In coastal sea water where red tide occurs frequently, marine bacteria play an important role for the interactions of phytoplankton, in the sense of decreasing and developing algal blooms. During the past decades, algicidal bacteria that can kill and lyse causative phytoplanktons of red tides could potentially be of great use in reducing and preventing harmful algal blooms. During the past decades, algicidal bacteria isolated from marine and coastal environments have been proposed to be of great use in reducing and preventing harmful algal blooms. During the past decades, algicidal bacteria isolated from marine and coastal environments have been assigned to the genera *Pseudomonas*, *Cytophaga*, *Flavobacterium*, *Pseudoalteromonas*, *Saprospira* and *Vibrio* (Baker & Herson, 1978; Ishio et al., 1989; Sawayama et al., 1991, 1993; Imai et al., 1993, 1995, 2001; Yoshinaga et al., 1995b; Lovejoy et al., 1998; Adachi et al., 2002).

In the screening of algicidal bacteria, a moderately halophilic, yellow-pigmented bacterium, designed OT-1<sup>T</sup>, was isolated from the surface water of Masan Bay, Korea, during an outbreak of red tide. This bacterium has the ability to kill and lyse several marine microalgal species. In this study, we report the taxonomic properties of strain OT-1<sup>T</sup>, for which the name *Kordia algicida* gen. nov., sp. nov. is proposed.

**Isolation**

A sea water sample was collected from a depth of 1 m in Masan Bay, Republic of Korea, in which algal bloom caused by a marine microalga, *Skeletonema costatum*, occurred. The sample was filtered through a 1-2 μm membrane filter, co-cultured with *S. costatum* and incubated at 20°C under cycling light (approx. 5000 lx for 14 h) and dark (10 h) periods. *S. costatum* cells in a co-culture tube were killed completely after 3 days. The bacterium that was responsible for killing *S. costatum*, designed OT-1<sup>T</sup>, was isolated from the co-culture tube by using ZoBell 2216e agar medium (ZoBell, 1946). The organism also showed algicidal activity against other algal species, namely *Thalassiosira* sp., *Heterosigma akashiwo* and *Cochlodinium polykrikoides*. The test
strain was maintained as a glycerol suspension (20%, w/v) at −80°C.

16S rDNA analysis

Genomic DNA was prepared by using a Wizard Genomic DNA Purification kit (Promega) according to the manufacturer’s instructions. 16S rDNA was enzymically amplified, purified and sequenced according to Chun & Goodfellow (1995). The sequence of strain OT-1T was aligned manually with representative sequences of the Cytophaga–Flavobacterium–Bacteroides (CFB) group that were obtained from GenBank. Phylogenetic trees were inferred by using the Fitch–Margoliash (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices for the neighbour-joining and Fitch–Margoliash methods were generated according to the model of Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out by using the PHYDIT (available at http://plaza.snu.ac.kr/~jchun/phydit/) and PAUP 4.0 (Swofford, 1998) programs, as described previously (Chun et al., 2000).

On the basis of 16S rDNA analysis, strain OT-1T showed low levels of sequence similarity to species with validly published names; highest similarities were found to the type strains of Cytophaga latercula (92.5%), Flexibacter trautwous (92.1%), Salegentibacter salegens (91.0%), Cellulophaga lytica (90.9%), Cellulophaga fucicola (90.7%), Tenacibaculum maritimum (90.5%) and Polaribacter franzmannii (90.1%). The position of strain OT-1T was not stable within the CFB complex, as the branching positions in the trees varied depending on the phylogenetic method employed (Fig. 1). However, it is evident from 16S rDNA analysis that our isolate is distant from all species with validly published names and represents a new phyletic lineage that merits novel genus status in the family Flavobacteriaceae.

Morphology and physiological properties

Tests for Gram stain, spore stain and motility were performed as described by Lewin & Lounsbury (1969). Gliding motility was observed by direct microscopic examination of the edge of colonies in the exponential phase on a plate that contained CY agar [3 g casitone, 1 g yeast extract, 1 g CaCl₂·2H₂O, 40 g artificial sea salts (Sigma) and 15 g agar in 1000 ml distilled water], according to Bernardet et al. (2002). The isolate was a Gram-negative, non-spore-forming, non-motile and non-gliding rod. For scanning electron microscopy, strain OT-1T was fixed for 3 h with 4% glutaraldehyde. After washing with 50 mM HEPES buffer (pH 7.5) and postfixation with 2% OsO₄ solution for 6–8 h, the material was dehydrated in a series of graded ethanol solutions. The specimen was freeze-dried, coated with gold in an ion sputter and examined with a scanning electron microscope. After 24 h growth in ZoBell 2216e broth at 25°C, single cells were 2–5 μm in length and 0.3–0.5 μm in diameter (Fig. 2). The colony colour of strain OT-1T was yellowish on ZoBell 2216e medium, but white on peptone/sea water agar medium. The bathychromatic shift test with 20% KOH revealed no flexirubin pigments. After 3 days cultivation, colonies of strain OT-1T were about 1.2 mm in diameter, slightly convex (elevation), entire (margin) and round (configuration).

Growth characteristics were examined by using ZoBell 2216e as the basal medium. Strain OT-1T grew at temperatures between 5 and 40°C; optimal growth occurred at 30°C. The pH range for growth was determined by measuring OD₆₆₀ after 24 and 48 h incubation at 25°C in ZoBell 2216e broth. The organism grew at pH values from 6 to 10; optimal growth occurred at around pH 7. Specific ion requirements and NaCl tolerance were tested by using synthetic ZoBell medium (5 g Bacto peptone, 1 g yeast

![Fig. 1. Neighbour-joining tree based on nearly complete 16S rDNA sequences, showing relationships between strain OT-1T and members of the CFB group. Numbers at nodes are levels of bootstrap support (%), based on neighbour-joining analyses of 1000 resampled datasets; solid circles indicate that the corresponding nodes (groupings) are also recovered in Fitch–Margoliash, maximum-likelihood and maximum-parsimony trees. Bar, 0·1 nucleotide substitution per position.](image-url)
urea, NH₄ or NO₃ (supplied as inorganic nitrogen sources), but grew when Casamino acid, sodium glutamate, peptone, tryptone or yeast extract were supplied instead of an inorganic nitrogen source.

The organism’s ability to oxidize various carbon compounds was examined by using a Biolog GN2 MicroPlate. The strain was grown for 2 days at 25 °C on trypticase soy agar (TSA; Difco) by using aged sea water. An inoculum was prepared in modified suspension solution (5 g MgCl₂·6H₂O, 1 g CaCl₂·2H₂O, 25 g NaCl in 1 l distilled water) instead of saline water. On the basis of the Biolog system, the test strain oxidized various carbon compounds, as given in the species description.

**Chemotaxonomy**

Fatty acid methyl esters were prepared from biomass that was scraped from TSA supplemented with 75 % aged sea water, incubated at 25 °C for 3 days and analysed by using GC, according to the instructions of the Microbial Identification system (MIDI). The organism had the following fatty acids: iso-C₁₅:0 (41·2 %), iso-C₁₇:0 3-OH (22·2 %), iso-C₁₅:0 3-OH (13·8 %), iso-C₁₆:0 3-OH (4·0 %), a mixture of iso-C₁₅:0 2-OH and C₁₆:0-7c (3·3 %); the MIDI system could not differentiate between these two fatty acids), anteiso-C₁₅:0 (2·8 %), iso-C₁₇:1-09c (2·3 %), C₁₅:0 (1·5 %) and unknown fatty acids (8·9 %). These levels of branched hydroxy fatty acids are often found in the CFB group (Gosink et al., 1998). Isoprenoid quinones were analysed by using HPLC, according to Minnikin et al. (1984) and Collins (1985). The predominant isoprenoid quinone of the test strain was menaquinone 6. The genera *Flavobacterium* (Bernardet et al., 1996), *Saledentibacter* (McCammon & Bowman, 2000) and *Tenacibaculum* (Suzuki et al., 2001) were also reported to have MK-6; this is different from the genera *Cytophaga* (Nakagawa & Yamasato, 1993; Nakagawa et al., 1997) and *Flexibacter* (Kleinig et al., 1974; Fujita et al., 1996), which contain MK-7 as their major isoprenoid quinone. DNA was prepared according to Wolff & Gemmill (1997) and DNA G + C contents were determined by HPLC of deoxyribonucleosides as described by Mshbah et al. (1989), using a reverse-phase column (Supelcosil LC-18-S; Supelco). The DNA G + C content of strain OT-1T was 34 mol%.

Pigment absorption spectra were determined by using a model UV-2401PC spectrophotometer (Shimadzu) after ethanol extraction (Lewin & Lounsbery, 1969). The resultant absorption spectrum, which included peaks at 448 and 475 nm and a shoulder at 425 nm, was similar to those of carotenoids.

**Taxonomic conclusions**

Very low 16S rDNA sequence similarity values (<93 %) to species with validly published names and the formation of an independent phyletic lineage indicate clearly that our isolate can be assigned to a novel genus in the CFB group and, in particular, to a clade whose members contain menaquinone 6 (Suzuki et al., 2001). In addition, several

Strain OT-1T was negative for catalase, urease, β-glucosidase (ascellulase), arginine dihydrolase, β-galactosidase, H₂S production, indole production, acid production from glucose and the O/F test (glucose). However, the isolate reduced NO₃ to N₂ and had oxidase activity. The test strain showed positive reactions for gelatin, skimmed milk and starch degradation, but was negative for degradation of cellulose, CM-cellulose, agar, chitin, alginate, pectin and inulin.

Utilization of nitrogen source was determined as described by Lewin & Lounsbery (1969). The strain did not grow on extract, 10 mg FePO₄ in 900 ml distilled water) that was supplemented with combinatorial artificial sea salts or NaCl. Growth in the synthetic medium was determined by measuring OD₆₆₀ after 24 and 48 h incubation at 25 °C. The strain showed absolute requirement for cations, namely Na⁺, Mg²⁺ and Ca²⁺, as no growth was observed in medium and had not been supplemented with any of these ions. This strain grew at NaCl concentrations between 1 and 5 %; optimal growth occurred in 3 % NaCl.

Physiological and biochemical characters were examined as described by Lewin & Lounsbery (1969). Liquefaction of gelatin (10 %) and degradation of agar (1 %), skimmed milk (20 %), starch (0·5 %), CM-cellulose (3 %), cellulose (Avicell, 1 %), chitin (2 %), inulin (1 %), alginate (3 %) and pectin (2 %) were examined. Additional biochemical tests were performed by using API 20NE strips (bioMérieux) following the manufacturer’s instructions, except that the solution used for bacterial suspension was the modified one (5 g MgCl₂·6H₂O, 1 g CaCl₂·2H₂O, 25 g NaCl in 1 l distilled water) instead of saline solution. As strain OT-1T was unable to grow in the Aux medium of the API 20NE test kit, no data were obtained from 12 carbohydrate assimilation and glucose acidification test cupsules. Thus, acid production from glucose and oxidation–fermentation (O/F; glucose) test media (Lewin & Lounsbery, 1969), supplemented with 50 % aged sea water, and a Biolog GN2 MicroPlate were used instead.

Strain OT-1T was negative for catalase, urease, β-glucosidase (as cellulase), arginine dihydrolase, β-galactosidase, H₂S production, indole production, acid production from glucose and the O/F test (glucose). However, the isolate reduced NO₃ to N₂ and had oxidase activity. The test strain showed positive reactions for gelatin, skimmed milk and starch degradation, but was negative for degradation of cellulose, CM-cellulose, agar, chitin, alginate, pectin and inulin.
phenotypic characters can be used to differentiate our isolate from its phylogenetic neighbours from marine sources (Table 1). On the basis of the polyphasic evidence presented in this study, we propose that the algicidal strain OT-1T should be classified in a novel genus in the family Flavobacteriaceae as *Kordia algicida* gen. nov., sp. nov.

**Description of *Kordia* gen. nov.**

*Kordia* (Kor’di.a. N.L. fem. n. *Kordia* arbitrary name derived from the abbreviation KORDI, which stands for Korea Ocean Research and Development Institute). Cells are strictly aerobic, Gram-negative, non-motile and non-gliding rods. Flexirubin-type pigments are present. Catalase is produced, but catalase is not. Several carbohydrates are used as sole carbon sources. Unable to grow in the absence of any of the ions Na⁺, Ca²⁺ and Mg²⁺. Requires 1–5 % (w/v) NaCl for growth, with optimal growth at 3 % NaCl. Gelatin, skimmed milk and starch are degraded. Major cellular fatty acids are saturated iso-branched and 3-hydroxy iso-branched fatty acids. Respiratory quinone is menaquinone 6. DNA G+C content is 34 mol%. The type and only species to date is *Kordia algicida*.

**Table 1.** Differential characteristics for strain OT-1T and phylogenetically related genera in the family Flavobacteriaceae

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*Kordia algicida* (al.gi’ci.da. L. fem. n. *alg.a -ae alga; L. suff. -cida* from L. *v. caedere* to cut or to kill; N.L. *algicida* alga-killer).

Cell widths range from 0·3 to 0·5 μm; cell lengths range from 2 to 5 μm. Resting stages are absent. Colonies on solid media (ZoBell 2216e and TSA plus sea water) are yellowish, slightly convex (elevation), entire (margin) and round (configuration). Urease and β-glucosidase (aesculinasan) are absent. H₂S and indole are not produced. Nitrate is reduced to nitrite. *x*-Cyclodextrin, dextrin, glycogen, *N*-acetyl-D-glucosamine, adonitol, *i*-erythritol, gentiobiose, *x*-D-glucose,
maltose, D-mannitol, D-mannose, D-raffinose, sucrose, citric acid, D-glucuronic acid, 3-ketogluartaric acid, DL-lactic acid, quinic acid, succinic acid, alaninamide, L-asparaginic acid, L-glutamic acid, glycol L-aspartic acid, glycyl L-glutamic acid, L-ornithine, L-proline, L-threonine, DL-carnitine, uridine, glucose-1-phosphate and glucose-6-phosphate are oxidized, but not Tween 40, Tween 80, N-acetyl-D-galactosamine, L-arabinose, D-arabitol, cellobiose, D-fructose, L-fucose, D-galactose, m-inositol, D-xylose, lactitol, D-melibiose, methyl β-D-glucoside, D-psicose, L-rihamnosse, D-sorbitol, D-trehalose, turanose, xylitol, methyl pyruvate, monomethyl succinate, acetic acid, cis-aconitic acid, formic acid, D-galactaronic acid lactone, D-galacturonic acid, D-glucaric acid, D-glucosaminic acid, 3-hydroxybutyric acid, DL-glycerol phosphate. Acid is not produced from glucose. Negative for O/F test (glucose). Does not utilize urea, NH4 or NO3 as sole nitrogen sources.

Acknowledgements

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