

## Supplementary Materials

International Journal of Systematic and Evolutionary Microbiology

### *Limobrevibacterium gyesilva* gen. nov., sp. nov., Isolated from Forest Soil

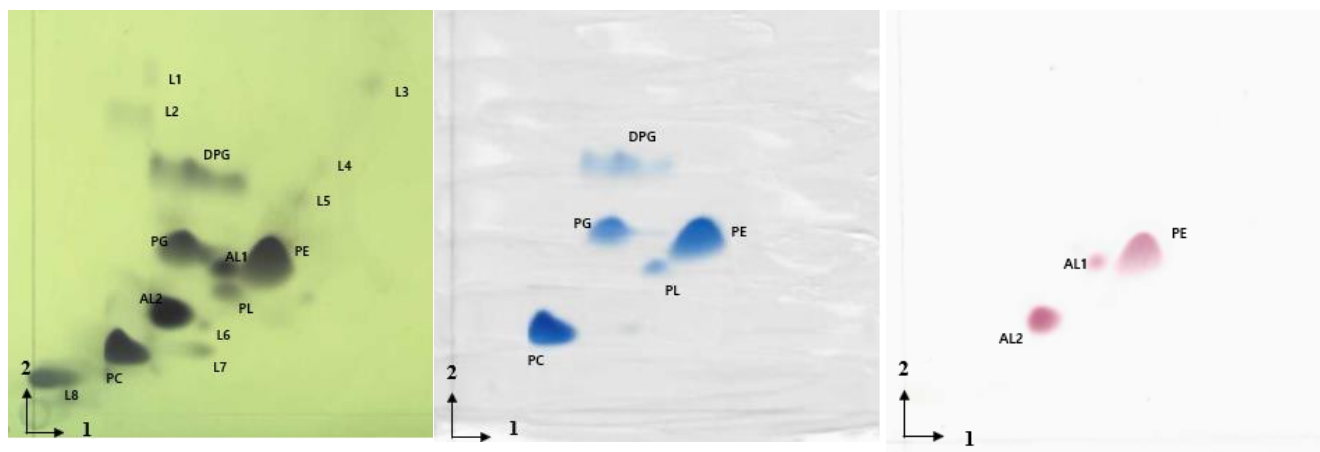
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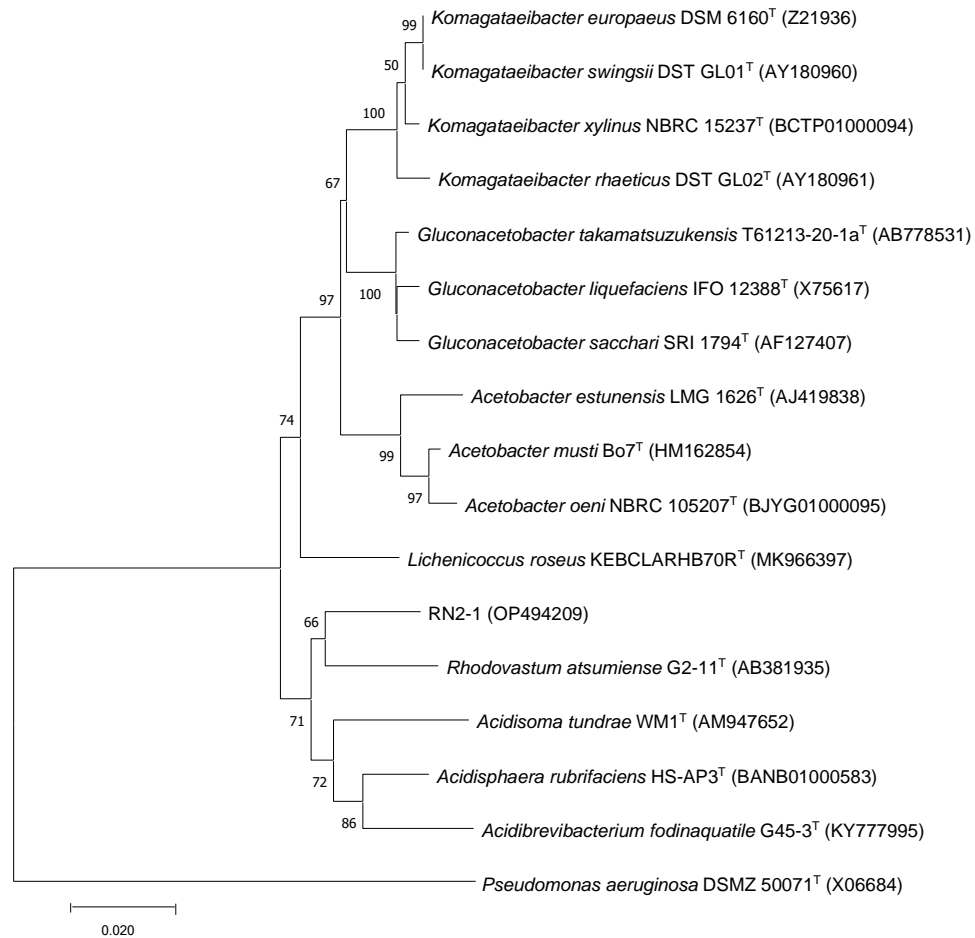
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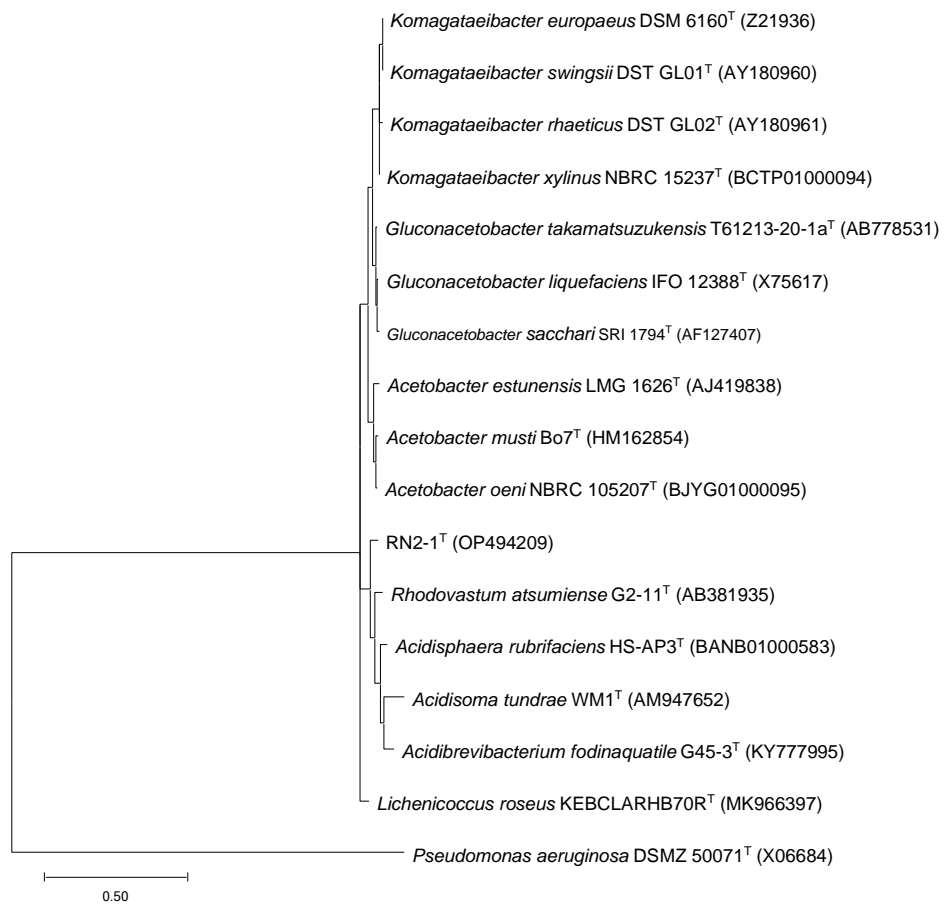


**Supplementary Figure S1.** Polar lipid profile of strain RN2-1<sup>T</sup>

PG - phosphatidylglycerol; DPG - diphosphatidylglycerol; PE - phosphatidylethanolamine; PC - phosphatidylcholine, PL - unidentified phospholipid, AL1-2 – unidentified aminolipid, L1-8 – unidentified polar lipids.



**Supplementary Figure S2.** Phylogenetic tree reconstructed with the Minimum Evolution method based on 16S rRNA gene sequences of strain RN2-1<sup>T</sup> and type species of the family *Acetobacteraceae*. *Pseudomonas aeruginosa* DSMZ 5071<sup>T</sup> was used as an outgroup. Numbers at branch nodes represent confidence levels (values>50% are shown) from 1000 replicate bootstrap samplings. GenBank accession numbers are given in parentheses. Bar, 0.02 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA X.



**Supplementary Figure S3.** Phylogenetic tree reconstructed with the Maximum Likelihood method based on 16S rRNA gene sequences of strain RN2-1<sup>T</sup> and type species of the family *Acetobacteraceae*. *Pseudomonas aeruginosa* DSMZ 5071<sup>T</sup> was used as an outgroup. Numbers at branch nodes represent confidence levels (values >50% are shown) from 1000 replicate bootstrap samplings. GenBank accession numbers are given in parentheses. Bar, 0.50 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA X.

**Supplemental Table S1.** Modified of MM2V medium.

	Composition	Capacity	Unit
1	Peptone	1.0	g/L
2	KH2PO4	0.2	g/L
3	NaCl	0.2	g/L
4	NH4NO3	0.2	g/L
5	MgSO4. 5H2O	0.04	g/L
6	CaCl2. 5H2O	0.04	g/L
7	Yeast extract	0.1	g/L
8	Vitamin solution*	1	ml/L
	pH	7.0-7.5	
	*Vitamin solution		
1	p-aminobenzoate	1.0	mg
2	Biotin	0.2	mg
3	Nicotinic acid	2.0	mg
4	Thiamin HCl	1.0	mg
5	Ca-pantothenate	0.5	mg
6	Pyridoxamine	5	mg
7	Vitamin B12	2	mg
8	Distilled water	100	mL

**Supplemental Table S2.** Results from API ZYM, API 20NE test. 1, RN2-1; 2, *Rhodovastum atsumiense*. All data are from the present study. +, positive; -, negative.

Characteristics	1	2
<b>Enzymatic reaction (API ZYM)</b>		
Alkaline phosphate	+	+
Esterase	+	+
Esterase lipase (C8)	-	-
Lipase (C14)	-	-
Leucine arylamidase	+	+
Valine arylamidase	-	-
Crystine arylamidase	-	-
Trypsin	-	-
Alpha chymotrypsin	-	+
Acid phosphatase	+	+
Naphthol-AS-BI- phosphate	+	+
Alpha galactosidase	-	-
Beta galactosidase	-	-
Beta glucuronidase	-	-
Alpha glucosidase	-	-
Beta glucosidase	-	-
N-acetyl beta glucosaminidase	-	-
Alpha monosidase	-	-
Alpha fucosidase	-	-
<b>API 20NE</b>		
Nitrate reduction	+	-
Indole production	-	-
Glucose fermentation	-	+
Arginine dihydrolase	-	+
Urease	-	+
Aesculin hydrolysis	-	-
Gelatin hydrolysis	-	+
β - Galactosidase	-	-

**Supplemental Table S3.** Results from carbon source utilization 1, RN2-1 (data from this study); 2, *Rhodovastum atsumiense* [5]; 3, *Acidisphaera rubrifaciens* [6]; 4, *Lichennicoccus roseus* [4]; 5, *Acidibrevibacterium fodinaquatile* [7]; 6, *Acidisoma tundrae* [8]. ++, good growth; +, moderate growth; +/-, little or ambiguous growth; -, no growth.

<b>Carbon-source utilization</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>
Acetate	-	++	-	-	nd	-
Lactate	-	+	+	-	nd	+
Mannose	+/-	+	-	+	-	+
Sorbitol	+/-	+	+	+	+	+
Glycerol	+/-	-	+	-	-	+
Asparagine	+	+	-	-	+	+
Aspartate	-	+	-	nd	+	-
Glutamate	-	++	-	-	nd	nd
Peptone	+	+	+/-	+	nd	+
Casamino acid	++	+	+/-	+	-	+
Methanol	+/-	-	-	-	+	-
Ethanol	+	+	+	+	+	+
Glutamine	++	++	-	-	nd	nd
Propanol	+	+	-	-	+	-
Alanine	+	nd	-	nd	+	-
Butanol	+/-	+	-	nd	-	-
Pyruvate	++	++	++	+	-	+
Fumarate	+	++	+	nd	-	-
Succinate	+	++	+	-	-	-
Formate	-	-	nd	nd	nd	-
Gluconate	+	++	++	-	nd	+
Propionate	-	-	+/-	-	nd	-
Butyrate	-	-	+/-	-	nd	+
Citrate	++	-	+/-	-	nd	-
Benzoate	-	-	+/-	nd	nd	-
Sucrose	+/-	-	+/-	+	nd	-
Malate	+	++	++	+	-	+
D-glucose	+/-	+	+	+	+	+
L-arabinose	+/-	+	+/-	+	+	nd
D-mannitol	+/-	+	nd	+	-	+

**Supplementary Table S4.** Detailed cellular fatty acid profiles (% of totals) of strain RN2-1<sup>T</sup> and closely related reference strains. Strains; 1, RN2-1; 2, *Rhodovastum atsumiense* KCTC5708; 3, *Acidisphaera rubrifaciens* JCM10600; 4, *Lichennicoccus roseus* KCTC72321; 5, *Acidibrevibacterium fodinaquatile* KCTC62275; 6, *Acidisoma tundrae* DSM19999. All data are from present study. Fatty acids that represent <0.1% of total in all strains are not shown; -, not detected.

Fatty acid	1	2	3	4	5	6
<b>Saturated</b>						
C <sub>12:0</sub>	-	-	-	0.21	-	-
C <sub>14:0</sub>	1.26	2.30	1.14	0.15	0.16	-
C <sub>16:0</sub>	6.54	20.47	20.07	7.82	22.22	10.33
C <sub>18:0</sub>	1.02	2.17	1.31	2.71	-	4.73
C <sub>20:0</sub>	-	0.13	-	-	-	-
10-methyl C <sub>17:0</sub>	-	0.68	-	-	-	-
10-methyl C <sub>19:0</sub>	-	0.22	-	-	-	-
anteiso -C <sub>11:0</sub>	-	0.14	-	-	0.14	-
anteiso C <sub>13:0</sub>	-	0.17	-	-	0.16	-
Cyclo-C <sub>17:0</sub>	5.62	-	-	-	1.06	-
<b>Unsaturated</b>						
C <sub>16:1</sub> ω5c	1.25	2.43	-	0.16	0.19	-
C <sub>16:1</sub> ω7c alcohol	-	0.19	-	-	-	-
C <sub>16:1</sub> ω9c	-	-	-	-	0.19	-
C <sub>17:1</sub> ω6c	-	0.31	-	-	-	-
C <sub>17:1</sub> ω7c	-	0.21	-	-	-	-
C <sub>18:1</sub> ω5c	-	0.74	-	0.62	-	-
C <sub>18:1</sub> ω9c	-	0.40	-	-	0.10	3.33
C <sub>20:1</sub> ω7c	-	0.58	-	-	-	-
cyclo C <sub>19:0</sub> ω8c	7.85	0.57	20.80	1.25	-	38.51
<b>Hydroxy</b>						
C <sub>16:0</sub> 2OH	-	0.37	-	-	0.14	-
C <sub>16:0</sub> 3OH	-	-	0.68	1.27	-	-
C <sub>18:0</sub> 3OH	0.68	1.56	0.81	1.64	-	9.72
C <sub>18:1</sub> 2OH	6.98	0.81	10.98	16.60	-	3.62
iso -C <sub>16:0</sub> 3OH	-	0.13	-	-	-	-
iso C <sub>18:1</sub> H	-	0.20	-	-	0.17	-
<b>Summed features*</b>						
Summed Feature 2	-	3.51	-	2.33	2.00	1.26
Summed Feature 3	7.65	9.69	0.58	0.51	0.72	-
Summed Feature 8	61.14	51.84	43.64	62.50	2.97	28.50

\*Summed features represent two or three fatty acids that can not be separated by the Microbial Identification System. Summed feature 2 consisted of C<sub>16:1</sub> iso I/C<sub>14:0</sub> 3OH; summed feature 3 consist of C<sub>16:1</sub> ω7c/C<sub>16:1</sub> ω6c summed feature 8 consist of C<sub>18:1</sub> ω7c/ C<sub>18:1</sub> ω6c.

**Supplementary Table S5.** Alcohol dehydrogenase proteins in RN2-1<sup>T</sup> genome.

Accession	Locus tag	Protein product	Length	Protein name
JAPDNT000000000.1	WP_264711838	WP_264711838.1	6.0	Methanol/ethanol family PQQ-dependent dehydrogenase
JAPDNT000000000.1	WP_264712314	WP_264712314.1	170	c-type cytochrome, methanol metabolism-related