion frequency from rod to resting coccoid cells is almost 100 % in the isolates and *Microbulbifer* reference strains, conversion from vegetative to resting cells in other bacteria is usually low and indefinite: less than 0.1 % in *Azotobacter* cysts (Sadoff, 1975), 5–95 % in *Methylosinus* exospores (Whittenbury *et al.*, 1970), 3–95 % in *Methylocystis* lipid cysts (Whittenbury *et al.*, 1970) and <5 to 30 % in *Bdellovibrio* bdelloccysts (Tudor & Conti, 1977). In *Myxococcus* myxospores, Dworkin (1996) reported a lowered rate due to autolysis of 65 to 90 % of the vegetative cells during the process of aggregation and early mount formation. The coccoid–ovoid cells of strains Ni-2088^T and F-104^T withstood heat treatment for 10 min at 75 and 70 °C, respectively, while the rod cells of both strains withstood 55 °C for 10 min. The heat resistance of the coccoid cells of all four *Microbulbifer* reference strains tested was 70 °C and that of rod cells was 55 °C for *M. maritimus* JCM 12187^T and 50 °C for the other three strains. The heat resistance of the coccoid cells of the new isolates and *Microbulbifer* reference strains is comparable to that of the resting cells of other bacteria: *Azotobacter* cysts (57 °C, 15 min; Socolofsky & Wyss, 1962), *Azospirillum* cysts (50–60 °C, 20 min; Sadasivan & Neyra, 1987), lipid cysts and *Azotobacter*-type cysts of *Methylocystis* (non-viable at 65 °C, 30 min; Whittenbury *et al.*, 1970), *Methylosoma* cysts (60–70 °C, 10 min; Rahalkar *et al.*, 2007), *Myxococcus* myxospores (killed slowly at 60 °C; Sudo & Dworkin, 1969) and *Bdellovibrio* bdelloccysts (60 °C, 15 min; Tudor & Conti, 1977). The difference in heat resistance (15–20 °C) between the coccoid and rod cells of the isolates and *Microbulbifer* reference strains is also comparable to the differences between the cysts and vegetative rod cells of other bacteria, including *Azotobacter* cysts (3 °C; Socolofsky & Wyss, 1962), *Azospirillum* cysts (<10 °C; Sadasivan & Neyra, 1987), *Methylosoma* cysts (<10 °C; Rahalkar *et al.*, 2007) and *Bdellovibrio* bdelloccysts (about 15 °C; Tudor & Conti, 1977). The higher heat resistance of coccoid cells over vegetative rod cells probably results from some structural component(s) of the coccoid cells, which may confer the ability to survive for extended periods. The role of coccoid cells in the life cycle appears to be survival during nutrient starvation, since the isolates do not require the ability to survive higher temperatures and desiccation stress in their

marine niches. This cycle of stages of vegetative rod and resting coccoid cells in association with the growth phase is the first such description in bacteria.

In addition to coccoid–ovoid cells, the formation of spherical bodies was observed in older cultures of the isolates. To observe spherical bodies, a drop of 75 % ASW was added to growth on an agar medium film of 1/10 MA that had incubated for 2 days. The slide was then covered with a coverslip and incubated for a further 24 h. Spherical bodies were also observed in cultures grown on MA and negatively stained with uranyl acetate. These were mostly formed at the tips of rod cells, were completely spherical when detached and had a smooth surface (Fig. 2o and Supplementary Fig. S5). Spherical bodies were occasionally ruptured in older cultures. There was a higher frequency of spherical bodies when isolates were grown on MA or in MB at 30 °C than on 1/10 MA at 20–25 °C, and their formation seemed to be enhanced under reduced aerobic conditions. Their size varied, but was generally greater than that of the coccoid cells, being 0.75–2.5 µm in diameter. The viability of spherical bodies remains unclear. Spherical bodies were also formed by the *Microbulbifer* reference strains (Supplementary Fig. S3i). They seem to have been formed by bulging out of the tips or other portions of rod cells. It appeared that the cell surface integrity was weakened in portions of the older rod cells and that these portions became swollen by internal cytoplasmic pressure. Formation of coccoid bodies, spherical bodies, spherical forms or round bodies in older cultures has been reported for various Gram-negative bacteria (Krieg, 1976, 1984). They exhibited unusual features: an unusual method of formation in '*Spirillum lunatum*' (Williams & Rittenberg, 1956) and '*Spirillum serpens*' (*Aquaspirillum serpens*; Terasaki, 1970), loss of viability in '*Desulfovibrio aestuarii*' (*Desulfovibrio desulfuricans* subsp. *aestuarii*; Levin & Vaughn, 1968) and *Vibrio* sp. (Baker & Park, 1975), loss or degradation of peptidoglycans in '*Spirillum itersonii*' (*Aquaspirillum itersonii* subsp. *itersonii*; Clark-Walker, 1969) and *Vibrio* sp. (Baker & Park, 1975) and association with aberrations in cell division and cell-wall formation (Felter *et al.*, 1969). The spherical bodies observed here in the new isolates and *Microbulbifer* reference strains are considered to be allied to these forms and are an aberrant form that does not constitute a stage in the life cycle.

Isoprenoid quinones extracted from cells grown in MB at 30 °C for 24 h were analysed according to the LC/MS method described by Nishijima *et al.* (1997). To investigate the cellular fatty acid composition, cells of the new isolates and reference strains were grown on MA plates at 25 °C for 2 days. Cellular fatty acid methyl esters were prepared and analysed by gas chromatography (HP6890 series GC system; Hewlett Packard) according to the instructions given for the MIDI Microbial Identification system. For the analysis of polar lipids, cells were grown in MB at 25 °C for 24 h with shaking on a reciprocal shaker. Polar lipids were extracted according to the method described by Minnikin *et al.* (1984). The two-dimensional TLC method described

by Komagata & Suzuki (1987) was performed to separate the polar lipid components, which were then identified on a chromatogram after detection using specific reagents: iodine vapour (total lipids), Dittmer–Lester reagent (phosphate), ninhydrin (free amino groups), periodate–Schiff reagent (α -glycols), Dragendorff reagent (quaternary nitrogen) and anisaldehyde/sulphuric acid (glycolipids).

The major respiratory quinone system of all the isolates was ubiquinone-8 (Q-8; 75–95 % peak ratio of the total), with Q-7 and Q-9 as minor components. The cellular fatty acid compositions of strains Ni-2088^T and F-104^T and the type strains of described *Microbulbifer* species are shown in Supplementary Table S2. Considerable differences in the fatty acid profiles were recognized between the new isolates and the seven *Microbulbifer* type strains. The new isolates possessed C_{18:1} ω 7c and C_{16:0} as the predominant fatty acids. The hydroxy acids that occurred in larger amounts were C_{10:0} 3-OH, C_{12:0} 3-OH and iso-C_{11:0} 3-OH. In contrast, the *Microbulbifer* reference strains have iso-C_{15:0} in common as one of the major fatty acids, C_{12:0} 3-OH is not present and iso-C_{11:0} 3-OH is present in larger amounts than in the new isolates. The differences in fatty acid composition between the new isolates and the *Microbulbifer* reference strains are exemplified by a dendrogram obtained by cluster analysis of fatty acid compositions (Supplementary Fig. S6). The new isolates and the *Microbulbifer* reference strains examined shared the major polar lipids phosphatidylethanolamine and phosphatidylglycerol, but the new isolates differed from the *Microbulbifer* species in possessing phosphatidylserine and an unidentified aminolipid (Supplementary Fig. S7).

For determination of the G+C content of the DNA and DNA–DNA hybridization experiments, genomic DNA was extracted and purified according to the combined methods of Marmur (1961) and Saito & Miura (1963). The genomic DNA was digested to nucleotides with nuclease P1 using a DNA–GC kit (Seikagaku Kogyo) according to the procedures described by Katayama–Fujimura *et al.* (1984). The G+C content of the DNA was determined by HPLC with a Develosil ODS–HG–5 column (4.6 $\times$ 250 mm; Nomura Chemical) and a UV 8010 detector (Tosoh) at 270 nm. DNA–DNA relatedness was determined by the fluorometric hybridization method on microdilution plates (Ezaki *et al.*, 1989). The G+C content of the DNA of the 15 isolates fell into a narrow range of 48.1–49.7 mol% (Supplementary Table S3), differing from the values of 55.2–59.9 mol% reported for *Microbulbifer* species (González *et al.*, 1997; Yoon *et al.*, 2003a, 2004, 2007; Miyazaki *et al.*, 2008; the value for *M. elongatus* ATCC 10144^T was 58.9 mol%, as determined in this study). The DNA–DNA relatedness between strain Ni-2088^T and the other pelagiomycin-producing strains ranged from 77 to 96 %, while the relatedness between strain F-104^T and the pelagiomycin-producing strains ranged from 6 to 27 % (Supplementary Table S3). This indicates that the pelagiomycin-producing strains constitute a single genomic species

and that strain F-104^T represents a separate genomic species.

The isolates shared almost identical characteristics and differed from described *Microbulbifer* species in the G+C content of the DNA (6–11 mol% different from the *Microbulbifer* species; Table 1), major cellular fatty acids and hydroxy fatty acids (Supplementary Table S2) and polar lipid composition (Supplementary Fig. S7). All the *Microbulbifer* species were similar in these traits. With the exception of *M. thermotolerans* and *M. maritimus*, this group corresponded to the phylogenetic cluster in the *Microbulbifer* clade obtained by sequence analysis of the 16S rRNA gene and concatenated *gyrB* and *ftsZ* genes. *M. thermotolerans* and *M. maritimus* formed a cluster with the new isolates and not with the *Microbulbifer* species in the phylogenetic tree based on 16S rRNA gene sequences, with a bootstrap value of 100 % (Fig. 1). The position of *M. maritimus* was indefinite in the phylogenetic clustering based on concatenated *gyrB* and *ftsZ* gene sequences (Supplementary Fig. S1). Although inconsistency amongst phylogenetic, genomic and chemotaxonomic traits has been recognized within the members of the *Microbulbifer* clade, we propose that the new isolates be placed in the genus *Microbulbifer* on the basis of phylogenetic affiliation, general accordance of taxonomic features and the possession of a vegetative rod and resting coccoid cell cycle.

The 16S rRNA gene sequence similarity, which was less than 98 % between the new isolates and *Microbulbifer* species (93.6–97.6 %), and the differences in DNA G+C content (6–11 mol%) exclude the assignment of the isolates to any of the extant species of this genus (Vandamme *et al.*, 1996; Stackebrandt & Goebel, 1994; Stackebrandt, 2006; Trüper & Schleifer, 2006). The 14 pelagiomycin-producing isolates constituted a single novel species based on the observations that they shared almost identical physiological, biochemical and chemotaxonomic characteristics and a high level of DNA–DNA relatedness. Strain F-104^T exhibited phenotypic features similar to those of the pelagiomycin-producing strains, but represents a distinct species showing low DNA–DNA relatedness (Supplementary Table S3). The phenotypic features that differ between these two species are the colour of the colonies, production of a water-soluble yellow–green pigment, aesculin hydrolysis, PNPG test, assimilation of acetate and L-arginine and α -mannosidase activity (Table 1; see species descriptions). The new isolates showed 16S rRNA gene sequence similarity to other gammaproteobacteria of less than 91 % and can be distinguished from other aerobic marine gammaproteobacteria by a combination of cell morphology, motility and flagellation, respiratory quinones, major fatty acid composition, polar lipid composition and DNA G+C content. Another distinguishing feature of these isolates is the possession of a vegetative rod–resting coccus cell cycle. Based on phylogenetic, genetic and chemotaxonomic data, the possession of a vegetative rod cell and resting coccus cell cycle and other phenotypic features, we propose to

include the 15 isolates in two novel species of the genus *Microbulbifer*, named *Microbulbifer variabilis* sp. nov. for the pelagiomycin-producing bacteria and *Microbulbifer epialgicus* sp. nov. for the UVAS-producing bacterial strain F-104^T.

Description of *Microbulbifer variabilis* sp. nov.

Microbulbifer variabilis (va.ri.a.bi'lis. L. masc. adj. *variabilis* changeable, variable, referring to the change of cell shape in the cell cycle).

Cells are Gram-negative, strictly aerobic, non-motile and non-spore-forming. Catalase- and oxidase-positive. Colonies on MA are irregular (young cultures), rhizoid (older cultures), umbonate, glossy and transparent (young cultures) to opaque (older cultures). Colour of colonies is milky white (young cultures) and yellow–green in the central portion (older cultures). Colonies on 1/10 MA are very thin and spreading. Produces water-soluble yellow–green pigment on MA and in MB. Does not produce fluorescent pigment on King's B medium. The organism possesses a rod–coccus cell cycle in association with the growth phase; cells are straight, slender, flexible rods in young cultures, and all convert to coccoid–ovoid cells when proliferation ceases by successive division and a probable simultaneous fragmentation of rod cells and rounding of each unit. Coccoid–ovoid cells are arranged in orderly chains, and revert to the rod form when transferred to fresh medium. The coccoid–ovoid cells are optically dense and have a coarse surface. They survive for extended periods and are considered to be a resting form. Spherical bodies (aberrant form) are formed. Rod cells of the type strain are 3.5–13.5 µm long and 0.42–0.63 µm wide on 1/10 MA in young cultures and the coccoid–ovoid cells are 0.40–0.85 µm in diameter and 0.6–1.1 and 0.4–0.5 µm along their major and minor axes, respectively, on 1/10 MA in older cultures. Rod cells possess fibrous peritrichous structures on the surface. Slightly halophilic; the range of NaCl concentration for growth is 0.5–7.0% (w/v), with optimum growth at 2.0% (w/v). No growth occurs in the absence of NaCl or in 7.5% NaCl. Requires Na⁺ and a bivalent cation, Mg²⁺ or Ca²⁺, for growth. Growth occurs at 12–42 °C, with an optimum of 30 °C, but not at 5 or 45 °C. Growth occurs at pH 6.0–9.0 and optimally at pH 7.5–8.0, but not at pH 5.5 or 9.5. Uses ammoniacal nitrogen; does not require growth factors and does not grow anaerobically in the presence of nitrate. Polyhydroxybutyrate is not accumulated. Nitrate reduction and PNPG tests are positive. Starch, gelatin, casein, DNA, Tween 80, aesculin and lecithin are hydrolysed. Agar and chitin are not hydrolysed. Negative for arginine dihydrolase, citrate utilization, urease, H₂S generation and indole production. D-Glucose, trehalose, cellobiose, methyl α-D-glucoside, N-acetyl-D-glucosamine, starch, acetate, propionate, L-alanine, L-glutamate, L-asparagine and L-arginine are used. L-Arabinose, D-xylose, D-fructose, D-galactose, sucrose, lactose, melezitose, glycerol, D-sorbitol, D-mannitol, myo-inositol, dulcitol, formate, fumarate, DL-

malate, malonate, succinate, citrate, gluconate, alginate, benzoate and DL-serine are not used. Mannose and maltose are used variably. Tests for the following enzymes are positive with the API ZYM gallery: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase and α-glucosidase. The major respiratory quinone is ubiquinone-8. The major cellular fatty acids are C_{18:1ω7c} and C_{16:0}. Hydroxy fatty acids are C_{10:0} 3-OH, C_{12:0} 3-OH and iso-C_{11:0} 3-OH. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and phosphatidylserine. The G+C content of the DNA is 48.1–49.7 mol%.

The type strain is Ni-2088^T (=MBIC01082^T =ATCC 700307^T). The type strain and 13 other strains were isolated from the surfaces of green, brown and red algae, a cyanobacterium and seagrass, collected in tropical and subtropical areas (Palau, Yap and Ishigaki Jima) in the Pacific Ocean.

Description of *Microbulbifer epialgicus* sp. nov.

Microbulbifer epialgicus (e.pi.al'gi.cus. Gr. prep. *epi* upon; N.L. masc. adj. *algicus* pertaining to water plants, to algae; N.L. masc. adj. *epialgicus* living on algae).

Cells are Gram-negative, strictly aerobic, non-motile and non-spore-forming. Catalase- and oxidase-positive. Colonies on MA are irregular (young cultures), convex, glossy, transparent to opaque (older cultures). Colour of colonies is pale brown, becoming browner in the central portion of older cultures. Colonies on 1/10 MA are very thin and spreading. Fluorescent pigment is not produced on King's B medium. The organism possesses a rod–coccus cell cycle in association with the growth phase; cells are straight, slender, flexible rods in young cultures, and all convert to coccoid–ovoid cells when proliferation ceases by successive division and a probable simultaneous fragmentation of rod cells and rounding of each unit. Coccoid–ovoid cells are arranged in orderly chains, and revert to the rod form when transferred to fresh medium. Coccoid–ovoid cells are optically dense and have a coarse surface. They survive for extended periods and are considered to be a resting form. Spherical bodies (aberrant form) are formed. The rod cells of the type strain are 3.0–13.5 µm long and 0.35–0.65 µm wide on 1/10 MA in young cultures and the coccoid–ovoid cells are 0.45–1.10 µm in diameter and 0.75–1.10 and 0.40–0.75 µm along their major and minor axes, respectively, on 1/10 MA in older cultures. The rod cells possess fibrous peritrichous structures on the surface. Slightly halophilic; the range of NaCl concentrations for growth is 0.5–6.5% (w/v) with an optimum of 2.0%. Does not grow in the absence of NaCl or in 7.0% NaCl. Requires Na⁺ and a bivalent cation, Mg²⁺ or Ca²⁺, for growth. Growth occurs at 10–40 °C with an optimum of 30 °C, but not at 5 or 45 °C. It grows at pH 6.0–9.0 and optimally at pH 7.0–8.0, but not at pH 5.5 or 9.5. Cells use

ammoniacal nitrogen; does not require growth factors and does not grow anaerobically in the presence of nitrate. Polyhydroxybutyrate is not accumulated. Nitrate is reduced. Starch, gelatin, casein, DNA, Tween 80 and lecithin are hydrolysed. Agar, chitin and aesculin are not hydrolysed. Negative for arginine dihydrolase, citrate utilization, urease, indole production and PNPG test. D-Glucose, mannose, trehalose, cellobiose, methyl α -D-glucoside, N-acetyl-D-glucosamine, starch, propionate, L-alanine, L-glutamate and L-asparagine are used. L-Arabinose, D-xylose, D-fructose, D-galactose, maltose, sucrose, lactose, melezitose, glycerol, D-sorbitol, D-mannitol, *myo*-inositol, dulcitol, formate, acetate, fumarate, DL-malate, malonate, succinate, citrate, alginate, benzoate, gluconate, DL-serine and L-arginine are not used. Tests for the following enzymes are positive with the API ZYM gallery: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase (weak), trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, N-acetyl- β -glucosaminidase, α -glucosidase and α -mannosidase. The major respiratory quinone is ubiquinone-8. The major cellular fatty acids are C_{18:1 ω 7c} and C_{16:0}. Hydroxy fatty acids are C_{10:0} 3-OH, C_{12:0} 3-OH and iso-C_{11:0} 3-OH. Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and phosphatidylserine. The G+C content of the DNA of the type strain is 49 mol%.

The type strain is F-104^T (=MBIC03330^T =DSM 18651^T), isolated from the surface of a green alga collected on the seashore in Palau in the Pacific Ocean.

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