**Litorisediminicola beolgyonensis** gen. nov., sp. nov., isolated from a coastal sediment

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A Gram-negative, non-spore-forming, aerobic, non-flagellated and rod- or oval-shaped bacterial strain, BB-MW24ᵀ, was isolated from a coastal sediment in South Korea. Strain BB-MW24ᵀ grew optimally at 30–37 °C, at pH 7.0–7.5 and in the presence of 2.0–3.0 % (w/v) NaCl. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences revealed that strain BB-MW24ᵀ clustered with *Ponticoccus litoralis* CL-GR66ᵀ and *Roseivivax lentus* S5-5ᵀ, with which it exhibited 96.0 and 96.2 % 16S rRNA gene sequence similarity, respectively. Strain BB-MW24ᵀ exhibited 85.2 % gyrB sequence similarity with *Sagittula stellata* DSM 11524ᵀ and 83.3 and 83.2 % gyrB sequence similarity with *P. litoralis* DSM 18986ᵀ and *R. lentus* S5-5ᵀ, respectively. Strain BB-MW24ᵀ contained Q-10 as the predominant ubiquinone and C₁₈:₁ω₇c as the predominant fatty acid. The polar lipid profile in strain BB-MW24ᵀ was similar to those of members of the genera *Ponticoccus* and *Roseivivax*, but one unidentified phospholipid was found as a major polar lipid only in strain BB-MW24ᵀ. The DNA G + C content was 68.9 mol%. The phylogenetic data and differential chemotaxonomic and phenotypic properties revealed that strain BB-MW24ᵀ represents a novel species in a new genus within the class Alphaproteobacteria, for which the name *Litorisediminicola beolgyonensis* gen. nov., sp. nov. is proposed; the type strain of *Litorisediminicola beolgyonensis* is BB-MW24ᵀ (=KCTC 32139ᵀ = CCUG 62953ᵀ).

A variety of tidal flats of western and southern coasts of the Korean peninsula have been proven to be good environments from which to isolate novel bacterial taxa (e.g. Kwon et al., 2006; Yoon et al., 2009, 2010; Baik et al., 2010; Lee et al., 2012). During the isolation of micro-organisms from a tidal flat of the southern coast of the peninsula, where cockles (*Tegillarca granosa*) are known to inhabit and high contents of germanium and selenium are found, many bacterial strains have been isolated and characterized taxonomically. One of these isolates, designated BB-MW24ᵀ, is described in this study. Comparative 16S rRNA gene sequence analysis showed that this strain was most phylogenetically closely related to the genera *Roseivivax* and *Ponticoccus* of the class Alphaproteobacteria. The genus *Roseivivax* was created by Suzuki et al. (1999) and, at the time of writing, includes five species with validly published names: *Roseivivax halodurans* and *Roseivivax halotolerans* (Suzuki et al., 1999), *Roseivivax lentus* (Park et al., 2010), *Roseivivax isoporae* (Chen et al., 2012) and *Roseivivax sediminis* (Xiao et al., 2012). The genus *Ponticoccus* was created by Hwang & Cho (2008) with the description of *Ponticoccus litoralis*, the sole recognized species of the genus until now. The aim of the present work was to determine the exact taxonomic position of strain BB-MW24ᵀ by using a polyphasic characterization that included determination of the phenotypic and chemotaxonomic properties and a detailed phylogenetic investigation based on 16S rRNA gene sequences.

Strain BB-MW24ᵀ was isolated by the dilution-plating technique on marine agar 2216 (MA; BD) at 25 °C and cultivated routinely on MA at 30 °C. Strain BB-MW24ᵀ was maintained on MA at 4 °C for short-term preservation and as a glycerol suspension (20 %, w/v) in distilled water at −80 °C for long-term preservation. *P. litoralis* DSM 18986ᵀ and *R. lentus* S5-5ᵀ were used as reference strains for phenotypic characterization, fatty acid analysis and sequencing of the DNA gyrase B subunit gene (gyrB). Cell morphology was examined by using light microscopy (BX51; Olympus) and transmission electron microscopy (CM-20; Philips). The latter technique was also used to assess the presence of flagella on cells from an exponentially increasing culture of strain BB-MW24ᵀ.
grown MA culture. For this purpose, cells were negatively
stained with 1% (w/v) phosphotungstic acid and 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. The presence
of poly-β-hydroxybutyrate granules was investigated by
epifluorescence microscopy (BX51) after staining with Nile
blue A as described by Ostle & Holt (1982). Growth under
anaerobic conditions was determined after incubation
in an anaerobic chamber (1029; Forma; N2/CO2/H2,
anaerobic conditions was determined after incubation
with RNA. The 16S rRNA gene was amplified by PCR
sequence was determined for both strands by
extension from vector-specific priming sites (T7 and SP-6 in
pGEM T-easy vector) according to the manufacturer’s
instructions. The gyrB sequence was determined for both strands by
extension from vector-specific priming sites (T7 and SP-6 in
pGEM T-easy vector). Phylogenetic analysis of the gyrB
sequence was performed as described by Yoon et al. (2007).

Isoprenoid quinones were extracted according to the
method of Komagata & Suzuki (1987) and analysed using
reversed-phase HPLC and a YMC ODS-A column
(250 x 4.6 mm). The isoprenoid quinones were eluted by
a mixture of methanol/2-propanol (2:1, v/v) at a flow rate
of 1 ml min⁻¹ at room temperature and detected by UV
absorbance at 275 nm. For fatty acid methyl ester analysis,
cell mass of strain BB-MW24T was harvested from MA
after incubation at 30 °C for 3 and 5 days, respectively, and
cell mass of P. litoralis DSM 18986T and R. lentus S5-5T was
harvested from MA after incubation at 30 °C for 3 days.
Fatty acids were saponified, methylated and extracted using
the standard protocol of the Sherlock Microbial
Identification System version 4.0 (MIDI), analysed by GC
(Hewlett Packard 6890) and identified using the TSBA40
database of the Microbial Identification System (Sasser,
1990). Polar lipids were extracted according to the
procedures described by Minnikin et al. (1984) and
separated by two-dimensional TLC using chloroform/
methanol/water (65:25:3.8, v/v) in the first dimension and
chloroform/methanol/acetic acid/water (40:7.5:6:1.8,
v/v) in the second dimension as described by Minnikin
et al. (1977). Individual polar lipids were identified by
spraying with ethanolic molybdoephosphoric acid, mol-
ydenum blue, ninhydrin and x-naphthol reagents
(Minnikin et al., 1984; Komagata & Suzuki, 1987) and
Dragendorff’s reagent (Sigma). The DNA G+C content
was determined by the method of Tamaoka & Komagata
(1984) with the modification that DNA was hydrolysed
and the resultant nucleotides were analysed by reversed-
phase HPLC equipped with a YMC ODS-A column
(250 x 4.6 mm). The nucleotides were eluted by a mixture
of 0.55 M NH4H2PO4 (pH 4.0) and acetonitrile (40:1, v/
v) at a flow rate of 1 ml min⁻¹ at room temperature and
detected by UV absorbance at 270 nm.

The morphological, cultural, physiological and biochemical
characteristics of strain BB-MW24T are given in the species
description and Table 1. The almost-complete 16S rRNA
gene sequence of strain BB-MW24T determined in this
study comprised 1382 nt, approximately 95% of the

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**Table 1.** Differential phenotypic characteristics of strain BB-MW24T and its closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell morphology</strong></td>
<td>Rods, ovals</td>
<td>Coci, short rods</td>
<td>Rods</td>
</tr>
<tr>
<td>Growth with 15% (w/v) NaCl</td>
<td>−</td>
<td>+*</td>
<td>−</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>−</td>
<td>+*</td>
<td>−</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>+*</td>
<td>−</td>
</tr>
<tr>
<td>Starch</td>
<td>−</td>
<td>+*</td>
<td>−</td>
</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>−*</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>w</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Maltose</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Enzyme activities (API ZYM)</td>
<td></td>
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<tr>
<td>Acid phosphatase</td>
<td>w</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>−</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Susceptibility to:</td>
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<td></td>
<td></td>
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<tr>
<td>Oleandomycin</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Penicillin G</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>68.9</td>
<td>67.9*</td>
<td>68.2</td>
</tr>
</tbody>
</table>

*Data for *P. litoralis* DSM 18986T from Hwang & Cho (2008).
The major polar lipids found in strain BB-MW24T were phosphatidylcholine, phosphatidylglycerol, phosphatidyldiyglycerol, one unidentified phospholipid and two unidentified lipids (Table 1). The DNA G+C content of strain BB-MW24T was 68.9 mol%, a similar value to those of the reference strains in that the major polar lipids were present (Fig. S1, available in IJSEM Online). The polar lipid profile of strain BB-MW24T was similar to those of the genus Roseivivax and representatives of some other related taxa. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood algorithm Stappia stellulata IAM 12621T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

From the phylogenetic analysis based on 16S rRNA gene sequences, the genus Roseivivax may not be monophyletic and may have to be taxonomically re-evaluated. However, it is likely that the re-evaluation should await additional phenotypic data or additional species.

The phylogenetic analyses based on 16S rRNA gene and gyrB sequences and the differential chemotaxonomic and phenotypic properties suggest that strain BB-MW24T represents a novel genus and species within the Alphaproteobacteria, for which the name Litorisediminicola beolgyonensis gen. nov., sp. nov. is proposed.

**Description of Litorisediminicola gen. nov.**

*Litorisediminicola* [Li.to.ri.se.di.mi.ni.co.la. L. n. *litus*-oris the seashore, coast; L. n. *sedimen*-inis sediment; L. suff. *cola* (from L. n. *incola*) inhabitant; N.L. masc. n. *Litorisediminicola* an inhabitant of coastal sediment, referring to the source of the organism].

Cells are aerobic, Gram-negative, non-spore-forming, non-motile and rod- or oval-shaped. Catalase- and oxidase-positive. Nitrate is not reduced to nitrite. The predominant ubiquinone is Q-10. The predominant fatty acid is C18 : 1ω7c.

Strain BB-MW24T was distinguishable from *P. litoralis* DSM 18986T and *R. lentus* SS-5T by differences in phenotypic properties, including cellular morphology, nitrate reduction, hydrolysis of and acid production from some substrates and susceptibility to antibiotics (Table 1).
Litorisediminicola beolgyonensis sp. nov.

Litorisediminicola beolgyonensis (be.ol.gy.o.nen'sis. N.L. masc. adj. beolgyonensis of or belonging to Beolgyo, from where the type strain was isolated).

Cells are aerobic, Gram-negative, non-flagellated and rod- or oval-shaped (0.3–0.9 × 0.8–6.0 μm). Several cells contain poly-β-hydroxybutyrate granules. Colonies on MA are circular, slightly convex, smooth, glistening, greyish yellow and 1.0–1.5 mm in diameter after incubation for 3 days at 30 °C. Optimal growth temperature is 30–37 °C; growth occurs at 10 and 40 °C, but not at 4 and 45 °C. Optimal pH for growth is pH 7.0–7.5; growth occurs at pH 5.5, but not at pH 5.0. Optimal growth occurs in the presence of 2.0–3.0 % (w/v) NaCl; growth occurs in the presence of 0.5–14.0 % (w/v) NaCl. Mg²⁺ ions are required for growth. Catalase- and oxidase-positive. Nitrate is not reduced to nitrite. Casein, gelatin, hypoxanthine, L-tyrosine and Tweens 20, 40, 60 and 80 are hydrolysed, but aesculin, starch and xanthine are not. Acid is produced from L-arabinose, cellobiose, D-fructose (weak), D-galactose, maltose (weak), D-ribose and D-xylose, but not from D-glucose, myo-inositol, lactose, D-mannitol, D-mannose, melibiose, melezitose, raffinose, L-rhamnose, D-sorbitol, sucrose or trehalose. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, oleandomycin and streptomycin and...
Table 2. Cellular fatty acid compositions (%) of strain BB-MW24 T and its closest phylogenetic neighbours

Strains: 1, Litorisidelimicola beolygonyensis gen. nov., sp. nov. BB-MW24 T; 2, L. beolygonyensis gen. nov., sp. nov. BB-MW24 T (5 days); 3, Ponticoccus litoralis DSM 18986 T; 4, Roseivivax lentus SS-5 T. All data were taken from this study after cultivation for 3 days unless otherwise indicated. Fatty acids that represented <0.5 % in all strains are not shown. tr, Trace (<0.5 %); −, not detected.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td><strong>Straight-chain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>1.7</td>
<td>2.1</td>
<td>6.6</td>
<td>7.2</td>
</tr>
<tr>
<td>C17:0</td>
<td>tr</td>
<td>−</td>
<td>−</td>
<td>1.9</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.5</td>
<td>4.7</td>
<td>5.3</td>
<td>5.1</td>
</tr>
<tr>
<td><strong>Unsaturated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:1ω7c</td>
<td>79.5</td>
<td>73.3</td>
<td>72.5</td>
<td>66.9</td>
</tr>
<tr>
<td>C20:1ω7c</td>
<td>tr</td>
<td>0.7</td>
<td>−</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>Hydroxy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12:0 3-OH</td>
<td>1.1</td>
<td>1.3</td>
<td>tr</td>
<td>1.4</td>
</tr>
<tr>
<td>C12:0 3-OH</td>
<td>−</td>
<td>−</td>
<td>1.3</td>
<td>0.6</td>
</tr>
<tr>
<td>C12:1 3-OH</td>
<td>2.2</td>
<td>3.2</td>
<td>2.8</td>
<td>2.1</td>
</tr>
<tr>
<td><strong>Cyclo C19:0 ω8c</strong></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>2.8</td>
</tr>
<tr>
<td>11-Methyl C18:1ω7c</td>
<td>9.3</td>
<td>12.0</td>
<td>10.7</td>
<td>10.3</td>
</tr>
<tr>
<td><strong>Summed feature 7</strong></td>
<td>1.2</td>
<td>0.8</td>
<td>−</td>
<td>tr</td>
</tr>
</tbody>
</table>

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 7 contained one or more of C19:1ω6c, cyclo C19:0ω10c and unknown fatty acid (equivalent chain-length 18.846).

weakly susceptible to tetracycline, but resistant to lincomycin, penicillin G and polymyxin B. With API ZYM, alkaline phosphatase, esterase (C4), esterase lipase (C8) and leucine arylamidase activities are present and acid phosphatase activity is weakly present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase, β-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. The predominant ubiquinone is Q-10. The predominant fatty acid is C18:1ω7c. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid, one unidentified lipid and one unidentified aminolipid; minor amounts of diphosphatidylglycerol, one unidentified phospholipid and two unidentified lipids are present.

The type strain, BB-MW24 T (=KCTC 32139 T =CCUG 62953 T), was isolated from coastal sediment at Beolgyo, South Korea. The DNA G+C content of the type strain is 68.9 mol% (HPLC).

Acknowledgements

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References


