Erythrobacter luteolus sp. nov., isolated from a tidal flat of the Yellow Sea in Korea

Jung-Hoon Yoon,¹ Kook Hee Kang,² Soo-Hwan Yeo³
and Tae-Kwang Oh¹

Correspondence

Jung-Hoon Yoon
jhyoon@kribb.re.kr

¹Korea Research Institute of Bioscience and Biotechnology (KRIBB), PO Box 115, Yusong, Taejeon, Korea

²Department of Food and Life Science, Sungkyunkwan University, Chuncheon-dong 300, Jangnan-gu, Suwon, Korea

³Center for Traditional Microorganism Resources, Keimyung University, Shindang-dong, Dalseo-gu, Daegu, Korea

A Gram-negative, non-spore-forming, yellow-pigmented, slightly halophilic bacterial strain, SW-109^T, was isolated from a tidal flat of the Yellow Sea in Korea, and subjected to a polyphasic taxonomic study. This isolate did not produce bacteriochlorophyll *a* and contained ubiquinone-10 as the predominant respiratory lipoquinone and C_{18:1}ω7c as the major fatty acid. The DNA G + C content was 60.3 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain SW-109^T is phylogenetically affiliated to the genus *Erythrobacter* of the family *Sphingomonadaceae*. Strain SW-109^T exhibited levels of 16S rRNA gene sequence similarity to the type strains of *Erythrobacter* species of 94.0–96.3%, making it possible to categorize strain SW-109^T as a species that is separate from previously recognized *Erythrobacter* species. On the basis of its phenotypic properties and phylogenetic distinctiveness, SW-109^T (=KCTC 12311^T=JCM 12599^T) was classified as the type strain of a novel *Erythrobacter* species, for which the name *Erythrobacter luteolus* sp. nov. is proposed.

The genus *Erythrobacter* was proposed by Shiba & Simidu (1982) and at the time of writing comprises seven species with validly published names: *Erythrobacter longus* (Shiba & Simidu, 1982), *Erythrobacter litoralis* (Yurkov *et al.*, 1994), *Erythrobacter citreus* (Denner *et al.*, 2002), *Erythrobacter flavus* (Yoon *et al.*, 2003), *Erythrobacter aquimaris* (Yoon *et al.*, 2004), *Erythrobacter seohaensis* (Yoon *et al.*, 2005) and *Erythrobacter gaetbuli* (Yoon *et al.*, 2005). The first two *Erythrobacter* species were orange- and red- or orange-pigmented and contained bacteriochlorophyll *a* (BChl *a*) (Shiba & Simidu, 1982; Yurkov *et al.*, 1994). However, two yellow-pigmented *Erythrobacter* species, *E. citreus* and *E. flavus*, lacking BChl *a*, have also been described (Denner *et al.*, 2002; Yoon *et al.*, 2003). In this study, we report on the taxonomic characterization of a slightly halophilic, yellow-pigmented *Erythrobacter*-like bacterial strain,

SW-109^T, which was isolated from sea water of a tidal flat of the Yellow Sea in Korea.

Strain SW-109^T was isolated by the usual dilution-plating technique on marine agar 2216 (MA; Difco) at 30 °C. Cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy (TEM). The presence of flagella was determined by using TEM with cells from exponentially growing cultures. For transmission electron microscopic observation, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after air-drying with a Philips CM-20 transmission electron microscope. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Growth in the absence of NaCl was investigated in trypticase soy broth without NaCl (Difco). Growth at various NaCl concentrations was investigated in marine broth 2216 (MB; Difco) or trypticase soy broth (Difco). Growth at various temperatures (4–40 °C) was measured on MA. 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 using the substrate concentrations described

Published online ahead of print on 21 January 2005 as DOI 10.1099/ijs.0.63522-0.

Abbreviation: BChl, bacteriochlorophyll.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SW-109^T is AY739662.

A table giving the percentage cellular fatty acid content of *Erythrobacter luteolus* SW-109^T and type strains of *Erythrobacter* species is available as supplementary material in IJSEM Online.

by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were investigated as described by Lanyi (1987), with the modification that artificial sea water was used for preparation of media. The artificial sea water contained (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). For *in vivo* pigment-absorption spectrum analysis, the strain was cultivated aerobically in the dark at 30 °C in MB and *Erythromicrobium/Roseococcus* medium (Yurkov *et al.*, 1994; DSMZ medium no. 767). *E. longus* DSM 6997^T was used as a positive control for the spectrum analysis. The cultures were washed twice by centrifugation using a MOPS buffer (MOPS/NaOH, 0.01 M; KCl, 0.1 M; MgCl_2 , 0.001 M; pH 7.5) and were disrupted by sonication with a Branson Sonifier 450. After removal of cell debris by centrifugation, the absorption spectrum of the supernatant was examined on a Beckman Coulter DU800 spectrophotometer. Susceptibility to antibiotics was detected on MA plates by using antibiotic discs with the following concentrations; 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) and tetracycline (30 µg). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of various substrates for growth was determined as described by Yurkov *et al.* (1994).

Cell biomass for respiratory lipoquinone analysis and for DNA extraction was obtained by cultivation in MB at 30 °C. Respiratory lipoquinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that ribonuclease T1 was treated in combination with ribonuclease A to minimize the contamination of RNA. For fatty acid methyl ester (FAME) analysis, cell mass of strain SW-109^T was harvested from agar plates after cultivation for 5 days at 30 °C on MA. The FAMES were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial

Table 1. Differential phenotypic characteristics of *Erythrobacter* species

Species: 1, *E. luteolus* sp. nov. ($n=1$); 2, *E. longus* ($n=11$) (data from Shiba & Simidu, 1982); 3, *E. litoralis* ($n=1$) (Yurkov *et al.*, 1994); 4, *E. citreus* ($n=2$) (Denner *et al.*, 2002; Vybiral *et al.*, 1999); 5, *E. flavus* ($n=2$) (Yoon *et al.*, 2003); 6, *E. aquimaris* ($n=3$) (Yoon *et al.*, 2004); 7, *E. seohaensis* ($n=1$) (Yoon *et al.*, 2005); 8, *E. gaetbuli* ($n=1$) (Yoon *et al.*, 2005). +, Positive reaction; −, negative reaction; w+, weakly positive reaction; ND, not determined; v, variable reaction. Data in parentheses are for the type strain. All species are rod-shaped, positive for catalase and oxidase, and susceptible to chloramphenicol (100 µg per disc; 30 µg per disc for *E. citreus*). All species are Gram-negative, non-sporulating and resistant to polymyxin B (100 U per disc; 300 U per disc for *E. citreus*).

Characteristic	1	2	3	4	5	6	7	8
Colony colour	Yellow	Orange	Red or orange	Yellow	Yellow	Orange	Orange–yellow	Orange–yellow
Motility	−	+	+	−	+	−	−	−
Presence of BChl <i>a</i>	−	+	+	−	−	−	−	−
Nitrate reduction	−	v (+)	−	+	−	−	−	−
Hydrolysis of:								
Gelatin	−	+	−	ND	−	−	−	−
Starch	w+	−	−	(−)	+	v (+)	−	−
Utilization of:								
Glucose	+	+	+	v (w+)	−	+	+	+
Fructose	−	ND	+	(−)	−	−	−	−
Acetate	−	+	+	+	+	+	+	+
Citrate	−	v (−)	−	v (w+)	−	−	−	−
Formate	−	v (−)	−	ND	−	−	−	−
Pyruvate	+	+	+	−	+	+	+	+
Glutamate	−	+	+	ND	−	−	−	−
Succinate	−	v (−)	+	ND	−	+	+	+
Malate	−	v (−)	−	−	−	+	−	+
Susceptibility to:								
Penicillin G (20 U; 10 U for <i>E. citreus</i>)	+	+	−	v (−)	−	−	−	−
Streptomycin (50 µg; 10 µg for <i>E. citreus</i>)	−	v (−)	−	v (−)	−	−	−	−
Optimum growth temperature (°C)	30	25–30	25–30	ND	30–37	30–37	30–35	30–37
DNA G+C content (mol%)	60.3	60–64 (60.7)	67	62.0–62.4 (62.0)	64.0–64.1 (64.0)	62.2–62.9 (62.2)	62.2	64.5

Identification System (Sasser, 1990). The DNA G + C content was determined by using the method of Tamaoka & Komagata (1984) with the modification that the DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The 16S rRNA gene was amplified by PCR using two universal primers, as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003).

The morphological, cultural, physiological and biochemical characteristics of strain SW-109^T are given in the species description (see below) or are shown in Table 1, together with those of some *Erythrobacter* species. The 16S rRNA gene sequence of strain SW-109^T determined in this study comprised 1443 nucleotides, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. Comparative 16S rRNA gene sequence analyses showed that strain SW-109^T is phylogenetically related to *Erythrobacter* species (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, strain SW-109^T joined *E. seohaensis* by a bootstrap confidence level of 62.7 % (Fig. 1). Similar tree topologies were found in trees generated with the maximum-likelihood and maximum-parsimony algorithms (data not shown). Levels of 16S rRNA gene sequence similarity between strain SW-109^T and the type strains of *Erythrobacter* species ranged from 96.3 % (with *E. seohaensis*) to 94.0 % (with *E. litoralis*). Sequence similarities with all other species of the family *Sphingomonadaceae* included in the phylogenetic analysis were lower than 94.1 % (Fig. 1).

Chemotaxonomic data support the result of monothetic phylogenetic classification of strain SW-109^T as a member of the genus *Erythrobacter*. Strain SW-109^T contained ubiquinone-10 (Q-10) as the predominant respiratory lipoquinone, at a peak area ratio of approximately 88 %. This predominant respiratory lipoquinone was the same as those of *Erythrobacter* species (Denner *et al.*, 2002; Yoon

et al., 2003, 2004, 2005). Strain SW-109^T had a cellular fatty acid profile that contained large amounts of unsaturated, straight-chain and hydroxyl fatty acids; the components (>1 %) were C_{18:1}ω7c (47.7 %), C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (13.9 %), C_{16:0} (9.0 %), 11-methyl-C_{18:1}ω7c (7.1 %), C_{17:1}ω6c (5.1 %), C_{16:0} 2-OH (3.6 %), C_{14:0} 2-OH (3.4 %), C_{18:1}ω5c (3.4 %), C_{16:1}ω5c (3.3 %) and iso-C_{16:0} 3-OH (1.0 %). This fatty acid profile was similar to those of the type strains of *Erythrobacter* species, particularly *E. citreus*, although there were differences in the proportion of some fatty acids (Denner *et al.*, 2002; Yoon *et al.*, 2003, 2004, 2005). The amounts of each hydroxyl fatty acid allowed differentiation of strain SW-109^T from *E. citreus* (Supplementary Table in IJSEM Online). The unsaturated fatty acid C_{17:1}ω6c was a minor component in yellow-pigmented *Erythrobacter* species as well as in strain SW-109^T, while it was a major component in the other *Erythrobacter* species, except *E. longus* (Supplementary Table in IJSEM Online). The DNA G + C content of strain SW-109^T was 60.3 mol%.

The phylogenetic distinctiveness is enough to categorize strain SW-109^T as a species that is distinct from the previously recognized *Erythrobacter* species (Stackebrandt & Goebel, 1994). There are differences between strain SW-109^T and other *Erythrobacter* species in some phenotypic characteristics (Table 1). Strain SW-109^T is distinguished from one or two yellow-pigmented *Erythrobacter* species by some phenotypic characteristics, including motility, nitrate reduction, starch hydrolysis and utilization of several substrates (Table 1). Therefore, on the basis of the data presented, strain SW-109^T should be placed in the genus *Erythrobacter* as a member of a novel species, for which the name *Erythrobacter luteolus* sp. nov. is proposed.

Description of *Erythrobacter luteolus* sp. nov.

Erythrobacter luteolus (lu.te'olus. L. adj. *luteolus* yellowish).

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence data showing the phylogenetic position of strain SW-109^T within the family *Sphingomonadaceae*. Bootstrap values (1000 replications) are shown as percentages at each node only if they are 50 % or greater. Bar, 0.01 substitution per nucleotide position. *Rhodospirillum rubrum* ATCC 11170^T was used as an outgroup.

Cells are rod-shaped, $0.6\text{--}0.8 \times 1.5\text{--}3.5\text{ }\mu\text{m}$ and non-spore-forming. Colonies on MA are smooth, glistening, circular, convex, yellow in colour and $1.0\text{--}1.5\text{ mm}$ in diameter after cultivation for 5 days at 30°C . Methanol-soluble pigment is characterized by absorption maxima at 332, 447 and 473 nm. Growth occurs at 4 and 36°C , but not at 37°C . Optimal pH for growth is pH 7.0–8.0; growth is observed at pH 5.5, but not at pH 5.0. Optimal growth occurs in the presence of 2% (w/v) NaCl; growth does not occur without NaCl or in the presence of >9% NaCl. Anaerobic growth does not occur on MA and on MA supplemented with nitrate. Urease-negative. Aesculin, casein, Tweens 20, 40, 60 and 80 and tyrosine are hydrolysed. Hypoxanthine and xanthine are not hydrolysed. H_2S is not produced. Acid is produced from D-cellobiose and D-xylose. Acid is not produced from L-arabinose, D-fructose, D-galactose, D-glucose, myo-inositol, lactose, maltose, D-mannitol, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, sucrose or D-trehalose. Maltose, sucrose and D-xylose are utilized; D-cellobiose is weakly utilized, but L-arabinose, D-galactose, D-mannose, D-trehalose, benzoate and salicin are not utilized. Susceptible to cephalothin, gentamicin and novobiocin, but not to ampicillin or tetracycline. The predominant respiratory lipoquinone is Q-10. The major fatty acid is $\text{C}_{18:1\omega7c}$. Hydroxy fatty acids are detected. The DNA G + C content is 60.3 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, SW-109^T (= KCTC 12311^T = JCM 12599^T), was isolated from a tidal flat of the Yellow Sea in Korea.

Acknowledgements

This work was supported by the 21C Frontier program of Microbial Genomics and Applications (grant MG02-0401-001-1-0-0) from the Ministry of Science and Technology (MOST) of the Republic of Korea and Faculty Research Fund of Sungkyunkwan University.

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.
- Denner, E. B. M., Vybiral, D., Koblížek, M., Kämpfer, P., Busse, H.-J. & Velimirov, B. (2002). *Erythrobacter citreus* sp. nov., a yellow-pigmented bacterium that lacks bacteriochlorophyll *a*, isolated from the western Mediterranean Sea. *Int J Syst Evol Microbiol* **52**, 1655–1661.
- Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–203.
- Lanyi, 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.
- Sasser, M. (1990). *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*. Newark, DE: MIDI.
- Shiba, T. & Simidu, U. (1982). *Erythrobacter longus* gen. nov., sp. nov., an aerobic bacterium which contains bacteriochlorophyll *a*. *Int J Syst Bacteriol* **32**, 211–217.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Vybiral, D., Denner, E. B. M., Haller, C. M., Busse, H.-J., Witte, A., Höfle, M. G. & Velimirov, B. (1999). Polyphasic classification of $0.2\text{ }\mu\text{m}$ filterable bacteria from the Western Mediterranean Sea. *Syst Appl Microbiol* **22**, 635–646.
- 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 intraspecific phylogenetic analysis of the genus *Nocardioideis* and related taxa based on 16S rDNA sequences. *Int J Syst Bacteriol* **48**, 187–194.
- Yoon, J.-H., Kim, H., Kim, I.-G., Kang, K. H. & Park, Y.-H. (2003). *Erythrobacter flavus* sp. nov., a slight halophile from the East Sea in Korea. *Int J Syst Evol Microbiol* **53**, 1169–1174.
- Yoon, J.-H., Kang, K. H., Oh, T.-K. & Park, Y.-H. (2004). *Erythrobacter aquimaris* sp. nov., isolated from sea water of a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **54**, 1981–1985.
- Yoon, J.-H., Oh, T.-K. & Park, Y.-H. (2005). *Erythrobacter seohaensis* sp. nov. and *Erythrobacter gaetbuli* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **55**, 71–75.
- Yurkov, V., Stackebrandt, E., Holmes, A. & 7 other authors (1994). Phylogenetic positions of novel aerobic, bacteriochlorophyll *a*-containing bacteria and description of *Roseococcus thio-sulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., sp. nov., and *Erythrobacter litoralis* sp. nov. *Int J Syst Bacteriol* **44**, 427–434.