Muricauda lutimaris sp. nov., isolated from a tidal flat of the Yellow Sea

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A Gram-negative, non-motile, rod-shaped bacterial strain, SMK-108^T, was isolated from a tidal flat of the Yellow Sea in Korea and was subjected to a polyphasic taxonomic investigation. Strain SMK-108^T grew optimally at pH 7.0–8.0 and at 30 °C. It contained MK-6 as the predominant menaquinone. The major fatty acids were iso-C_{17:0} 3-OH, iso-C_{15:1} and iso-C_{15:0}. The DNA G+C content was 41.1 mol%. Comparative 16S rRNA gene sequence analysis showed that strain SMK-108^T was related most closely to members of the genus *Muricauda*, exhibiting 96.6–98.8% sequence similarity to the type strains of recognized *Muricauda* species. Strain SMK-108^T was distinguishable from recognized *Muricauda* species on the basis of differential phenotypic characteristics, levels of DNA–DNA relatedness and phylogenetic distinctiveness. This organism is thus considered to represent a novel species of the genus *Muricauda*, for which the name *Muricauda lutimaris* sp. nov. is proposed. The type strain is SMK-108^T (=KCTC 22173^T =CCUG 55324^T).

The genus *Muricauda*, a member of the family *Flavobacteriaceae* in the phylum *Bacteroidetes*, was proposed by Bruns *et al.* (2001) for a single species isolated from intertidal sediment, *Muricauda ruestringensis*. Subsequently, *Muricauda flavescens* and *Muricauda aquimarina* were described from a salt lake (Yoon *et al.*, 2005). In the present study, we report on the taxonomic characterization of a bacterial strain (designated SMK-108^T) isolated from a tidal flat sediment of the Yellow Sea in Korea and displaying the basic characteristics of *Muricauda* strains.

A tidal flat sediment sample collected from Saemankum on the Yellow Sea, Korea, was used as a source for the isolation of bacterial strains. Strain SMK-108^T was isolated by the dilution plating technique on marine agar 2216 (MA; Difco) plates incubated at 25 °C. The type strains of the three recognized *Muricauda* species were used as reference strains for DNA–DNA hybridization experiments and for investigation of enzyme activities. *M. ruestringensis* DSM 13258^T was obtained from the DSMZ, while the type strains of *M. flavescens* and *M. aquimarina* were from our own institute (Yoon *et al.*, 2005). The morphological, physiological and biochemical characteristics of strain SMK-108^T were investigated by using routine cultivation on MA at 30 °C. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy (Philips CM-20). The latter technique was

also used to search for flagella or the kind of appendages reported for the three recognized *Muricauda* species (Bruns *et al.*, 2001; Yoon *et al.*, 2005). Cells from exponentially growing cultures were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with potassium nitrate (0.1%, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. Growth in the absence of NaCl was investigated by using trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded. Growth with NaCl at various concentrations (1–15%, w/v, at 1% intervals) was investigated in marine broth 2216 (MB; Difco) or trypticase soy broth (Difco). Growth at various temperatures (4, 10, 15, 20, 25 and 28 °C, and 30–40 °C at 1 °C intervals) was measured on MA. The pH range for growth was determined in MB that was adjusted to various pH values (4.5–9.5 at intervals of 0.5 pH units). Catalase and oxidase activities and hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA by using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were investigated as described by Lányi (1987) with the modification that artificial seawater was used for preparation of media. The artificial seawater contained

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(per litre distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5.94 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Bruns *et al.*, 2001). H_2S production was tested as described by Bruns *et al.* (2001). The presence of flexirubin-type pigments was investigated as described by Reichenbach (1992). Susceptibility to antibiotics was investigated on MA plates by using discs (Advantec) containing the following compounds: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U),

chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), neomycin (30 µg) and carbenicillin (100 µg). Acid production from carbohydrates was tested as described by Leifson (1963). Utilization of various substrates for growth was determined according to Yurkov *et al.* (1994). Enzyme activities were determined by using the API ZYM system (bioMérieux).

Table 1. Differential phenotypic characteristics between strain SMK-108^T and recognized *Muricauda* species

Taxa: 1, strain SMK-108^T; 2, *M. ruestringensis* (data from Bruns *et al.*, 2001; Yoon *et al.*, 2005); 3, *M. flavescens* (Yoon *et al.*, 2005); 4, *M. aquimarina* (Yoon *et al.*, 2005). +, Positive; −, negative; w, weakly positive. All are Gram-negative, non-spore-forming rods. All are positive for the following characteristics: presence of oxidase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, α-glucosidase and N-acetyl-β-glucosaminidase activities, hydrolysis of aesculin, tyrosine and Tween 20, utilization of fructose, cellobiose, sucrose and mannose and acid production from D-fructose, melibiose, D-glucose, D-mannose, cellobiose, lactose, sucrose, maltose, trehalose and raffinose. All are negative for the following characteristics: growth without NaCl and at 4 °C, nitrate reduction, H_2S production, presence of lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase activities, hydrolysis of hypoxanthine, starch, urea and xanthine, utilization of acetate, pyruvate, glutamate, butyrate, citrate, lactate, formate, methanol, ethanol, benzoate, L-serine and mannitol and acid production from D-sorbitol, myo-inositol, D-ribose, D-mannitol and L-rhamnose.

Characteristic	1	2	3	4
Catalase activity	+	−*	+	+
Facultative anaerobic growth	+	+	−	−
Optimal growth temperature (°C)	30	20–30	30–37	30–37
Hydrolysis of:				
Casein	+	−	−	−
Gelatin	+	−	−	−
Tweens 40, 60 and 80	−	+	+	+
Enzyme activity (API ZYM)†				
Esterase (C4)	+	−	+	+
Trypsin	−	−	w	+
α-Chymotrypsin	+	−	+	+
Acid phosphatase	+	+	−	+
Naphthol-AS-BI-phosphohydrolase	+	+	−	+
β-Glucosidase	+	+	−	+
Utilization of:				
Glucose	+	−	+	+
Lactose	−	+	+	+
Raffinose	−	+	+	+
Malate	−	+	−	−
Succinate	−	+	+	+
Alanine	−	+	−	−
L-Arginine	−	+	−	−
Acid production from:				
D-Xylose	−	+	−	−
L-Arabinose	+	+	−	−
Melezitose	+	+	+	−
D-Galactose	+	+	−	−
DNA G + C content (mol%)	41.1	41	45.2–45.4	44.1–44.2

*Negative in Bruns *et al.* (2001), but positive in Yoon *et al.* (2005).

†All API ZYM data are for the type strains from the present study.

Table 2. Cellular fatty acid compositions (% of total) of strain SMK-108^T and recognized *Muricauda* species

Strains: 1, SMK-108^T; 2, *M. ruestringensis* DSM 13258^T; 3, *M. flavescens* SW-62^T; 4, *M. aquimarina* SW-63^T. Data for reference strains are from Yoon *et al.* (2005). —, Not detected. Fatty acids representing less than 0.5% in all strains were omitted. All strains were cultivated for 3 days on MA at 30 °C. ECL, Equivalent chain length.

Fatty acid	1	2	3	4
Straight-chain				
C _{15:0}	7.6	13.2	12.4	5.9
C _{16:0}	0.4	0.4	0.6	0.4
Branched				
iso-C _{14:0}	—	—	—	0.6
iso-C _{15:0}	14.5	14.7	16.4	23.7
iso-C _{15:1} G	21.3	20.5	19.9	21.6
anteiso-C _{15:0}	1.4	1.1	2.1	2.0
iso-C _{16:0}	0.3	—	0.6	0.5
iso-C _{17:1} ω9c	1.8	1.4	1.3	1.5
Unsaturated				
C _{15:1} ω6c	0.6	0.9	0.9	0.9
C _{17:1} ω6c	0.9	1.0	0.5	0.5
C _{17:1} ω8c	0.5	0.7	0.4	—
Hydroxy				
C _{15:0} 2-OH	0.4	0.5	0.6	0.2
C _{15:0} 3-OH	2.6	1.8	1.0	1.4
iso-C _{15:0} 3-OH	7.8	4.6	4.7	5.2
C _{16:0} 3-OH	0.5	0.4	0.8	0.6
iso-C _{16:0} 3-OH	4.0	1.7	2.9	4.0
C _{17:0} 2-OH	1.4	0.7	1.3	0.7
C _{17:0} 3-OH	0.8	1.3	0.7	0.3
iso-C _{17:0} 3-OH	24.6	20.9	19.9	17.3
Summed feature 3*	3.3	4.2	4.1	2.3
Unknown				
ECL 11.543	0.2	0.6	—	0.8
ECL 13.565	1.7	6.5	5.4	4.8
ECL 16.582	1.9	1.7	1.6	1.3

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 comprised C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

glistening, yellow and 0.8–1.2 mm in diameter after 7 days incubation at 30 °C. Optimal growth occurs at 30 °C. Growth occurs at 10–38 °C, but not at 4 or 39 °C. Optimal pH for growth is 7.0–8.0. Growth occurs at pH 5.0, but not at pH 4.5. Growth occurs in the presence of up to 10% (w/v) NaCl, but not in the absence of NaCl or in the presence of more than 11% (w/v) NaCl. Anaerobic growth occurs on MA and on MA supplemented with potassium nitrate. Flexirubin-type pigments are not produced. L-Arabinose, maltose, trehalose and salicin are utilized, but D-galactose and D-xylose are not. Susceptible to chloramphenicol, novobiocin, tetracycline, carbenicillin, lincomycin and oleandomycin, but not to ampicillin, cephalothin, gentamicin, kanamycin, neomycin, polymyxin

B, penicillin G or streptomycin. The predominant menaquinone is MK-6. The major fatty acids (>10% of the total) are iso-C_{17:0} 3-OH (24.6%), iso-C_{15:1} (21.3%) and iso-C_{15:0} (14.5%). The DNA G+C content is 41.1 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, SMK-108^T (=KCTC 22173^T =CCUG 55324^T), was isolated from a sediment sample collected from a tidal flat at Saemankum, on the Yellow Sea, Korea.

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References

- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* **51**, 1997–2006.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Reichenbach, H. (1992). The order Cytophagales. In *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 2nd edn, pp. 3631–3675. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.
- Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998). Inter- and intra-specific phylogenetic analysis of the genus *Nocardioide*s and related

taxa based on 16S rDNA sequences. *Int J Syst Bacteriol* **48**, 187–194.

Yoon, J.-H., Kim, I.-G., Shin, D.-Y., Kang, K. H. & Park, Y.-H. (2003). *Microbulbifer salipaludis* sp. nov., a moderate halophile isolated from a Korean salt marsh. *Int J Syst Evol Microbiol* **53**, 53–57.

Yoon, J.-H., Lee, M.-H., Oh, T.-K. & Park, Y.-H. (2005). *Muricauda flavescens* sp. nov. and *Muricauda aquimarina* sp. nov., isolated from a salt lake near Hwajinpo Beach of the East Sea in Korea, and

emended description of the genus *Muricauda*. *Int J Syst Evol Microbiol* **55**, 1015–1019.

Yurkov, V., Stackebrandt, E., Holmes, A., Fuerst, J. A., Hugenholtz, P., Golecki, J., Gad'on, N., Gorlenko, V. M., Kompantseva, E. I. & Drews, G. (1994). Phylogenetic positions of novel aerobic, bacteriochlorophyll *a*-containing bacteria and description of *Roseococcus thiosulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., sp. nov., and *Erythrobacter litoralis* sp. nov. *Int J Syst Bacteriol* **44**, 427–434.