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Luteimonas aestuarii sp. nov., Isolated from Tidal Flat Sediment

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A novel bacterium B9^T was isolated from tidal flat sediment. Its morphology, physiology, biochemical features, and 16S rRNA gene sequence were characterized. Colonies of this strain are yellow and the cells are Gram-negative, rod-shaped, and do not require NaCl for growth. The 16S rRNA gene sequence similarity indicated that strain B9^T is associated with the genus *Lysobacter* (≤ 97.2%), *Xanthomonas* (≤ 96.8%), *Pseudomonas* (≤ 96.7%), and *Luteimonas* (≤ 96.0%). However, within the phylogenetic tree, this novel strain shares a branching point with the species *Luteimonas composti* CC-YY255^T (96.0%). The DNA-DNA hybridization experiments showed a DNA-DNA homology of 23.0% between strain B9^T and *Luteimonas mephitis* B1953/27.1^T. The G+C content of genomic DNA of the type strain is 64.7 mol% (SD, 1.1). The predominant fatty acids are iso-$C_{11:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, iso-$C_{17:1}$ *ω9c*, and iso-$C_{11:0}$ 3-OH. Combined analysis of the 16S rRNA gene sequences, fatty acid profile, and results from physiological and biochemical tests indicated that there is genotypic and phenotypic differentiation of the isolate from other *Luteimonas* species. For these reasons, strain B9^T was proposed as a novel species, named *Luteimonas aestuarii.* The type strain of the new species is B9^T (= KCTC 22048^T, DSM 19680^T).

***Keywords*:** *Luteimonas aestuarii* sp. nov., taxonomy

The genus *Luteimonas* belongs to the γ-subclass *Proteobacteria*, as first proposed by Finkmann *et al.* (2000). This genus currently contains only two species (1997): *Luteimonas mephitis* B1953/27.1^T that was isolated from an experimental biofilter (Finkmann *et al.*, 2000) and *Luteimonas composti* CC-YY255^T that was isolated from food waste (Young *et al.*, 2007). This paper aims to establish the taxonomic position of strain B9^T isolated from a marine environment, through phenotypic, genetic, and chemotaxonomic analyses.

Materials and Methods

Bacterial strains

The novel strain B9^T was isolated from tidal flat sediment in Yeosu (34°47'26" N 127°34'01" E), Republic of Korea. The novel strain was isolated on TSBA [tryptic soy broth solidified with 20.0 g agar per liter (Difco)], followed by repeated restreaking to obtain a pure culture. For broth cultivation, TSB medium was used. Bacterial cultures of the isolates were stored at -80°C in the presence of 20% (v/v) glycerol. The novel strain was deposited into the KCTC (Korean Collection for Type Cultures) as KCTC 22048^T as well as the DSMZ (German Collection of Microorganisms and Cell Cultures) as DSM 19680^T. Reference strains used for DNA-DNA homology tests included *Luteimonas mephitis* B1953/27.1^T, obtained from CRBIP (Centre de Ressources Biologiques de l'Institut Pasteur), and *Lysobacter gummosus* UASM 402^T from KCTC.

Morphology and physiological characteristics

The cell morphology was examined by light microscopy (ECLIPSE 80*i*, Nikon) and Gram staining was performed by Gram staining method (Gram, 1884) and the non-staining method described by Buck (Buck, 1982). Catalase activity was determined by observing bubble production in a 3% (v/v) hydrogen peroxide solution. Growth under different temperatures (10, 15, 20, 25, 30, 34, 37, 40, and 42°C) and pH (4.0∼13.0 at intervals of 0.5 pH units) were assessed on TSB medium. The requirements and tolerance of various NaCl (0.0, 1.0, 3.0, 5.0, 7.5, 10.0, 15.0, and 20.0%, w/v) concentrations were determined in R2A broth medium supplemented with the appropriate concentrations of NaCl. API 20NE, API ZYM test strips (bioMérieux), and Biolog GN plates with GN/GP inoculating fluid were used to investigate enzyme activity and substrate utilization from sole carbon sources.

Determination of 16S rRNA gene sequencing, phylogenetic analysis, and DNA-DNA hybridization

Chromosomal DNA was extracted and purified using a DNA Extraction Kit (G-spin™, iNtRON Biotechnology). Determination of 16S rRNA gene sequencing, phylogenetic analysis, and DNA-DNA hybridization were performed as described previously (Roh *et al.*, 2008). The identification

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Table 1. Characteristics that differentiate *Luteimonas aestuarii* sp. nov. from its closely related species
Taxa: 1, *Luteimonas aestuarii* B9^T sp. nov.; 2, *L. composti* CC-YY255^T (data from Young *et al.*, 2007); 3, *L. mephitis* B1953/27.1^T (Finkmann *et al.*, 2000). +, positive; -, negative; w, weak; NR, not reported.

Characteristic	1	2	3
Growth at 6% NaCl	-	+	NR
NaCl range (%) for growth	0~3	0~6	NR
Growth at 37°C	+	+	-[a]
Nitrate reduction	-	+	-
Aesculin hydrolysis	+	+	-
Utilization of			
D-Glucose	+	+	w[a]
D-Galactose	-	+	-[a]
D-Gluconate	+	-	+[a]
Enzyme activities			
Esterase (C4)	-	+	NR
β-Galactosidase	-	+	NR
α-Glucosidase	-	+	NR
β-Glucosidase	+	+	-
DNA G+C content	64.7	68.1	NR

[a] Data from Young *et al.* (2007)

of phylogenetic neighbors was initially carried out by the BLAST (Altschul *et al.*, 1997) and FASTA (Pearson and Lipman, 1988) programs against the database of type strains of validly named species (Chun *et al.*, 2007). The 50 sequences with the highest scores were then selected for the calculation of pairwise sequence similarity using global alignment algorithm, which was implemented at the EzTaxon server version 1 [http://www.eztaxon.org/; (Chun *et al.*, 2007)].

Nucleotide sequence accession number
The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence of strain B9^T is EF660758.

Determination of G+C content and analysis of cellular fatty acid
The G+C content was determined through triplicate experiments by a fluorimetric method using SYBR Green and a real-time PCR thermocycler (Gonzalez and Saiz-Jimenez, 2002). For quantitative analysis of cellular fatty acid composition, the novel isolate was cultivated on TSBA at 28°C for 2 days and cells were harvested. Cellular fatty acids were saponified, methylated, extracted, as described by Sherlock Microbial Identification Systems (MIDI), and analyzed by gas chromatography (Hewlett Packard 6890) and identified using the Microbial Identification software package (Sasser, 1990).

Results and Discussion

Morphological and physiological characteristics
Strain B9^T is Gram-negative, catalase-, and oxidase-positive, and can grow at up to 3% (w/v) NaCl and 40°C. Aesculin hydrolysis and gelatin hydrolysis occured. A detailed species description is presented below and Table 1 shows a comparison between strain B9^T and closely related strains.

Phylogenetic analysis and DNA-DNA hybridization
The 16S rRNA gene sequence similarity indicated that strain B9^T is associated with the genus *Lysobacter* (≤ 97.2%), *Xanthomonas* (≤ 96.8%), *Pseudomonas* (≤ 96.7%), and *Luteimonas* (≤ 96.0%). However, within the phylogenetic tree with 16S rRNA gene sequences from members of the genus *Lysobacter*, *Xanthomonas*, *Pseudomonas*, *Luteimonas*, and other genus groups, which were selected according to the 16S rRNA gene sequence similarity, this novel strain falls within the cluster of *Luteimonas* species (Fig. 1) and is closely related to the type strains *Luteimonas composti* CC-YY255^T (96.0%), and *Luteimonas mephitis* B1953/27.1^T (95.5%). The tree topology is also supported from different tree-making algorithms using minimum evolution, maximum-parsimony, and maximum-likelihood method. The result of DNA-DNA relatedness showed a DNA-DNA homology of 23.0% and 15.7% between strain B9^T and *Luteimonas mephitis* B1953/27.1^T and *Lysobacter gummosus* UASM 402^T, respectively. The characteristics of 16S rRNA gene sequence similarity and DNA-DNA relatedness values below the threshold of 70% (Wayne *et al.*, 1987) indicate that strain B9^T represents a distinct genospecies. The molecular phylogenetic analysis shows that strain B9^T has the highest 16S rRNA gene sequence similarity with *Lysobacter gummosus* UASM 402^T (97.2%). However, strain B9^T is clustered with *Luteimonas* species in the phylogenetic tree regardless of different tree-making algorithms. Despite the each low bootstrap values (53~66%) of the four treeing methods used, supporting the branching point between the major *Luteimonas* cluster and the strain B9^T, most of the branching points remained unchanged regardless of the treeing methods, supporting the affiliation of the strain B9^T with the genus *Luteimonas*.

G+C content and analysis of cellular fatty acid
The G+C content of genomic DNA of the strain B9^T is 64.7 mol% (SD, 1.1). The value of genomic DNA G+C content in the validated *Luteimonas* species, *Luteimonas composti* CC-YY255^T is 68.1 mol% (Young *et al.*, 2007), which is similar to the value of novel strain. The predominant cellular fatty acids in strain B9^T were iso-$C_{17:1}$ ω9*c* (19.2%), iso-$C_{15:0}$ (16.8%), iso-$C_{11:0}$ (16.0%), iso-$C_{17:0}$ (10.7%), iso-$C_{11:0}$ 3-OH (10.1%), and iso-$C_{16:0}$ (8.7%). $C_{17:0}$ cyclo and iso-$C_{13:0}$ 3-OH were absent or rare as described by Finkmann *et al.* (2000) in the description of *Luteimonas mephitis* B1953/27.1^T. There were no $C_{18:1}$ ω7*c* and iso-$C_{17:0}$ 3-OH in the genus *Luteimonas*, although these two fatty acids were detected in the genus *Lysobacter*. *Luteimonas* strains had higher contents of iso-$C_{11:0}$, anteiso-$C_{15:0}$, and iso-$C_{17:0}$ than *Lysobacter* (Young *et al.*, 2007; Park *et al.*, 2008). The detailed fatty-acid composition of strain B9^T is shown in Table 2. In addi-

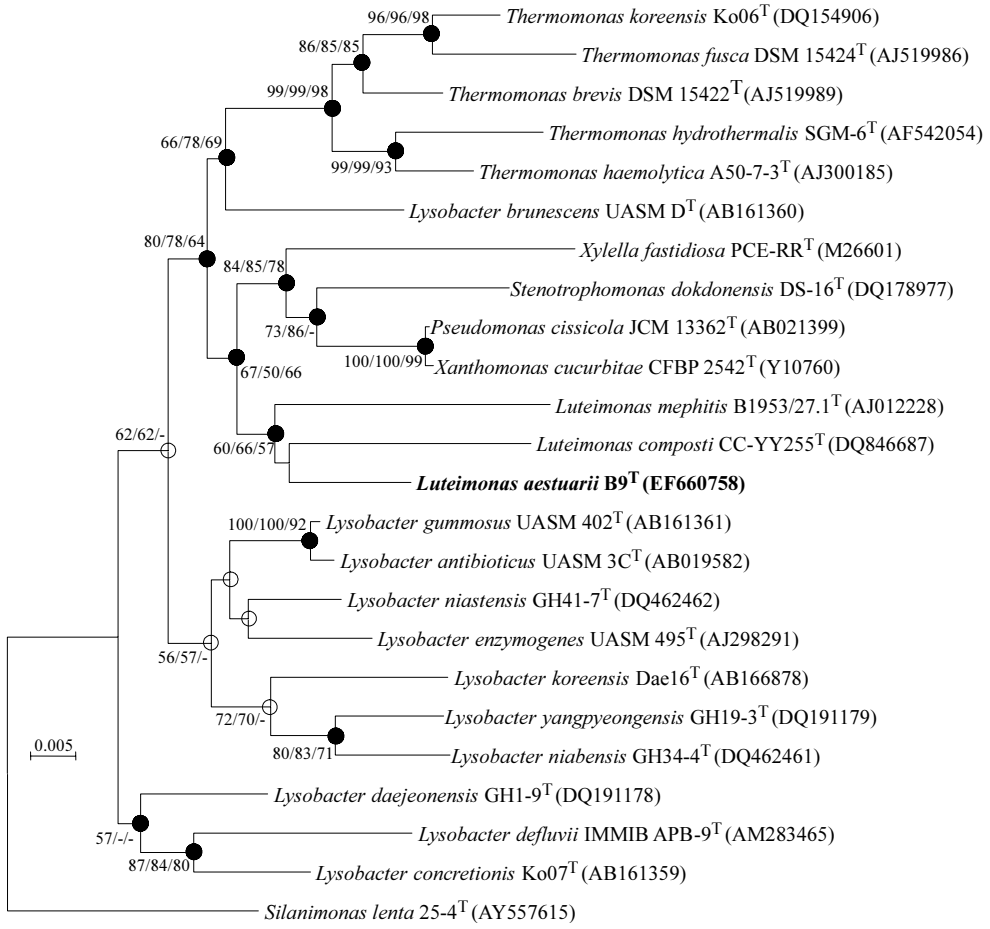

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences. The position of strain B9^T is shown with respect to other species of the genus *Luteimonas.* The phylogenetic tree was generated by the neighbor-joining method. Filled circles and open circles indicate generic branches that were also recovered using the minimum evolution and maximum-parsimony algorithm, and using the minimum evolution algorithm, respectively. Numbers at nodes indicate bootstrap values (1,000 replications) as calculated by neighbor-joining/minimum evolution/maximum-parsimony probabilities in percent. Bootstrap values greater than 50% are shown at the branch points. GenBank accession no. are shown in parentheses. Bar, 0.005 accumulated changes per nucleotide.

tion to the phylogenetic tree, the major fatty acid components of strain B9^T confirm that this novel strain belongs to the genus *Luteimonas*.

Results from 16S rRNA gene sequence analysis, and physiological and biochemical tests indicated that there are genotypic and phenotypic differences between strain B9^T and other *Luteimonas* species. Thus, on the basis of genetic, chemotaxonomic, and phenotypic comparisons to previously described taxa, strain B9^T is the type strain of a novel species of the genus *Luteimonas*, for which the name *Luteimonas aestuarii* sp. nov. is proposed.

Description of *Luteimonas aestuarii* sp. nov.

Luteimonas aestuarii (a.es.tu.a.ri'i. L. gen. n. aestuarii, of a tidal flat)

Cells are Gram-negative and rod-shaped (0.5×1.5~2.0 μm). The colonies are yellow-colored and circular. Growth occurs in 0~3% (w/v) NaCl, at temperatures ranging from 15 to 40°C, and in the pH range of 6.5 to 11.0. Optimal growth conditions are temperatures ranging from 34 to 37°C, a pH of 8.0, and NaCl concentration of 0%. The strain cannot reduce nitrate to nitrite, indole is not produced and D-glucose fermentation does not occur. L-Arginine dihydrolase-, urease-negative, and oxidase-, catalase-positive. Aesculin hydrolysis and gelatin hydrolysis occurs but PNPG (p-nitrophenyl-β-D-galactopyranoside) hydrolysis does not occur. According to growth on Biolog GN plates, the strain assimilates α-cyclodextrin, dextrin, glycogen, Tween 80, *N*-acetyl-D-glucosamine, D-cellobiose, i-erythritol, D-fructose, gentiobiose, α-D-glucose, maltose, D-melibiose, D-trehalose, turanose, xylitol, acetic acid, D-gluconic acid, D-glucosaminic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-keto glutaric acid, α-keto valeric acid, D,L-lactic acid, L-alaninamide, L-alanine, L-alanylglycine, L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, L-leucine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, inosine, and uridine as sole carbon sources, but not other carbon sources on Biolog GN plates. Positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phospatase, naph-

Table 2. Fatty acid contents (% of the total fatty acids) of strain B9^T and closely related *Luteimonas* and *Lysobacter* species
Taxa: 1, *Luteimonas aestuarii* B9^T; 2, *Luteimonas composti* CC-YY255^T; 3, *Luteimonas mephitis* B1953/27.1^T; 4, *Lysobacter capsici* YC5194^T; 5, *Lysobacter gummosus* KCTC 12132^T; 6, *Lysobacter antibioticus* KCTC 12129^T; 7, *Lysobacter enzymogenes* KCTC 12131^T; 8, *Lysobacter koreensis* KCTC 12204^T; 9, *Lysobacter daejeonensis* DSM 17634^T; 10, *Lysobacter yangpyeongensis* DSM 17635^T. Data in column 2 and 3 are from Young *et al.* (2007) and in column 4∼10 are from Park *et al.* (2008). All strains were grown on TSBA at 28°C for 2 days. tr, trace (less than 1.0%); -, not detected.

Fatty acid	1	2	3	4	5	6	7	8
Saturated (straight-chain)								
$C_{10:0}$	tr	-	tr	tr	tr	tr	-	tr
$C_{14:0}$	1.7	-	tr	1.9	1.3	1.2	4.1	1.7
$C_{16:0}$	3.6	4.7	1.6	10.8	17.6	15.4	21.5	18.8
Saturated (branched)								
iso-$C_{10:0}$	tr	tr	tr	-	-	-	-	-
iso-$C_{11:0}$	16.0	6.5	5.2	2.3	tr	tr	-	tr
anteiso-$C_{11:0}$	tr	tr	tr	-	-	-	-	-
iso-$C_{13:0}$	tr	-	tr	tr	-	-	-	-
iso-$C_{14:0}$	tr	1.9	1.5	tr	-	tr	tr	-
iso-$C_{15:0}$	16.8	25.5	47.4	23.3	tr	4.8	tr	tr
anteiso-$C_{15:0}$	4.0	2.7	3.0	tr	-	-	tr	-
iso-$C_{16:0}$	8.7	13.8	5.6	-	-	-	tr	-
iso-$C_{17:0}$	10.7	5.5	5.5	3.7	-	-	tr	-
Unsaturated								
iso-$C_{17:1}$ $\omega 9c$	19.2	25.5	18.7	-	-	-	-	-
$C_{18:1}$ $\omega 7c$	-	-	-	6.5	22.6	18.3	13.6	22.1
Hydroxy								
iso-$C_{11:0}$ 3-OH	10.1	5.5	5.3	3.8	-	-	-	-
iso-$C_{17:0}$ 3-OH	-	-	-	tr	4.6	2.7	3.6	4.1
Summed feature 3[a]	tr	5.5	tr	20.4	14.4	19.5	26.9	16.4
Cyclopropane acids								
$C_{17:0}$ cyclo	-	-	tr	tr	6.9	9.2	10.8	8.1

[a] Summed feature 3 comprised $C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2-OH

tol-AS-BI-phosphohydrolase, and *N*-acetyl-β-glucosaminidase; and negative for esterase (C4), lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, and α-fucosidase. Predominant fatty acids are iso-$C_{17:1}$ $\omega 9c$, iso-$C_{15:0}$, iso-$C_{11:0}$, iso-$C_{17:0}$, iso-$C_{11:0}$ 3-OH, and iso-$C_{16:0}$. The G+C content of genomic DNA of the type strain is 64.7 mol% (SD, 1.1).

The type strain is B9^T (= KCTC 22048^T, DSM 19680^T) and was isolated from tidal flat sediment in Yeosu (34°47'26" N 127°34'01" E), Republic of Korea.

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