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Limibacillus halophilus gen. nov., sp. nov., a moderately halophilic bacterium in the family *Rhodospirillaceae* isolated from reclaimed land

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A Gram-stain-negative, aerobic, non-motile, non-spore-forming and short rod-shaped bacterial strain, designated CAU 1121^T, was isolated from reclaimed land in the Republic of Korea and its taxonomic position was investigated using a polyphasic approach. The bacterium grew optimally at 37 °C, at pH 6.5 and in the presence of 2 % (w/v) NaCl. Based on 16S rRNA gene sequence similarity, the novel isolate belonged to the family Rhodospirillaceae within the class Alphaproteobacteria and formed an independent lineage within the evolutionary radiation encompassed by the phylum Proteobacteria. Strain CAU 1121^T exhibited very low levels of 16S rRNA gene sequence similarity with its phylogenetic neighbours Pelagibius litoralis (similarity, 92.5 %), Fodinicurvata fenggangensis (similarity, 91.4 %), Fodinicurvata sediminis (similarity, 90.7 %) and *Tistlia consotensis* (similarity, 91.0 %). Strain CAU 1121^T contained ubiquinone-10 as the only respiratory quinone and $C_{18:1}\omega7c$ as the major cellular fatty acid. The DNA G+C content of the strain was 65 mol%. On the basis of phylogenetic inference, and physiological and chemotaxonomic data, it is proposed that strain CAU 1121^T represents a novel genus and novel species in the family Rhodospirillaceae, for which the name Limibacillus halophilus gen. nov., sp. nov. is suggested. The type strain is CAU 1121^T (=KCTC 42420^T=CECT 8803^T=NBRC 110928^T).

The family Rhodospirillaceae comprises 34 genera at the time of writing, namely Azospirillum, Caenispirillum, Conglomeromonas, Constrictibacter, Defluviicoccus, Desertibacter, Dongia, Elstera, Ferrovibrio, Fodinicurvata, Inquilinus, Insolitispirillum, Limimonas, Magnetospira, Magnetospirillum, Magnetovibrio, Marispirillum, Nisaea, Novispirillum, Oceanibaculum, Pelagibius, Phaeospirillum, Phaeovibrio, Rhodocista, Rhodospira, Rhodospirillum, Rhodovibrio, Roseospira, Skermanella, Telmatospirillum, Thalassobaculum, Thalassospira, Tistlia and Tistrella (http://www.bacterio.net). Members of the family include photo- and chemoheterotrophs, with varying metabolic and nutritional properties (Nissen & Dundas, 1984; Mack et al., 1993; Garrity et al., 2005). Some members of the family Rhodospirillaceae are known to change nutritional mode depending on the concentration of oxygen and carbon sources and the availability of light. This is demonstrated by members of the genera Rhodovibrio, Rhodospirillum, Rhodospira, Rhodocista, *Roseospira* and *Phaeospirillum* (Kawasaki *et al.*, 1992; Pfennig *et al.*, 1997; Imhoff *et al.*, 1998). Some members of the family *Rhodospirillaceae*, such as those of the genus *Azospirillum*, have the ability to fix atmospheric nitrogen (Xie & Yokota, 2005a).

Among the 92 recognized species in the family Rhodospirillaceae, only 15 species related to the genera Marispirillum (Lai et al., 2009a), Nisaea (Urios et al., 2008), Oceanibaculum (Lai et al., 2009b; Dong et al., 2010), Pelagibius (Choi et al., 2009), Rhodospirillum (Drews, 1981), Rhodospira (Pfennig et al., 1997), Rhodovibrio (Mack et al., 1993; Imhoff et al., 1998), Roseospira (Kalyan Chakravarthy et al., 2007), Thalassobaculum (Zhang et al., 2008; Urios et al., 2010) and Thalassospira (López-López et al., 2002; Liu et al., 2007; Kodama et al., 2008; Tsubouchi et al., 2014) have been found in marine environments. During the screening of microorganisms with biotechnological potential from the marine environments on the west coast of the Korean peninsula, a novel moderately halophilic bacterial strain, designated CAU 1121^T, was isolated. The objective of this study was to establish the taxonomic position of strain CAU 1121^T using a polyphasic taxonomic approach that included the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU 1121^{T} is KP233090.

One supplementary figure is available with the online Supplementary Material.

determination of phenotypic and chemotaxonomic properties, and 16S rRNA gene sequence analysis.

Strain CAU 1121^T was isolated from a sample of reclaimed land in Modo $(37^{\circ} 32' 12.28'' \text{ N} 126^{\circ} 24' 51.47'' \text{ E})$, Republic of Korea, using the dilution plating technique described by Gordon & Mihm (1962). Sample dilutions were plated on marine agar 2216 (MA; Difco) and incubated at 30 °C for 10 days under aerobic conditions. Single colonies were purified through subculture plating and preserved at -80 °C in marine broth (MB; Difco) containing 25 % (v/v) glycerol.

Morphological, physiological and biochemical characteristics of strain CAU 1121^T were investigated using cells cultivated on MA at 30 °C. Cell morphology was observed using a light microscope (model DM 1000, Leica Microsystems). Transmission electron microscopy (model JEM 1010, JEOL) was used to determine the presence of flagella of cells from an exponentially growing culture. The Gram reaction was determined using the bioMérieux Gram staining kit, according to the manufacturer's instructions. Motility was assessed by the hanging-drop method. Growth at 4-45 °C was investigated on MA using an aerobic incubator (model MIR-253, Sanyo Electric Biomedical) and a Bactron anaerobic chamber (Sheldon Manufacturing). The optimal pH for growth was determined by culturing at 37 °C in MB with a pH range of 4.5-10.0. pH levels were adjusted at 0.5 unit increments using sodium acetate/acetic acid and Na₂CO₃ buffers. Growth in the presence of 0-15.0 % (w/v; increased at 1 % increments) NaCl was investigated by culturing in MB prepared according to the Difco formula, except that NaCl was excluded and that 0.45 % (w/v) MgCl₂. 6H₂O and 0.06 % (w/v) KCl were added. Catalase production was determined by gas production by cells treated with 3 % hydrogen peroxide. Oxidase production was examined using 1 % (w/v) tetramethyl-p-phenylenediamine (Cappuccino & Sherman, 2002). Hydrolysis of casein, gelatin, starch, aesculin, citrate and Tween 80 was examined as described by Lányí (1987) and Smibert & Krieg (1994). In addition, acid production from carbohydrate metabolism, and other physiological and biochemical characteristics were examined using the API 20E and API 50CH systems (bioMérieux). Enzymic activities were analysed using API ZYM systems (bioMérieux), according to the manufacturer's instructions. API 20E and API ZYM strips were read after 24 h and API 50CH strips after 48 h.

PCR amplification of the 16S rRNA gene of strain CAU 1121^T was carried out as described by Cho *et al.* (2008). The amplified 16S rRNA gene was sequenced by using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems Life Technologies) and an automatic DNA sequencer (model 3730, Applied Biosystems). The nearly complete 16S rRNA gene sequence of strain CAU 1121^T (1447 bp) was established and compared with reference sequences from members of the family *Rhodospirillaceae* available in the GenBank database. Multiple alignment of the 16S rRNA gene sequence with recognized species in the

family Rhodospirillaceae and calculation of sequence similarity levels were performed by using the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon) and CLUSTAL X 2.1 program (Larkin et al., 2007). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximumlikelihood (Felsenstein, 1981) algorithms in the PHYLIP package (Felsenstein, 1989). Evolutionary distance matrices were generated by the neighbour-joining method described by Jukes & Cantor (1969). The phylogenetic tree topology was assessed by the bootstrap resampling method (Felsenstein, 1985), based on 1000 replicates of the neighbour-joining dataset using the SEQBOOT and CONSENSE programs from the PHYLIP package. The G + C content (mol%) of the genomic DNA was determined following a modification of the method of Tamaoka & Komagata (1984) with the modification that reversed-phase HPLC was used.

For the determination of fatty acid composition, the cell mass of strain CAU 1121^T was harvested from MA (Difco) after cultivation for 3 days at 30 °C. The physiological age of the biomass harvested for fatty acid analysis was standardized by observing growth development during incubation of the cultures and choosing the moment of harvesting according to the standard MIDI Sherlock Microbial Identification System method. Cellular fatty acid methyl esters were obtained by using the method of Minnikin et al. (1980) and separated in an automated gas chromatograph (model 6890N, Agilent Technologies) fitted with a 7683 autosampler (Agilent). Peaks were detected by using the Microbial Identification software package (MOORE library version 5.0; MIDI database TSBA6). Menaquinones were isolated according to the method of Komagata & Suzuki (1987) and were separated by HPLC using an isocratic solvent system [methanol/isopropyl ether (3:1, v/v)] and a flow rate of 1 ml min⁻¹.

Detailed morphological, cultural, physiological and biochemical characteristics are presented in Table 1 and the species description. Strain CAU 1121^T was Gram-stainnegative, non-spore-forming, strictly aerobic and nonmotile. Cells were rod-shaped, approximately 0.3-0.45 µm in diameter and 1.0-2.0 µm in length. Flagella were not observed (Fig. S1, available in the online Supplementary Material). Strain CAU 1121^T differed from its relatives in the genera Pelagibius, Tistlia, Fodinicurvata, Rhodovibrio, Nisaea, Thalassobaculum and Azospirillum by the absence of flagella. Colonies were cream in colour, entire, circular and convex with irregular margins after 5 days of cultivation on MA at 30 °C. Growth of strain CAU 1121^T was observed over a temperature range of 20–40 °C (optimum, 37 °C), over a pH range from pH 6.5 to 10.5 (optimum, pH 6.5). Because CAU 1121^T was isolated from a brackish environment, it required NaCl for growth and grew with up to 5 % (w/v) NaCl (optimum, 2 %). The growth temperature range distinguished strain CAU 1121^T (20-40 °C) from members of the genera Pelagibius (15-33 °C), Fodinicurvata (15-42 °C), Rhodovibrio (25-47 °C), Nisaea (15-44 °C), Thalassobaculum (10-35 °C) and Azospirillum (4-41 °C). In addition, the salt tolerance range distinguished

Table 1. Phenotypic characteristics of strain CAU 1121^T and the most closely related genera of the family *Rhodospirillaceae*

Strains: 1, CAU 1121^T (data from this study); 2, *Pelagibius litoralis* CL-UU02^T (Choi *et al.*, 2009); 3, *Tistlia consotensis* USBA 355^T (Díaz-Cárdenas *et al.*, 2010); 4, *Fodinicurvata sediminis* YIM D82^T (Wang *et al.*, 2009); 5, *Rhodovibrio salinarum* ATCC 35394^T (Imhoff *et al.*, 1998; Garrity *et al.*, 2005); 6, *Nisaea denitrificans* DR41_21^T (Urios *et al.*, 2008); 7, *Thalassobaculum litoreum* CL-GR58^T (Zhang *et al.*, 2008); 8, *Azospirillum lipoferum* 59b^T (Tarrand *et al.*, 1978; Lavrinenko *et al.*, 2010). All taxa are Gram-stain-negative, and positive for oxidase and nitrate reduction (the following data were not available: oxidase for the genera *Tistlia* and *Rhodovibrio*; nitrate reduction for the genus *Rhodovibrio*). +, Positive; –, negative; V, variable; ND, no data available.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---------------------------|--|---|--|--|---|---|---|---|
| Habitat | Reclaimed land | Coastal seawater | Saline spring | Deposit of salt mine | Seawater | Seawater | Coastal seawater | Wheat root |
| Colony colour | Cream | Cream | ND | Cream-white | Pink | Cream | Cream-yellow | Pink |
| Cell shape | Short rod | Slightly curved rod | Slightly curved rod | Rod and vibrioid | Vibrioid, spiral | Rod | Slightly curved rod and straight rod | Vibrioid |
| Flagella | _ | + | _ | _ | + | + | + | + |
| Temperature (°C) | | | | | | | | |
| Range | 20-40 | 15-33 | 20-40 | 15-42 | 25-47 | 15-44 | 10-35 | 4-41 |
| Optimum | 37 | 28-30 | 30 | 28 | 35-42 | 30 | 30-35 | 37 |
| рН | | | | | | | | |
| Range | 6.5-10.5 | 6.0-11.0 | 5.0-8.0 | 6.5-8.5 | 7.0-8.0 | 5.0-9.0 | 7.0-9.0 | 5.7-6.8 |
| Optimum | 6.5 | 7.0-8.0 | 6.5-6.7 | 7.5 | 7.0 | 6.0 | 8.0 | ND |
| Salt tolerance | 0-5 | 2-6 | 0-4 | 1.5-20 | 3-24 | 0–6 | 1-10 | 0-4 |
| (%, w/v) | | | | | | | | |
| Catalase | + | + | — | + | ND | ND | + | + |
| Utilization | | | | | | | | |
| L-Arabinose | _ | + | + | + | - | - | + | + |
| D-Glucose | + | + | + | + | - | + | - | + |
| Inositol | _ | + | + | - | ND | ND | - | _ |
| D-Mannitol | — | + | ND | + | — | + | - | _ |
| D-Rhamnose | _ | — | + | - | ND | ND | - | _ |
| D-Ribose | _ | — | - | - | + | - | + | + |
| Sucrose | _ | — | ND | + | - | - | + | - |
| Major quinone | Q-10 | Q-10 | Q-10, Q-9 | Q-10 | Q-10, MK-10 | Q-10 | Q-10 | Q-10 |
| Major fatty acids | С _{18 : 1} <i>w</i> 7 <i>c</i> , | С _{18 : 1} <i>w</i> 7 <i>c</i> , | $C_{19:0}$ cyclo $\omega 8c$, | С _{18 : 1} <i>w</i> 7 <i>с</i> , | C _{18 : 1} , C _{18 : 0} , | С _{18 : 1} <i>w</i> 7 <i>c</i> , С _{16 : 1} <i>w</i> 7 <i>c</i> , | C _{18 : 1} ω7 <i>c</i> , | C _{18 : 1} ω7 <i>c</i> , C _{16 : 1} ω7 <i>c</i> , |
| | C _{19 : 0} cyclo <i>ω</i> 8 <i>c</i> | C _{18:0} 3-OH | $C_{18:1}\omega$ 7 <i>c</i> , $C_{18:0}$ | C _{18 : 1} 2-OH, C _{16 : 0} | $C_{16:0}, C_{14:0}$ | C _{16 : 0} | $C_{16:0}, C_{17:0}$ | C _{16 : 0} , C _{14 : 0} 3-OH, C _{16 : 0} 3-OH |
| DNA G+C content (mol%) | 65 | 66.3 | 71 | 61.5 | 66–67 | 60 | 68 | 69–70 |

strain CAU 1121^T (0–5 %) from members of the genera *Pelagibius* (2–6 %), *Fodinicurvata* (1.5–20 %), *Rhodovibrio* (3–24 %) and *Thalassobaculum* (1–10 %). Strain CAU 1121^T hydrolysed gelatin and had enzyme activities for esterase (C4), esterase lipase (C8) and trypsin; it was weakly positive for α -chymotrypsin and acid phosphatase. However, strain CAU 1121^T differed from its close relative *Pelagibius litoralis* CL-UU02^T (Choi *et al.*, 2009) by its ability to hydrolyse gelatin, positive reactions for arginine dihydrolase and urease, and esterase lipase (C8) and trypsin enzymic activity. In addition, strain CAU 1121^T differed from members of the genera *Nisaea, Fodinicurvata* and *Thalassobaculum* by its negative reactions for alkaline phosphatase and leucine arylamidase.

In the phylogenetic tree based on the neighbour-joining algorithm, strain CAU 1121^T formed a distinct lineage within the evolutionary radiation encompassed by the family Rhodospirillaceae, its closest relatives were members of the genera Pelagibius, Tistlia, Fodinicurvata and Rhodovibrio (Fig. 1). The trees obtained with the maximum-likelihood and leastsquares algorithms also supported the same topology. The cluster comprising the novel strain joined the phylogenetic lineage of *Pelagibius litoralis* CL-UU02^T at a bootstrap resampling value of 82 %, but strain CAU 1121^T exhibited a very low level of 16S rRNA gene sequence similarity (92.5 %) to Pelagibius litoralis CL-UU02^T. Strain CAU 1121^T exhibited very low levels of 16S rRNA gene sequence similarity (85.8-91.4 %) to other type strains of genera in the family *Rhodos*pirillaceae. The G+C content of the DNA of strain CAU 1121^T was 65 mol%, which compares with a range of



Fig. 1. Neighbour-joining phylogenetic tree derived from nearly complete 16S rRNA gene sequences showing the position of strain CAU 1121^T among members of the family *Rhodospirillaceae*. *Azorhizobium caulinodans* LMG 6465^T (AP009384) was used as an outgroup organism. Only bootstrap values >70 % are given (1000 replications). Filled circles indicate that the corresponding nodes were also recovered with the least-squares and maximum-likelihood algorithms. Bar, 0.1 substitutions per nucleotide position.

66.2–71.0 mol% for the type strains of the recognized bacterial species in Fig. 1.

Overall, the chemotaxonomic data were in agreement with previously published data for the class *Alphaproteobacteria* (Labrenz *et al.*, 2000). The predominant isoprenoid quinone detected in strain CAU 1121^T was ubiquinone 10 (Q-10), in line with most members of the family *Rhodospirillaceae* (Xie & Yokota, 2005b), which is consistent with data for the type species of the genus, *Pelagibius litoralis* CL-UU02^T (Choi *et al.*, 2009) and *Fodinicurvata fenggangensis* YIM D812^T (Wang *et al.*, 2009). The fatty acid profile of strain CAU 1121^T is shown in Table 2, together with those of members of the genera *Pelagibius* (Choi *et al.*, 2009), *Tistlia* (Díaz-Cárdenas *et al.*, 2010), *Fodinicurvata*

Table 2. Cellular fatty acid content (%) of strain CAU1121^T and members of the most closely related genera of the family *Rhodospirillaceae*

Strains: 1, CAU 1121^T (data from this study); 2, *Pelagibius litoralis* CL-UU02^T (Choi *et al.*, 2009); 3, *Tistlia consotensis* USBA 355^T (Díaz-Cárdenas *et al.*, 2010); 4, *Fodinicurvata sediminis* YIM D82^T (Wang *et al.*, 2009); 5, *Rhodovibrio salinarum* ATCC 35394^T (Imhoff *et al.*, 1998; Garrity *et al.*, 2005). TR, Trace (<1.0 %); –, not detected; summed feature 3, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; ELC, equivalent chain-length.

| Fatty acid | 1 | 2 | 3 | 4 | 5 |
|---|------|------|------|------|------|
| Saturated | | | | | |
| C _{13:0} | TR | - | _ | - | _ |
| C _{14:0} | TR | - | - | 1.4 | 1.0 |
| C _{16:0} | 4.5 | 1.5 | - | 11.8 | 7.4 |
| C _{17:0} | 6.9 | TR | - | TR | - |
| C _{18:0} | 5.2 | 3.0 | 18.0 | 9.3 | 23.0 |
| C _{19:0} | TR | - | - | - | - |
| Unsaturated | | | | | |
| $C_{17:1}\omega 8c$ | 1.0 | - | - | - | - |
| $C_{18:1}\omega7c$ | 34.5 | 48.5 | 26.0 | 48.6 | 35.2 |
| $C_{18:1}\omega 5c$ | TR | - | - | - | - |
| $C_{18:1}\omega 9c$ | - | 1.6 | - | 2.0 | - |
| $C_{20:1}\omega7c$ | TR | - | - | - | - |
| C _{20 : 2} <i>w</i> 6,9 <i>c</i> | - | - | 1.2 | TR | - |
| 10-Methyl C _{19 : 0} | - | 4.6 | - | - | - |
| 11-Methyl C _{18 : 1} ω7c | 7.5 | 7.0 | TR | 1.0 | - |
| C _{17:0} cyclo | TR | - | - | - | - |
| C _{19:0} cyclo ω8c | 20.3 | 10.3 | 38.0 | 7.9 | - |
| Branched-chain | | | | | |
| iso-C _{18:0} | _ | - | 1.0 | - | _ |
| Hydroxy | | | | | |
| C _{15:0} 2-OH | TR | - | - | - | - |
| C _{16:0} 2-OH | 1.2 | TR | - | - | - |
| C _{17:0} 2-OH | TR | - | _ | - | _ |
| C _{17:0} 3-OH | TR | TR | _ | - | _ |
| C _{18 : 1} 2-OH | 3.7 | 3.1 | 3.7 | 12.2 | _ |
| C _{18:0} 3-OH | 1.0 | 17.5 | 3.6 | 1.3 | - |
| Summed feature 3 | 10.4 | - | 1.5 | TR | TR |
| ECL 14.959 | - | 1.2 | _ | - | - |
| | | | | | |

(Wang et al., 2009) and Rhodovibrio (Nissen & Dundas, 1984; Mack et al., 1993). The strain contained saturated, unsaturated and hydroxy fatty acids. The major fatty acids (>10 %) were $C_{18:1}\omega7c$ (34.5 %), $C_{19:0}$ cyclo $\omega8c$ (20.3 %) and $C_{16:1}\omega 7c/C_{16:1}\omega 6c$ (10.4 %). Other fatty acids (>1 %) were 11-methyl $C_{18:1}\omega$ 7c (7.5 %), $C_{17:0}$ (6.9 %), $C_{18:0}$ (5.2 %), $C_{16:0}$ (4.5 %), $C_{18:1}$ 2-OH (3.7 %), $C_{16\,:\,0}$ 2-OH (1.2 %), $C_{18\,:\,0}$ 3-OH (1.0 %) and $C_{17:1}\omega 8c$ (1.0 %). Trace amounts of $C_{17:0}$ 2-OH (0.8 %), $C_{17:0}$ cyclo (0.7 %), $C_{18:1}\omega 5c$ (0.5 %), $C_{14:0}$ (0.4 %), $C_{15:0}$ 2-OH (0.4 %), $C_{20:1}\omega7c$ (0.4 %), $C_{17:0}$ 3-OH $(0.3 \%), C_{19:0}(0.3 \%) \text{ and } C_{13:0}(0.2 \%) \text{ were also present.}$ The fatty acid $C_{18:1}\omega7c$ was the major component in strain CAU 1121^T and the members of closely related genera, except Tistlia consotensis USBA 355^T (Table 2), which is characteristic of members of the class Alphaproteobacteria (Labrenz et al., 2000), although there were differences in the proportions of some fatty acids. However, strain CAU 1121^T could be distinguished from the members of the genus Rhodovibrio by the higher content of $C_{19:0}$ cyclo $\omega 8c$ (20.4 %), and from those of the other genera by the presence of $C_{17:0}$ (6.9 %) and summed feature 3 (10.4 %).

The predominant ubiquinone (Q-10) detected in strain CAU 1121^T was the same as that in all members of the class Alphaproteobacteria used in the phylogenetic analysis except the members of the genera Phaeospirillum (Tarrand et al., 1978; Schleifer et al., 1991; Imhoff et al., 1998; López-López et al., 2002; Zhang et al., 2008; Choi et al., 2009; Wang et al., 2009; Díaz-Cárdenas et al., 2010). Strain CAU 1121^T could not be clearly differentiated from the members of the genus Pelagibius based on chemotaxonomic characteristics, but their fatty acid profiles exhibited differences in the proportions of the predominant fatty acids compared with other closely related genera (Zhang et al., 2008; Choi et al., 2009; Wang et al., 2009; Díaz-Cárdenas et al., 2010). Strain CAU 1121^T was clearly distinguishable from members of the most closely related genus, Pelagibius, on the basis of temperature range and salt tolerance for growth. The low level of 16S rRNA gene sequence similarity between strain CAU 1121^T and the reference type strains of the family Rhodospirillaceae, together with the differential phenotypic characteristics, differentiate this strain.

On the basis of the phenotypic, chemotaxonomic and genotypic data presented, strain CAU 1121^T represents a novel species of a new genus, for which the names *Limibacillus* gen. nov., and *Limibacillus halophilus* sp. nov. are proposed.

Description of Limibacillus gen. nov.

Limibacillus (Li.mi.ba.cil'lus. L. masc. n. *limus* mud; L. masc. n. *bacillus* rod; N.L. masc. n. *Limibacillus* a rod-shaped bacterium from mud, referring to the typical habitat of the family and isolation of the type strain from coastal soil).

Cells are aerobic, short rods which do not form spores. Catalase- and oxidase-positive. Gram reaction is negative. Q-10 is the predominant ubiquinone and $C_{18:1}\omega7c$ and $C_{19:0}$ cyclo $\omega8c$ are the dominant fatty acids. Phylogenetically, 16S rRNA gene sequence analysis classifies the genus *Limibacillus* in the family *Rhodospirillaceae*. The type species is *Limibacillus halophilus*.

Description of Limibacillus halophilus sp. nov.

Limibacillus halophilus (ha.lo'phi.lus. Gr. n. *hals halos* salt; Gr. adj. *philos* friendly, loving; N.L. masc. adj. *halophilus* salt loving, referring to the requirement for salt).

Cells are Gram-stain negative and non-spore-forming. Cells are approximately 0.3-0.45 µm in diameter and 1.0-2.0 µm in length, and show non-motility. Optimally, growth occurs at 37 °C (temperature range for growth 20-40 °C), pH 6.5 (pH range for growth pH 6.5–10.5) and with optimum NaCl of 2 % (w/v) (NaCl range for growth 0-5 %). Catalase- and oxidase-positive. Nitrate is reduced. Gelatin and urea are hydrolysed, but casein and aesculin are not. H₂S is not produced. Growth occurrs on D-glucose and maltose as sole carbon source. According to API ZYM, positive for esterase (C4), esterase lipase (C8) and trypsin, weakly positive for α-chymotrypsin and acid phosphatase, but negative for alkaline phosphate, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. According to the API 20NE system, positive for nitrate reduction, glucose fermentation, assimilation of maltose and L-arginine, weakly positive for assimilation of 4-nitrophenyl- β -D-galactopyranoside, but negative for assimilation of L-tryptophan, Larabinose, D-mannitol and potassium gluconate.

The type strain is CAU 1121^{T} (=KCTC 42420^{T} =CECT 8803^{T} -NBRC 110928^{T}), isolated from reclaimed land in Modo in South Korea. The DNA G+C content of the type strain is 65 mol%.

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