# Emended descriptions of the genus *Lewinella* and of *Lewinella cohaerens*, *Lewinella nigricans* and *Lewinella persica*, and description of *Lewinella lutea* sp. nov. and *Lewinella marina* sp. nov.

Shams Tabrez Khan, Yukiyo Fukunaga, Yasuyoshi Nakagawa and Shigeaki Harayama

Correspondence
Shams Tabrez Khan
shams-tabrez-khan@nite.go.jp

NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), 2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan

Two strains, MKG-38$^T$ and FYK2402M69$^T$, were isolated from a marine sediment sample and a sea snail, respectively, both collected on the Pacific coast of Japan. Phylogeny of these new isolates based on 16S rRNA gene sequences indicated that they are members of the genus *Lewinella*. Morphological, physiological and biochemical properties of these two isolates, together with the type strains of the three previously described species of the genus *Lewinella*, were characterized. The new isolates were Gram-negative, aerobic, rod-shaped, chemo-organotrophic and able to degrade starch and CM-cellulose. A comparative polyphasic study showed that these two isolates represent two novel species of the genus *Lewinella*, for which the names *Lewinella marina* sp. nov. (type strain, MKG-38$^T$=NBRC 102633$^T$=NCIMB 14312$^T$) and *Lewinella lutea* sp. nov. (type strain, FYK2402M69$^T$=NBRC 102634$^T$=NCIMB 14313$^T$) are proposed. Emended descriptions of the genus *Lewinella* (Sly *et al.* 1998) and of *Lewinella cohaerens*, *Lewinella nigricans* and *Lewinella persica* are also proposed.

Lewin (1970) described three novel marine species of the genus *Herpetosiphon*, namely *Herpetosiphon cohaerens*, *Herpetosiphon nigricans* and *Herpetosiphon persicus*, based on phenotypic characteristics. Later, 16S rRNA gene sequences of these strains were determined and a novel genus, *Lewinella*, in the family '*Saprospiraceae*' was created for these strains (Sly *et al.*, 1998). In this paper, we describe the characterization of two strains isolated on the Pacific coast of Japan; strain MKG-38$^T$ was isolated from a marine sediment sample collected at Kamogawa, whilst strain FYK2402M69$^T$ was isolated from a sea snail (*Nodilittorina trochoides*) collected from Mikurajima. Polyphasic characterization of the new isolates, together with the type strains of previously described species of the genus *Lewinella*, was performed. Based on their phenotypic traits and phylogenetic positions, we propose that the two new isolates represent novel species of the genus *Lewinella*.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Lewinella marina* MKG-38$^T$, *Lewinella lutea* FYK2402M69$^T$, *Lewinella cohaerens* II-2$^T$, *Lewinella nigricans* SS-2$^T$ and *Lewinella persica* T-3$^T$ are AB301495, AB301494, AB301614, AB301615 and AB301616, respectively.

Electron micrographs showing morphology of members of the genus *Lewinella* and a supplementary table showing pairwise 16S rRNA gene sequence similarities are available with the online version of this paper.

Strains MKG-38$^T$ and FYK2402M69$^T$ formed light-orange and dark-orange colonies, respectively, on half-strength marine agar (HSMA) plates (Marine Agar 2216; Difco) after 3–4 days incubation at 25 °C. Strains were maintained on HSMA plates and stored at −80 °C in artificial seawater (ASW; Naigai Chemicals) containing 20 % (v/v) glycerol.

For 16S rRNA gene sequencing, template DNA was prepared by using Prepman Ultra (Applied Biosystems). The 16S rRNA gene was amplified by using a universal set of primers (27f and 1492r) and sequenced directly, using a BigDye Terminator v3.1 cycle sequencing kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). By BLAST search (Altschul *et al.*, 1990) against the sequences available in GenBank/DDBJ, *Lewinella persica* (accession no. AF039295) was identified as the closest relative of strains MKG-38$^T$ and FYK2402M69$^T$. Thus, pairwise comparison of the 16S rRNA gene sequences of strains MKG-38$^T$ and FYK2402M69$^T$ with those of type strains of species of the genus *Lewinella* and other related genera of the family '*Saprospiraceae*' were performed by using the Needleman–Wunsch alignment algorithm (Needleman & Wunsch, 1970; http://www.ebi.ac.uk/emboss/align/). Strains MKG-38$^T$ and FYK2402M69$^T$ shared sequence similarity of 86.4–92.7 % with the members of the genus *Lewinella*, whereas they shared <85 % sequence similarity

  

with members of other related genera (see Supplementary Table S1, available in IJSEM Online). The 16S rRNA gene sequences of the strains shown in Fig. 1 were aligned by using the CLUSTAL_X program (Thompson *et al.*, 1997), and a phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987). The robustness of the tree topology was calculated from bootstrap analysis using 1000 resamplings of the sequences (Felsenstein, 1985). Strains MKG-38$^T$ and FYK2402M69$^T$ clustered with the members of the genus *Lewinella*. *L. persica* was the closest relative of both strains, and the grouping was supported by high bootstrap values (Fig. 1).

Morphological, physiological and biochemical traits of strains MKG-38$^T$ and FYK2402M69$^T$, *Lewinella cohaerens* NBRC 102661$^T$, *Lewinella nigricans* NBRC 102662$^T$ and *L. persica* NBRC 102663$^T$ were characterized. The morphology of Gram-stained and unstained cells was observed under a light microscope, and that of cells negatively stained with 1 % (w/v) phosphotungstic acid was observed under a transmission electron microscope (H7600; Hitachi).

For most of the tests, cells grown on HSMA plates for 3–5 days at 25 °C were used unless stated otherwise. Gram reactions were performed as described by Cowan & Steel (1993). The method described by Buck (1982) was also used for the differentiation of Gram-positive and Gram-negative bacteria. Gliding motility was examined in hanging drops of Marine Broth 2216 (MB; Difco) and on HSMA plates (Perry, 1973). Catalase activity was tested by mixing cells from colonies grown on HSMA plates with 3 % (v/v) hydrogen peroxide on a glass slide, whilst oxidase activity was tested by spotting the cells onto a cytochrome oxidase strip (Nissui Pharmaceuticals). Absorption spectra (260–700 nm) of acetone extracts of cells were recorded spectroscopically to examine the presence of carotenoid-type pigments. Bathochromic shift tests with 20 % (w/v) KOH were performed to test for the presence of flexirubin-type pigments (Fautz & Reichenbach, 1980). Growth at different temperatures (4, 10, 15, 20, 25, 30, 35, 37, 40, 42 and 45 °C) was assessed on HSMA plates, whilst growth at different pH values was examined on HSMA plates or in MB with the final pH adjusted with either HCl (pH 5 and 6) or NaOH (pH 8–10). Growth at high NaCl concentrations [4–8 % (w/v) NaCl] was tested in MB. Requirement of NaCl for growth was tested in one-fifth strength Luria–Bertani (LB) medium containing 0, 1 or 2 % (w/v) NaCl (Khan *et al.*, 2007). Utilization of different nitrogen sources [sodium nitrate (7.8 g $l^{-1}$), ammonium sulphate (7.8 g $l^{-1}$), sodium glutamate (20 g $l^{-1}$), Casamino acids (10 g $l^{-1}$) and peptone (10 g $l^{-1}$)] was tested in medium N (1.0 g glucose and 0.2 g $NaHCO_3$ dissolved in 1 l ASW, pH 7.0). Methods described in detail elsewhere (Cowan & Steel, 1993; Lewin & Lounsbery, 1969; Smibert & Krieg, 1981; Khan *et al.*, 2007) were used to test the abilities of the strains to hydrolyse agar, carrageenan (type I; Sigma), casein, cellulose, chitin, CM-cellulose (High Viscosity; Sigma), DNA, gelatin, starch and Tweens 20, 40 and 80. Indole production from tryptophan, acid production from glucose, hydrolysis of urea, aesculin and gelatin, and reduction of nitrate and nitrite by these strains were tested with the API 20NE system (bioMérieux), whilst their abilities to acidify different carbon sources were tested with the API 50CH system (bioMérieux) according to the

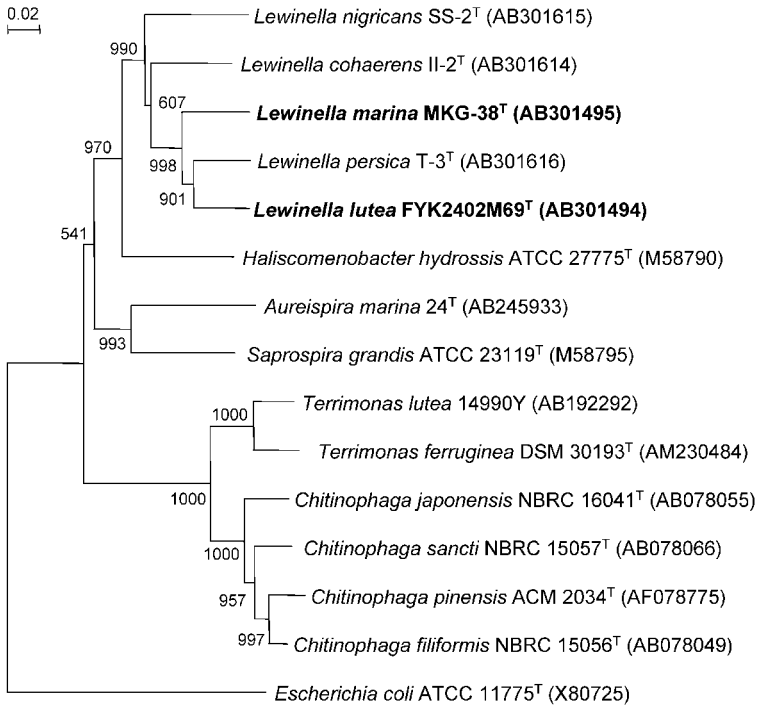


**Fig. 1.** Neighbour-joining tree showing the positions of strains MKG-38$^T$ and FYK2402M69$^T$ within the radiation of the genus *Lewinella*. Analysis was based on almost-complete 16S rRNA gene sequences. Numbers at nodes represent bootstrap values calculated from 1000 resamplings. Bar, 0.02 $K_{nuc}$.

manufacturer's instructions, except that inocula for API 20NE were prepared in ASW, whereas those for API 50CH were made in a 1 : 1 mixture of CHB medium (bioMérieux) and ASW. The API strips were incubated at 25 °C for 3–4 days before test results were scored.

For fatty acid analysis, strains were grown on Marine Agar (MA; Difco) for 3–4 days at 25 °C. Fatty acid profiles were determined by using the Sherlock microbial identification system (MIDI) according to the manufacturer's protocol (Sasser, 1990). Respiratory quinones were analysed by using the protocol of Nakagawa & Yamasato (1993). Exponentially growing cells of strains MKG-38$^T$ and FYK2402M69$^T$ in MB were used to prepare genomic DNA as described by Minamisawa (1990); the HPLC method of Mesbah *et al.* (1989) was used to determine the G+C content of the DNA samples.

Morphological, physiological and chemotaxonomic characteristics of the strains are summarized in Tables 1 and 2 and in the genus and species descriptions. Although strains MKG-38$^T$ and FYK2402M69$^T$ formed a monophyletic clade with *L. persica* (Fig. 1), low 16S rRNA gene sequence similarities (<92.7 %) and differences in phenotypic characteristics indicated clearly that strains MKG-38$^T$ and FYK2402M69$^T$ cannot be assigned to any of the previously described species in the genus *Lewinella*. Therefore, the names *Lewinella marina* sp. nov. and *Lewinella lutea* sp. nov. are proposed to include strains MKG-38$^T$ and FYK2402M69$^T$, respectively.

Originally, members of the genus *Lewinella* were described to be 2–3 μm in length (Sly *et al.*, 1998). Strains MKG-38$^T$ and FYK2402M69$^T$, however, were smaller than 2 μm in size (see Supplementary Fig. S1, available in IJSEM Online, and novel species descriptions below). In addition, members of the genus *Lewinella* have been described to be unable to degrade starch and cellulose (Sly *et al.*, 1998). In contrast to the description, all strains studied were able to degrade starch, and strains MKG-38$^T$ and FYK2402M69$^T$ were able to degrade CM-cellulose. Gliding motility has previously been observed in members of the genus *Lewinella* (Sly *et al.*, 1998). However, in the present study, no gliding motility was observed in any of the strains studied. Thus, an emended description of the genus *Lewinella* is proposed. We also emend the species descriptions of *L. cohaerens*, *L. nigricans* and *L. persica*,

**Table 1.** Differentiating characteristics of the novel isolates and other species of the genus *Lewinella*

Taxa: 1, *L. marina* MKG-38$^T$; 2, *L. lutea* FYK2402M69$^T$; 3, *L. cohaerens* NBRC 102661$^T$; 4, *L. nigricans* NBRC 102662$^T$; 5, *L. persica* NBRC 102663$^T$. All strains tested positive for the presence of carotenoid-type pigments, degradation of DNA, starch, casein, gelatin, Tweens 20, 40 and 80, β-galactosidase and β-glucosidase, growth at 15–30 °C, growth at pH 6–10, growth in 1–5 % (w/v) NaCl and growth in 30–100 % (v/v) seawater. All strains tested negative for flexirubin-type pigments, degradation of agar, cellulose, chitin and urea, reduction of nitrate and nitrite, indole production from tryptophan, acid production from glucose, arginine dihydrolase activity, growth at 45 °C and growth in 8 % NaCl (w/v). +, Positive; −, negative; (+), weakly positive; v, variable; tr, trace (<1 % of total).

| Characteristic | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Cell shape | Short to medium rods | Short to elongated rods | Short to elongated rods | Short to elongated rods | Short to elongated rods; clumps are formed |
| Pigment | Dull orange | Dark orange | Light orange | Diffusible black | Dark orange |
| CM-cellulose | + | + | − | − | − |
| Casein | (+) | (+) | + | + | + |
| Growth at: | | | | | |
| pH 5.0 | v | − | − | − | − |
| pH 9.0 | + | + | (+) | (+) | (+) |
| pH 10.0 | + | (+) | (+) | (+) | (+) |
| 4 °C | + | − | − | − | − |
| 35 °C | + | + | − | + | − |
| 37 °C | + | − | − | − | − |
| 42 °C | + | − | − | − | − |
| Growth in 7 % NaCl | + | − | − | − | − |
| Cellular fatty acids (% of total) | | | | | |
| C15 : 0 | tr | tr | 13 | 6 | tr |
| i-C17 : 1ω9*c* | 23 | 9 | tr | 2 | 10 |
| Summed feature 3* | 23 | 53 | tr | 9 | 30 |
| DNA G+C content (mol%) | 61 | 56 | 45† | 53† | 53† |

*Summed feature 3 comprises i-C15 : 0 2-OH and/or C16 : 1ω7*c*.
†DNA G+C content data for *L. cohaerens*, *L. nigricans* and *L. persica* are from Sly *et al.* (1998).

**Table 2.** Comparision of cellular fatty acid profiles of novel strains isolated during this study and of previously reported species of the genus *Lewinella*

Taxa: 1, *L. marina* MKG-38$^T$; 2, *L. lutea* FYK2402M69$^T$; 3, *L. cohaerens* NBRC 102661$^T$; 4, *L. nigricans* NBRC 102662$^T$; 5, *L. persica* NBRC 102663$^T$. tr, Trace (<1 % of total).

| Fatty acid | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| C15 : 0 | tr | tr | 13 | 6 | tr |
| i-C15 : 0 | 22 | 16 | 30 | 32 | 28 |
| i-C15 : 0 3-OH | 2 | 4 | 3 | 2 | 2 |
| i-C15 : 1 | 3 | 2 | 29 | 19 | 4 |
| C15 : 1$\omega$6*c* | 1 | 2 | – | – | 2 |
| C15 : 1$\omega$8*c* | – | – | – | – | 2 |
| C16 : 0 | 4 | 3 | tr | 7 | 2 |
| i-C16 : 0 | – | – | 3 | 3 | tr |
| i-C16 : 1 | – | 1 | 1 | – | tr |
| C16 : 1$\omega$5*c* | – | – | 2 | – | tr |
| C16 : 1$\omega$7*c* alcohol | tr | – | – | 1 | tr |
| C16 : 1$\omega$11*c* | – | – | – | 2 | – |
| i-C17 : 0 | 4 | 1 | 2 | tr | tr |
| i-C17 : 0 3-OH | 6 | 4 | 3 | 6 | 5 |
| C17 : 1$\omega$6*c* | 3 | 2 | tr | tr | 5 |
| C17 : 1$\omega$9*c* | – | – | – | 1 | – |
| i-C17 : 1$\omega$9*c* | 23 | 9 | tr | 2 | 10 |
| C18 : 1$\omega$7*c* | 2 | – | – | – | 1 |
| C19 : 0 10-methyl | 1 | – | – | – | 2 |
| Summed feature 3* | 23 | 53† | tr | 9 | 30 |
| Summed feature 4* | tr | – | 2 | – | tr |
| Unknown | 2 | 3 | 3 | 2 | 3 |

*Summed feature 3 comprises i-C15 : 0 2-OH and/or C16 : 1$\omega$7*c*; summed feature 4 comprises i-C17 : 1 and/or a-C17 : 1.
†Two peaks for summed feature 3 were detected.

because detailed phenotypic characteristics of these strains were obtained for the first time in this study.

## Emended description of the genus *Lewinella* Sly *et al.* 1998

*Lewinella* (Le.wi.nel′la. L. dim. ending *-ella*; N.L. fem. dim. n. *Lewinella* named after Professor Ralph Lewin, who first isolated these organisms).

Cells are Gram-negative, aerobic, chemo-organotrophic, asporogenic rods that are 0.7–3.0 μm in length and 0.3–0.7 μm in width; often elongated to a filament of 5–25 μm in length. Carotenoid-type pigments are produced; flexirubin-type pigments are not. Oxidase- and catalase-positive. Major respiratory quinone is MK-7. Major cellular fatty acids are i-C15 : 0, either i-C15 : 1 or i-C17 : 1$\omega$9*c* and either C15 : 0 or summed feature 3 (i-C15 : 0 2-OH and/or C16 : 1$\omega$7*c*). Members of the genus are marine bacteria that require NaCl for growth. Nitrate and nitrite are not reduced. Indole is not produced from tryptophan. The DNA G+C content is 45–61 mol%.

The type species of the genus is *Lewinella cohaerens*.

## Emended description of *Lewinella cohaerens* (Lewin 1970) Sly *et al.* 1998

*Lewinella cohaerens* (co.hae′rens. L. part. adj. *cohaerens* coherent, uniting together).

The species possesses the following characteristics in addition to those described for the genus. Cells are rod-shaped, 1.2–2.0 μm in length and 0.3–0.5 μm in width, often elongated to a long filament of 5–15 μm in length. Colonies on MA are light orange. Growth occurs at 10–30 °C (optimally at 25 °C). Growth does not occur at 4 °C or at 35 °C or higher. Growth occurs at pH 6–8 (optimally at pH 7). Weak growth is observed at pH 9 and 10. Growth does not occur at pH 5. Growth occurs with 2–4 % (w/v) NaCl; weak growth is observed with 5 % (w/v) NaCl. Growth does not occur with 0, 1 or 6 % (w/v) NaCl or higher. Positive for degradation of casein, DNA, gelatin, starch and Tweens 20, 40 and 80. Negative for degradation of agar, carrageenan, cellulose, chitin, CM-cellulose and urea. $\beta$-Galactosidase and $\beta$-glucosidase are positive, but arginine dihydrolase is negative. Casamino acids and peptone are utilized as nitrogen sources, but ammonium sulphate, sodium glutamate and sodium nitrate are not. Acid is produced from D-cellobiose, D-glucose, D-lactose, D-lyxose, D-turanose, aesculin and gentiobiose. Acid production is weak from amygdalin, arbutin, D-fucose, D-melezitose, D-tagatose, L-fucose and methyl-α-D-glucopyranoside. Major cellular fatty acids are i-C15 : 0, i-C15 : 1 and C15 : 0. The DNA G+C content is 45 mol%.

The type strain is II-2$^T$ (=NBRC 102661$^T$=NCIMB 12855$^T$). Isolated from beach sand at Biarritz, France.

## Emended description of *Lewinella nigricans* (Lewin 1970) Sly *et al.* 1998

*Lewinella nigricans* (ni′gri.cans. L. part. adj. *nigricans* blackening).

The species possesses the following characteristics in addition to those described for the genus. Cells are rod-shaped, 1.5–3.0 μm in length and 0.3–0.6 μm in width, often elongated to a filament of 5–25 μm in length. Colonies on MA produce black pigments. Growth occurs at 15–37 °C (optimally at 25–30 °C). Growth does not occur at 4, 10 or 40 °C or higher. Growth occurs at pH 6–8 (optimally at pH 7). Weak growth is observed at pH 9 and 10. Growth does not occur at pH 5. Growth occurs with 2–4 % (w/v) NaCl; weak growth is observed with 5 % (w/v) NaCl. Growth does not occur with 0 or 1 % (w/v) NaCl. Positive for degradation of casein, DNA, gelatin, starch and Tweens 20, 40 and 80. Negative for degradation of agar, carrageenan, cellulose, chitin, CM-cellulose and urea. $\beta$-Galactosidase and $\beta$-glucosidase are positive, but arginine dihydrolase is negative. Casamino acids and peptone are utilized as nitrogen sources, but ammonium sulphate, sodium glutamate and sodium nitrate are not. Acid is produced from starch, D-arabinose, D-cellobiose, D-galactose, D-glucose, D-lactose, D-maltose, D-melibiose,

D-melezitose, D-raffinose, D-sucrose, D-trehalose, D-turanose, D-xylose, aesculin, glycogen, L-arabinose, L-rhamnose, salicin and xylitol. Acid production is weak from amygdalin, arbutin, D-lyxose, D-fucose, D-mannose, L-fucose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside and *N*-acetylglucosamine. Major fatty acids are i-C15 : 0, i-C15 : 1 and summed feature 3 (i-C15 : 0 2-OH and/or C16 : 1*ω7c*). The DNA G+C content is 53 mol%.

The type strain is SS-2$^T$ (=NBRC 102662$^T$=NCIMB 1420$^T$). Isolated from beach sand near Lagos, Nigeria.

## Emended description of *Lewinella persica* (Lewin 1970) Sly *et al.* 1998

*Lewinella persica* [per′si.ca. L. adj. *persica* Persian (of peach), i.e. peach-coloured].

The species possesses the following characteristics in addition to those described for the genus. Cells are rod-shaped, 1.5–3.0 μm in length and 0.3–0.5 μm in width, often elongated to a filament of 5–17 μm in length. Cells adhere to each other, forming clumps. Colonies on MA are dark orange. Growth occurs at 4–30 °C (optimally at 25 °C). Growth does not occur at 35 °C or higher. Growth occurs at pH 6–8 (optimum at pH 7). Weak growth is observed at pH 9 and 10. Growth does not occur at pH 5. Growth occurs with 2–4 % (w/v) NaCl; weak growth is observed with 5 % (w/v) NaCl. Growth does not occur with 0, 1 or 6 % (w/v) NaCl or higher. Positive for degradation of casein, DNA, gelatin, starch and Tweens 20, 40 and 80. Negative for degradation of agar, carrageenan, cellulose, chitin and urea. β-Galactosidase and β-glucosidase are positive, but arginine dihydrolase is negative. Casamino acids and peptone are used as nitrogen sources, but ammonium sulphate, sodium glutamate and sodium nitrate are not. Acid is produced from D-galactose, D-raffinose, L-rhamnose, aesculin and gentiobiose. Acid production is weak from amygdalin, D-adonitol, D-cellobiose, D-fructose, D-glucose, D-lactose, D-mannose, D-ribose, D-sucrose, erythritol, L-fucose, methyl-α-D-glucopyranoside and methyl-β-D-xylopyranoside. Major cellular fatty acids are i-C15 : 0, summed feature 3 (i-C15 : 0 2-OH and/or C16 : 1*ω7c*) and i-C17 : 1*ω9c*. The DNA G+C content is 53 mol%.

The type strain is T-3$^T$ (=NBRC 102663$^T$=NCIMB 1396$^T$). Isolated from brown mud, Galway, Ireland.

## Description of *Lewinella marina* sp. nov.

*Lewinella marina* (ma.ri′na. L. fem. adj. *marina* of the sea, marine).

The species possesses the following characteristics in addition to those described for the genus. Cells are short rods, 0.6–1.0 μm in length and 0.4–0.5 μm in width. Colonies on MA are semi-dry and dull orange. Growth occurs at 4–42 °C (optimally at 25–30 °C). Growth does not occur at 45 °C. Growth occurs at pH 6–10 (optimally at pH 7–8). Weak growth is observed at pH 5. Growth is observed with 1–7 % (w/v) NaCl [optimally with 2–4 % (w/v) NaCl]. Positive for degradation of DNA, CM-cellulose, gelatin, starch and Tweens 20, 40 and 80. Casein degradation activity is weak. Negative for degradation of agar, carrageenan, cellulose, chitin and urea. β-Galactosidase and β-glucosidase are positive, but arginine dihydrolase is negative. Casamino acids and peptone are utilized as nitrogen sources, but ammonium sulphate, sodium glutamate and sodium nitrate are not. Acid is produced from amygdalin, arbutin, D-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, D-lyxose, D-maltose, D-melibiose, D-mannose, D-melezitose, D-raffinose, D-sucrose, D-trehalose, D-turanose, D-xylose, gentiobiose, aesculin, L-arabinose, L-fucose, L-rhamnose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, *N*-acetylglucosamine and salicin. Acid production is weak from starch, D-fucose, D-ribose, D-tagatose, L-sorbose, L-xylose and inulin. The major cellular fatty acids are i-C15 : 0, summed feature 3 (i-C15 : 0 2-OH and/or 16 : 1*ω7c*) and i-C17 : 1*ω9c*. The DNA G+C content is 61 mol%.

The type strain is MKG-38$^T$ (=NBRC 102633$^T$=NCIMB 14312$^T$). Isolated from a marine sediment sample of Kamogawa city, Japan.

## Description of *Lewinella lutea* sp. nov.

*Lewinella lutea* (lu.te′a. L. fem. adj. *lutea* orange-coloured).

The species possesses the following characteristics in addition to those described for the genus. Cells are short rods, 0.6–1.0 μm in length and 0.3–0.5 μm in width, often elongated to a filament of 5–20 μm in length. Colonies on MA are dark orange. Growth occurs at 10–35 °C (optimally at 25 °C). Growth does not occur at 4 or 37 °C or higher. Growth occurs at pH 6–8 (optimally at pH 7). Growth does not occur at pH 4 or 5. Weak growth is observed at pH 9 and 10. Growth occurs with 2–4 % (w/v) NaCl; weak growth is observed with 5 % (w/v) NaCl. Growth does not occur with 6 % (w/v) NaCl or higher. Positive for the degradation of CM-cellulose, DNA, gelatin, starch and Tweens 20, 40 and 80. Casein degradation activity is weak. Negative for degradation of agar, carrageenan, chitin, cellulose and urea. β-Galactosidase and β-glucosidase are positive, but arginine dihydrolase is negative. Casamino acids and peptone are utilized as nitrogen sources, but ammonium sulphate, sodium glutamate and sodium nitrate are not. Acid is produced from amygdalin, arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannose, D-melibiose, D-raffinose, D-sucrose, D-trehalose, D-turanose, D-xylose, gentiobiose, L-rhamnose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, *N*-acetylglucosamine and salicin. Acid production is weak from starch, D-fructose, D-melezitose and L-fucose. The major cellular fatty acids are summed feature 3 (i-C15 : 0 2-OH and/or

C16 : 1$\omega$7*c*), i-C15 : 0 and i-C17 : 1$\omega$9*c*. The DNA G+C content is 56 mol%.

The type strain is FYK2402M69$^T$ (=NBRC 102634$^T$= NCIMB 14313$^T$). Isolated from a marine snail collected from Mikurajima island, Japan.

## Acknowledgements

This work was supported by the New Energy and Industrial Technology Development Organization (NEDO; grant no. 04000182-0). The authors would like to thank Ms Mai Takahashi for providing 16S rRNA gene sequences of *Lewinella* species and Mr Motoyuki Ohuchi for technical assistance.

## References

**Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990).** Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

**Buck, J. D. (1982).** Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.

**Cowan, S. T. & Steel, K. J. (1993).** *Manual for the Identification of Medical Bacteria*, 3rd edn. London: Cambridge University Press.

**Fautz, E. & Reichenbach, H. (1980).** A simple test for flexirubin-type pigments. *FEMS Microbiol Lett* **8**, 87–91.

**Felsenstein, J. (1985).** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

**Khan, S. T., Nakagawa, Y. & Harayama, S. (2007).** *Sediminibacter furfurosus* gen. nov., sp. nov. and *Gilvibacter sediminis* gen. nov., sp. nov., novel members of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **57**, 265–269.

**Lewin, R. A. (1970).** New *Herpetosiphon* species (Flexibacterales). *Can J Microbiol* **16**, 517–520.

**Lewin, R. A. & Lounsbery, D. C. (1969).** Isolation, cultivation and characterization of flexibacteria. *J Gen Microbiol* **58**, 145–170.

**Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

**Minamisawa, K. (1990).** Division of rhizobitoxine-producing and hydrogen-uptake positive strains of *Bradyrhizobium japonicum* by *nifDKE* sequence divergence. *Plant Cell Physiol* **31**, 81–89.

**Nakagawa, Y. & Yamasato, K. (1993).** Phylogenetic diversity of the genus *Cytophaga* revealed by 16S rRNA sequencing and menaquinone analysis. *J Gen Microbiol* **139**, 1155–1161.

**Needleman, S. B. & Wunsch, C. D. (1970).** A general method applicable to the search for similarities in the amino acid sequence of two proteins. *J Mol Biol* **48**, 443–453.

**Perry, L. B. (1973).** Gliding motility in some non-spreading flexibacteria. *J Appl Bacteriol* **36**, 227–232.

**Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

**Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc. http://www.midi-inc.com/media/pdfs/TechNote_101.pdf

**Sly, L. I., Taghavi, M. & Fegan, M. (1998).** Phylogenetic heterogeneity within the genus *Herpetosiphon*: transfer of the marine species *Herpetosiphon cohaerens*, *Herpetosiphon nigricans* and *Herpetosiphon persicus* to the genus *Lewinella* gen. nov. in the *Flexibacter–Bacteroidetes–Cytophaga* phylum. *Int J Syst Bacteriol* **48**, 731–737.

**Smibert, R. M. & Krieg, N. R. (1981).** General characterization. In *Manual of Methods for General Bacteriology*, pp. 409–443. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.

**Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.