

Report of 21 unrecorded bacterial species in Korea belonging to Betaproteobacteria and Epsilonproteobacteria

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During the extensive survey of the prokaryotic species diversity in Korea, bacterial strains belonging to Betaproteobacteria and Epsilonproteobacteria were isolated from various sources including freshwater, sediment, soil and fish. A total of 23 isolates were obtained, among which 22 strains were assigned to the class Betaproteobacteria and one strain to the class Epsilonproteobacteria. The 22 betaproteobacterial strains were further assigned to *Comamonadaceae* (11 strains), *Burkholderiaceae* (6 strains), *Oxalobacteraceae* (2 strains), *Neisseriaceae* (1 strain) and unclassified family groups (2 strains). For the strains of *Burkholderiaceae*, 3 strains were identified as 3 species of *Burkholderia*, and 2 strains were as 2 species of *Cupriavidus*. For the strains of *Comamonadaceae*, 4 strains were identified as 2 species of the genus *Hydrogenophaga*, 2 strains as 2 species of *Acidovorax*, 2 strains as 2 species of *Limnochabitans*, and each of the remaining strains as single species of *Comamonas*, *Curvibacter* and *Rhodoferax*, respectively. For the strains of *Oxalobacteraceae*, 1 strain was identified as a species of *Undibacterium*, and the other strain as a species of *Herbaspirillum*. The strain belonging to *Neisseriaceae* was identified as a species of *Iodobacter*. The remaining strains of Betaproteobacteria were identified as species of *Sphaerotilus* and *Methylibium* respectively (family unassigned). The epsilonproteobacterial strain was identified as a species of *Arcobacter* of the family *Campylobacteraceae*. The detailed description of each unrecorded species is provided.

Keywords: 16S rRNA, API20NE, Betaproteobacteria, Epsilonproteobacteria, Fatty acid, unrecorded species

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DOI:10.12651/JSR.2017.6.1.015

INTRODUCTION

The understanding on the prokaryotic diversity on earth is still at large despite the fact that more than 600 new species are published every year (Euzéby, 2016). The study on prokaryotic diversity is significant in taxonomic and ecological aspects as well as in industrial aspects as an invaluable source of useful bioactive compounds.

The class Betaproteobacteria is one of the representative and largest Gram negative bacterial groups currently encompassing 7 orders, namely *Burkholderiales*,

Hydrogenophilales, *Methylophilales*, *Neisseriales*, *Nitrosomonadales*, “*Procabacteriales*” and *Rhodocyclales*, 13 families, *Alcaligenaceae*, *Burkholderiaceae*, *Comamonadaceae*, *Oxalobacteraceae*, *Sutterellaceae*, *Hydrogenophilaceae*, *Methylophilaceae*, *Neisseriaceae*, *Gallionellaceae*, *Nitrosomonadaceae*, *Spirillaceae*, “*Procabacteriaceae*” and *Rhodocyclaceae*, and 161 genera (Garrity *et al.*, 2005a; Euzéby, 2016).

The Epsilonproteobacteria is a relatively small group currently containing 2 orders, *Campylobacterales* and *Nautiliales*, 4 families, *Campylobacteraceae*, *Helicobacteraceae*, “*Hydrogenimonaceae*” and *Nautiliaceae*,

and 17 genera (Garrity *et al.*, 2005a; Euzéby, 2016).

Betaproteobacteria include physiologically wide group of organisms, for example phototrophs, animal or plant pathogens, hydrogen bacteria, nitrifying bacteria, nitrogen fixing bacteria and methylophs (Garrity *et al.*, 2005a). Some betaproteobacteria are known as parasites or symbionts to invertebrates (Gruwell *et al.*, 2010; Lund *et al.*, 2010), and as degraders of recalcitrants (Aguirre de Cárcer *et al.*, 2007; Callaghan *et al.*, 2009; Heinzl *et al.*, 2009). Epsilonproteobacteria include intestinal pathogens such as *Campylobacter* and *Helicobacter*, but a number of species have also been found in natural environment (Garrity *et al.*, 2005b; Euzéby, 2016).

In this study, bacterial strains belonging to Betaproteobacteria and Epsilonproteobacteria were isolated from various sources including freshwater, sediment, soil and fish during the extensive survey of the prokaryotic species diversity in Korea. As a result, a total of 23 strains are proposed to represent 21 unrecorded species of Ko-

rea, and the detailed descriptions are given below.

MATERIALS AND METHODS

A total of 23 bacterial strains assigned to the classes Betaproteobacteria and one strain assigned to Epsilonproteobacteria were isolated from diverse environmental samples collected from soil, sediment, freshwater, seawater and fish gut (Table 1). The samples were processed or treated separately, diluted and spread onto diverse culture media R2A, marine agar (MA), tryptic soy agar (TSA) and nutrient agar (NA) (BD), and incubated at 25-30°C for 2-5 days. The designated strain IDs, sources of isolation, culture media, and incubation conditions are provided in the description and Table 1. All strains were purified as single colonies and stored as 10-20% glycerol suspension at -80°C as well as lyophilized ampoules.

Colony characteristics of the isolates were observed

Table 1. List of strains, isolation sources and taxonomic affiliations.

Strain ID	NIBR no.	Isolation source	Culture medium (temperature)	Closest match (Identification)	16S rRNA gene similarity (%)
Betaproteobacteria					
<i>Burkholderiales</i>					
<i>Burkholderiaceae</i>					
LB-1	BA0000114802	Artificial pond	TSA (30°C)	<i>Burkholderia caryophylli</i>	98.8
R1-16	BA0000114814	soil	R2A (30°C)	<i>Burkholderia jiangsuensis</i>	99.9
RG 3Y-10-2	BA0000114876	Field soil	R2A (30°C)	<i>Burkholderia phytofirmans</i>	98.8
NGS 3Y-15-3	BA0000114886	Field soil	R2A (30°C)	<i>Cupriavidus campinensis</i>	99.9
Oil1-9	BA0000114824	Oil-contaminated soil	R2A (25°C)	<i>Cupriavidus necator</i>	99.3
IMCC26232	BA0000114870	Freshwater	R2A (20°C)	<i>Polynucleobacter necessarius</i> subsp. <i>asymbioticus</i>	99.8
<i>Comamonadaceae</i>					
WS97	BA0000115011	Freshwater	R2A (25°C)	<i>Acidovorax delafieldii</i>	99.3
61DPR29	BA0000114796	Freshwater	R2A (25°C)	<i>Acidovorax radialis</i>	99.3
WS99	BA0000115015	Freshwater	R2A (25°C)	<i>Comamonas jiangduensis</i>	98.9
HMF2824	BA0000115005	Sediment	R2A (30°C)	<i>Curvibacter fontanus</i>	98.9
MG2F 9	BA0000114784	Freshwater	R2A (25°C)	<i>Hydrogenophaga atypica</i>	99.0
03SU8	BA0000115023	Freshwater	R2A (25°C)	<i>Hydrogenophaga atypica</i>	99.4
MR22	BA0000114927	Fish gut	R2A (25°C)	<i>Hydrogenophaga taeniospiralis</i>	98.1
WS11	BA0000115016	Freshwater	R2A (25°C)	<i>Hydrogenophaga taeniospiralis</i>	99.3
LIN8	BA0000114804	Freshwater	R2A (25°C)	<i>Limnohabitans curvus</i>	99.9
63ED25-2	BA0000114801	Freshwater	R2A (25°C)	<i>Limnohabitans parvus</i>	99.4
MC2F19	BA0000114774	Freshwater	R2A (25°C)	<i>Rhodiferax saidenbachensis</i>	99.7
<i>Oxalobacteraceae</i>					
LR-14	BA0000114808	Freshwater pond	TSA (30°C)	<i>Herbaspirillum aquaticum</i>	99.2
WA5	BA0000114770	Freshwater	R2A (25°C)	<i>Undibacterium pigrum</i>	99.1
<i>Unclassified Burkholderiales</i>					
HMF2787	BA0000115004	Sediment	R2A (30°C)	<i>Methylibium petroleiphilum</i>	99.9
HMF2472	BA0000114996	Freshwater	R2A (25°C)	<i>Sphaerotilus natans</i> subsp. <i>natans</i>	99.9
<i>Neisseriales</i>					
<i>Neisseriaceae</i>					
61DPR38	BA0000114797	Freshwater	R2A (25°C)	<i>Iodobacter arcticus</i>	99.7
Epsilonproteobacteria					
<i>Campylobacteriales</i>					
<i>Campylobacteraceae</i>					
63ED13	BA0000114800	Freshwater	R2A (25°C)	<i>Arcobacter butzleri</i>	100

on the same agar media for cultivation. Cellular morphology and cell size were examined using either transmission electron microscope or scanning electron microscope. Gram staining was performed using the standard procedures. Biochemical characteristics were tested by using API 20NE galleries (bioMérieux) according to the manufacturer's instructions.

The extraction of genomic DNA, PCR amplification of 16S rRNA gene and sequencing were performed using the standard procedures as described previously (Shin *et al.*, 2011; Choi *et al.*, 2015). The 16S rRNA gene sequences of the strains assigned to the Betaproteobacteria and Epsilonproteobacteria were compared with the reference strains using the EzBioCloud (Kim *et al.*, 2012). Phylogenetic trees were generated by using neighbor-joining (Saitou and Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms using MEGA 6.0 (Tamura *et al.*, 2013). The phylogenetic trees were evaluated using bootstrap analyses based on 1,000 resampled dataset.

RESULTS AND DISCUSSION

The bacterial strains belonging to Betaproteobacteria and Epsilonproteobacteria were isolated from sources

including freshwater, sediment, soil and fish. As a result, a total of 22 isolates belonging to the class Betaproteobacteria and one strain belonging to the class Epsilonproteobacteria were isolated (Table 1). The 22 betaproteobacterial strains were further assigned to *Comamonadaceae* (11 strains), *Burkholderiaceae* (6 strains), *Oxalobacteraceae* (2 strains) and *Neisseriaceae* (1 strain), but 2 strains could not be assigned to any known family. All families belong to the order *Burkholderiales* except for *Neisseriaceae*, which belongs to the order *Neisseriales*. For the strains of *Burkholderiaceae*, 3 strains were identified as 3 species of *Burkholderia*, 2 strains as 2 species of *Cupriavidus*, and 1 strain as a species of *Polynucleobacter* (Fig. 1). For the strains of *Comamonadaceae*, 4 strains were identified as 2 species of the genus *Hydrogenophaga*, 2 strains as 2 species of *Acidovorax*, 2 strains as 2 species of *Limnohabitans*, and each of the remaining strains as single species of *Comamonas*, *Curvibacter* and *Rhodoferax*, respectively (Fig. 2). For the strains of *Oxalobacteraceae*, 1 strain was identified as a species of *Undibacterium*, and the other as a species of *Herbaspirillum* (Fig. 3). The remaining strains were identified as single species of *Sphaerotilus* and *Methylibium* respectively, which were unassigned members of the order *Burkholderiales* (Fig. 3). For the strain belonging to *Neisseriales*, the

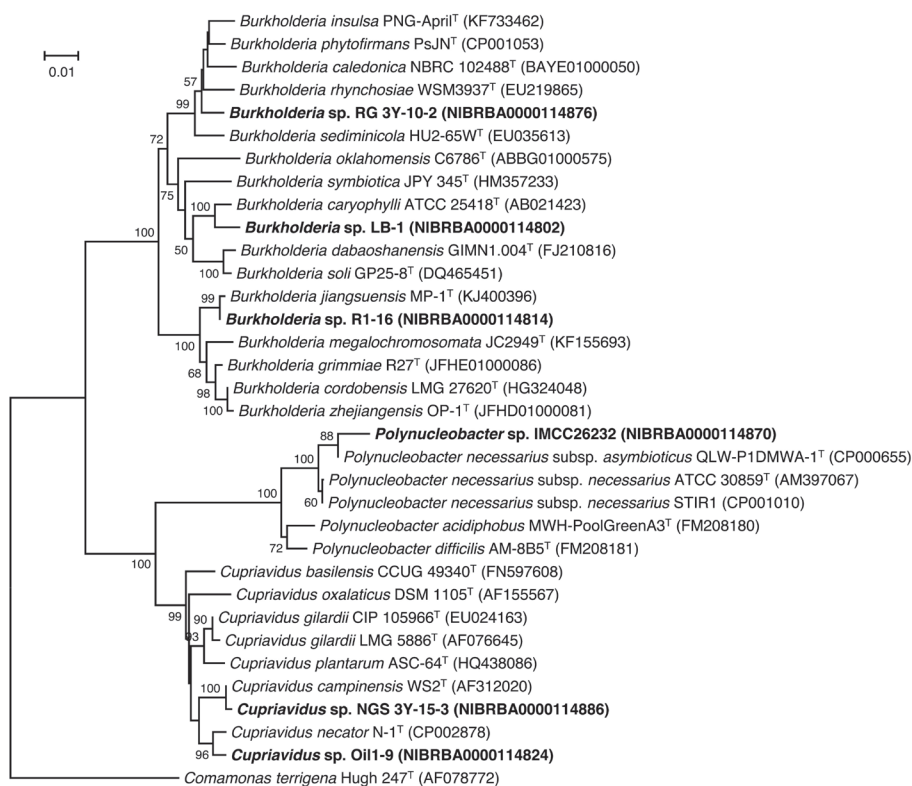


Fig. 1. Neighbor-joining tree of the isolates and related taxa belonging to the family *Burkholderiaceae*. Numbers at nodes indicate level of bootstrap support (%) based on 1,000 resamplings. Branches Scale bar, 0.01 substitutions per nucleotide position.

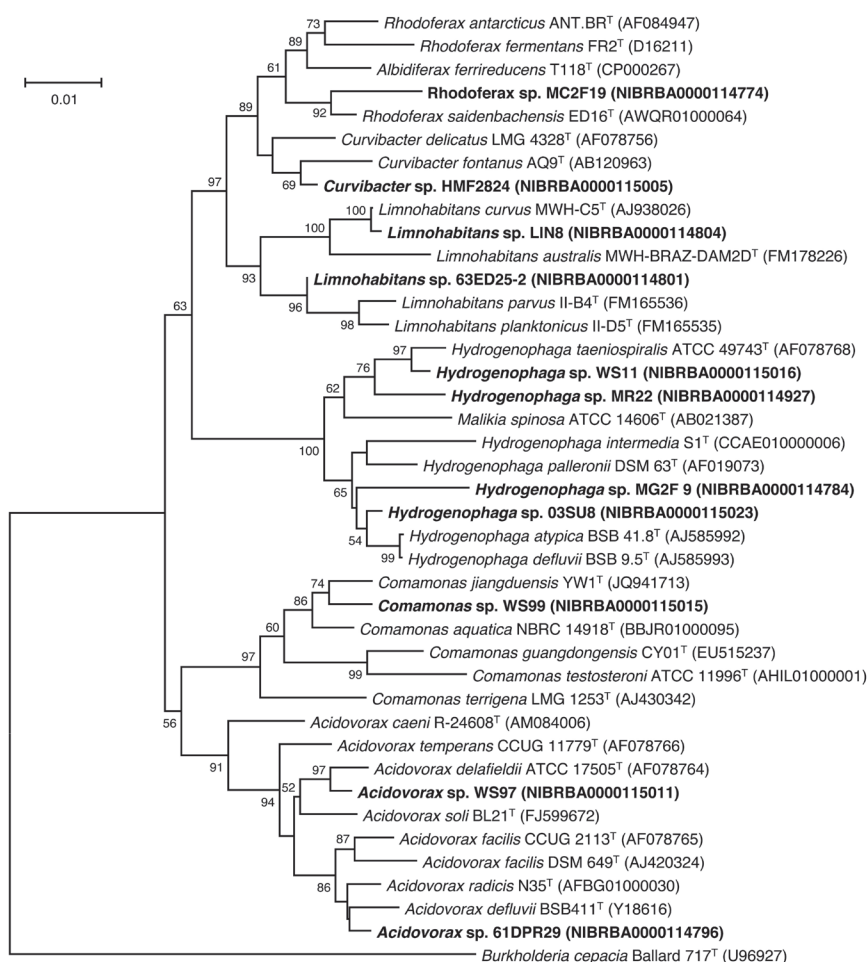


Fig. 2. Neighbor-joining tree of the isolates and related taxa belonging to the family *Comamonadaceae*. Numbers at nodes indicate level of bootstrap support (%) based on 1,000 resamplings. Scale bar, 0.01 substitutions per nucleotide position.

isolate was identified as a species of *Iodobacter* (Fig. 4). The epsilonproteobacterial strain was identified as a species of *Arcobacter* of the family *Campylobacteraceae* (Fig. 5). The detailed description of each unrecorded species is as given below. The electron micrographic images of each isolate were also provided in Fig. 6.

Family *Burkholderiaceae*

Description of *Burkholderia caryophylli* LB-1

Cells are Gram negative and rod shaped. Grows on TSA at 30°C. The colonies are light brown colored, and circular, glistening and moist. Diffusible pigment is not produced. Based on API20NE, cytochrome oxidase, β -glucosidase, protease and β -galactosidase activities are positive, but nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease activities are negative. D-Glucose, L-arabinose, D-mannitol, D-maltose and potassium gluconate are assimilated, but not D-mannose, *N*-acetyl-glucosamine, capric acid, adipic

acid, malic acid, trisodium citrate and phenylacetic acid. Strain LB-1 (= NIBRBA0000114802) was isolated from artificial pond. The 16S rRNA gene sequence accession number is KP182159.

Description of *Burkholderia jiangsuensis* R1-16

Cells are Gram negative and rod shaped. Grows on R2A at 30°C. The colonies are bright yellow colored, and circular, convex with entire margin. Diffusible pigment is not produced. Based on API20NE, nitrate reduction is positive, but indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, and phenylacetic acid are assimilated, but not L-arabinose, D-maltose, capric acid, adipic acid and trisodium citrate. Strain R1-16 (= NIBRBA0000114814) was isolated from soil. The 16S rRNA gene sequence accession number is KP182171.

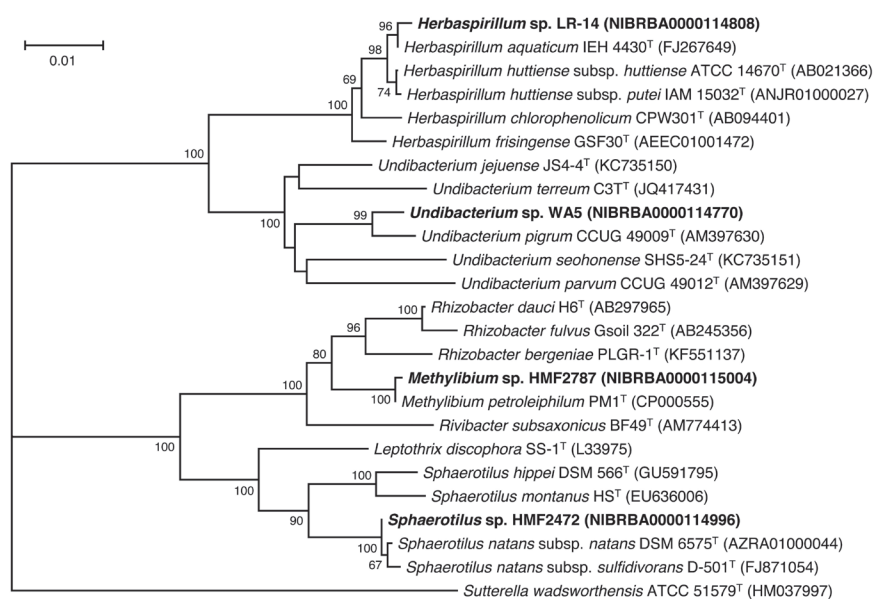


Fig. 3. Neighbor-joining tree of the isolates and related taxa belonging to the family *Comamonadaceae*. Numbers at nodes indicate level of bootstrap support (%) based on 1,000 resamplings. Scale bar, 0.01 substitutions per nucleotide position.

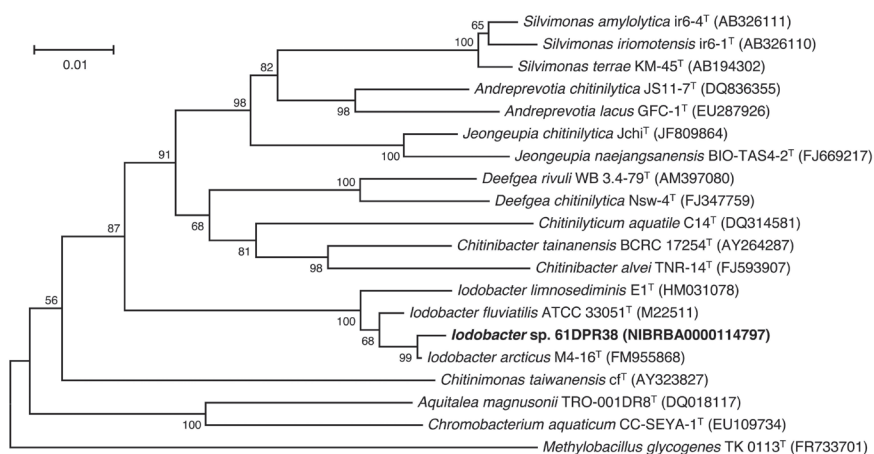


Fig. 4. Neighbor-joining tree of the isolate and related taxa belonging to the family *Neisseriaceae*. Numbers at nodes indicate level of bootstrap support (%) based on 1,000 resamplings. Scale bar, 0.01 substitutions per nucleotide position.

Description of *Burkholderia phytofirmans* RG 3Y-10-2

Cells are Gram negative. Grows on R2A at 30°C. Motile by flagella. The colonies are pale yellow colored, and circular, convex with entire margin. Based on API20NE, β -galactosidase activity is positive, but cytochrome oxidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase and protease activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are assimilated,

but not maltose. Strain RG 3Y-10-2 (= NIBRBA0000114876) was isolated from agricultural field soil. The 16S rRNA gene sequence accession number is KP126810.

Description of *Cupriavidus campinensis* NGS 3Y-15-3

Cells are Gram negative and rod shaped. Grows on R2A at 30°C. Colonies are white colored, and punctiform, raised with entire margin. Based on API20NE, nitrate reduction is positive, but cytochrome oxidase, indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase activities are negative. Potassium gluconate, capric acid,

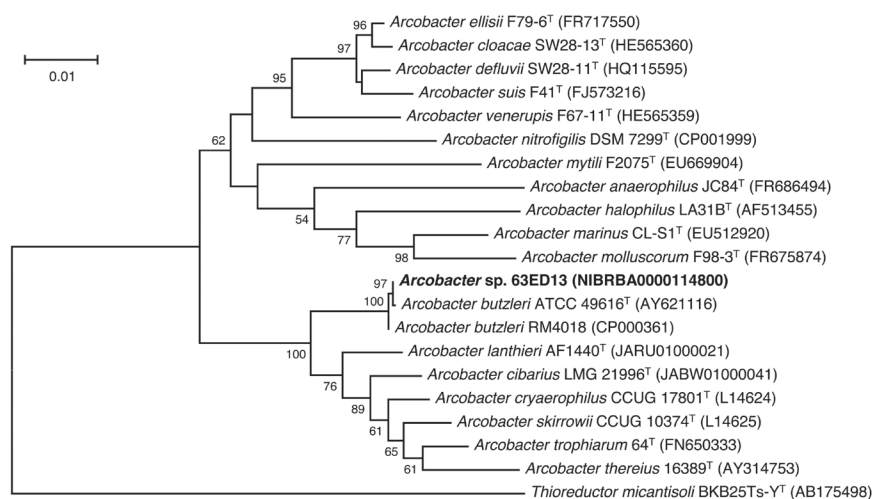


Fig. 5. Neighbor-joining tree of the isolate and related taxa belonging to the family *Campylobacteraceae*. Numbers at nodes indicate level of bootstrap support (%) based on 1,000 resamplings. Scale bar, 0.01 substitutions per nucleotide position.

adipic acid, malic acid, citrate and phenylacetic acid are assimilated, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose and trisodium citrate. Strain NGS 3Y-15-3 (= NIBRBA0000114886) was isolated from agricultural field soil. The 16S rRNA gene sequence accession number is KP126819.

Description of *Cupriavidus necator* Oil1-9

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are white colored, and circular, convex with entire margin. Diffusible pigment is not produced. Based on API20NE, cytochrome oxidase, nitrate reduction, arginine dihydrolase and urease activities are positive, but indole production, glucose fermentation, β-glucosidase, protease and β-galactosidase activities are negative. Potassium gluconate, capric acid, adipic acid, malic acid, citrate and phenylacetic acid are assimilated, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose and trisodium citrate. Strain Oil1-9 (= NIBRBA0000114824) was isolated from oil-contaminated soil.

Description of *Polynucleobacter necessarius* subsp. *asymbioticus* IMCC26232

Cells are Gram negative and rod shaped. Grows on R2A at 20°C. Colonies are cream colored, and circular, convex and smooth. Diffusible pigment is not produced. Based on API20NE, cytochrome oxidase activity is positive, but nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β-glucosidase, protease and β-galactosidase activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-

glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not assimilated. Strain IMCC26232 (= NIBRBA0000114870) was isolated from freshwater.

Comamonadaceae

Description of *Acidovorax delafieldii* WS97

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are beige colored, and round, circular and convex. Diffusible pigment is not produced. Based on API20NE, cytochrome oxidase and nitrate reduction activities are positive, but indole production, glucose fermentation, arginine dihydrolase, urease, β-glucosidase, protease and β-galactosidase activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid and malic acid are assimilated, but not *N*-acetyl-glucosamine, D-maltose, adipic acid, trisodium citrate and phenylacetic acid. Strain WS97 (= NIBRBA0000115011) was isolated from freshwater.

Description of *Acidovorax radialis* 61DPR29

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are light brown colored, and circular, smooth with entire margin. Diffusible pigment is not produced. Based on API20NE, cytochrome oxidase, urease and protease activities are positive, but indole production, glucose fermentation, arginine dihydrolase, β-glucosidase and β-galactosidase activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, adipic acid and malic acid are assimilated, but not *N*-acetyl-glucosamine, D-maltose, capric

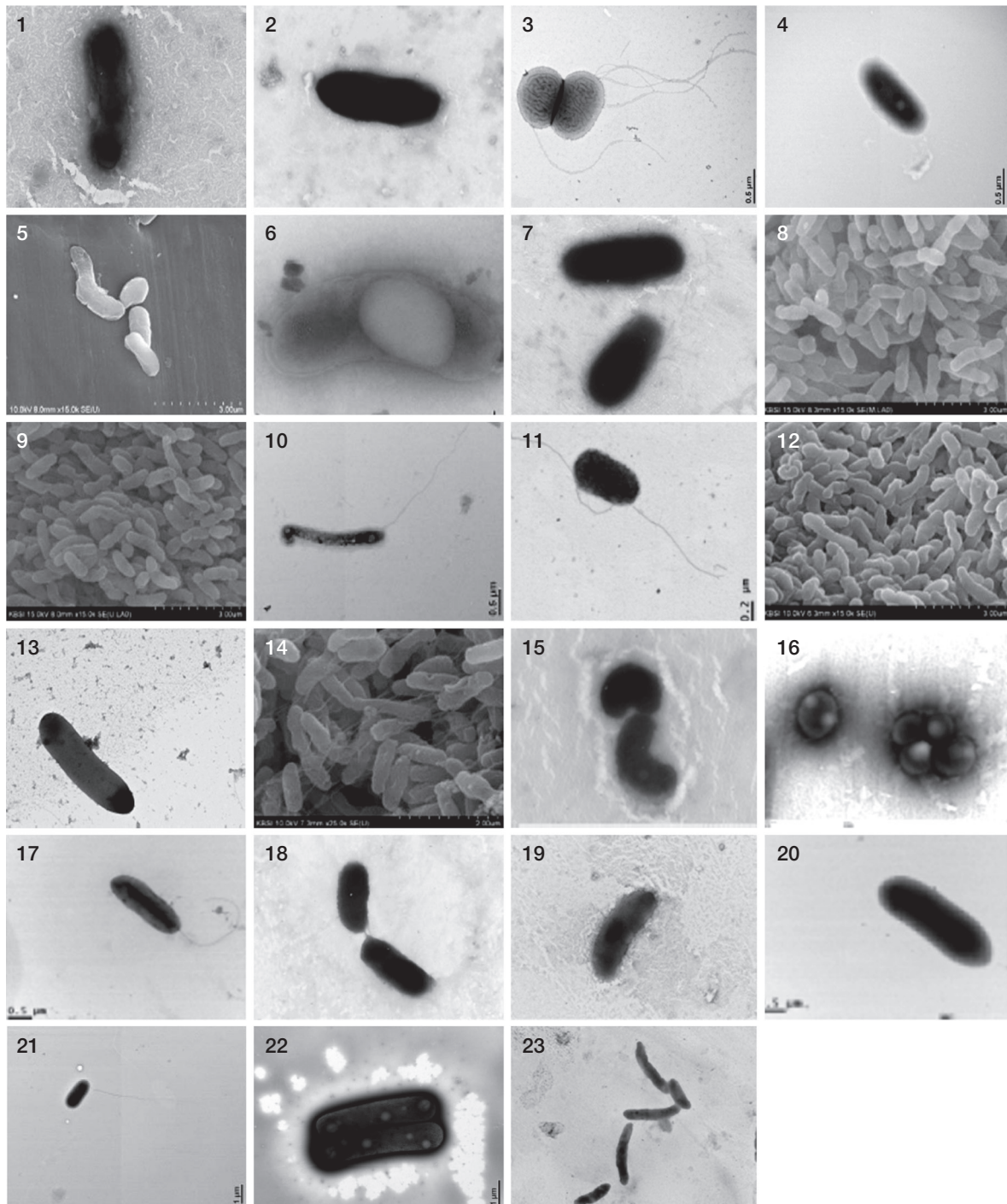


Fig. 6. Electron micrographic images of cells. Strains: 1, LB-1; 2, R1-16; 3, RG 3Y-10-2; 4, NGS 3Y-15-3; 5, Oii1-9; 6, IMCC26232; 7, 61DPR29; 8, WS97; 9, WS99; 10, HMF2824; 11, MG2F 9; 12, 03SU8; 13, MR22; 14, WS11; 15, LIN8; 16, 63ED25-2; 17, MC2F19; 18, 61DPR38; 19, LR-14; 20, WA5; 21, HMF2787; 22, HMF2472; 23, 63ED13.

acid, trisodium citrate and phenylacetic acid. Strain 61DPR29 (=NIBRBA0000114796) was isolated from fresh-water. The 16S rRNA gene sequence accession number is KP182153.

Description of *Comamonas jiangduensis* WS99

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are white colored, and irregular, umbonate and lobate. Diffusible pigment is not produced.

Based on API20NE, cytochrome oxidase and nitrate reduction activities are positive, but indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase activities are negative. Capric acid and malic acid are assimilated, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, trisodium citrate and phenylacetic acid. Strain WS99 (=NIBRBA0000115015) was isolated from freshwater. The 16S rRNA gene sequence accession number is KP099964.

Description of *Curvibacter fontanus* HMF2824

Cells are Gram negative and rod shaped. Grows on R2A at 30°C. Colonies are beige colored, and circular, convex with entire margin. Diffusible pigment is not produced. Based on API20NE, cytochrome oxidase and urease activities are positive, but nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose and potassium gluconate are assimilated, but not L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain HMF2824 (=NIBRBA0000115005) was isolated from sediment. The 16S rRNA gene sequence accession number is KP099964.

Description of *Hydrogenophaga atypica* MG2F 9

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are cream colored, and circular, raised with entire margin. Based on API20NE, cytochrome oxidase activity is positive, but nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not assimilated. Strain MG2F 9 (=NIBRBA0000114784) was isolated from freshwater. The 16S rRNA gene sequence accession number is KP196831.

Description of *Hydrogenophaga atypica* 03SU8

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are white colored, and raised-circular, smooth, convex. Diffusible pigment is not produced. Based on API20NE, cytochrome oxidase, nitrate reduction and glucose fermentation activities are positive, but indole production, arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose, D-mannitol, potassium gluco-

nate and malic acid are assimilated, but not L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain 03SU8 (=NIBRBA0000115023) was isolated from freshwater.

Description of *Hydrogenophaga taeniospiralis* MR22

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are cream colored and circular. Based on API20NE, cytochrome oxidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not assimilated. Strain MR22 (=NIBRBA0000114927) was isolated from the gut of *Mugil cephalus* (grey mullet). The 16S rRNA gene sequence accession number is KP 172206.

Description of *Hydrogenophaga taeniospiralis* WS11

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are pale yellow colored, and circular, slightly convex, smooth with entire margin. Diffusible pigment is not produced. Based on API20NE, nitrate reduction is positive, but cytochrome oxidase, indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not assimilated. Strain WS11 (=NIBRBA0000115016) was isolated from freshwater.

Description of *Limnohabitans curvus* LIN8

Cells are Gram negative and vibroid shaped. Grows on R2A at 25°C. Colonies are beige colored, and punctiform, smooth with entire margin. Diffusible pigment is not produced. Based on API20NE, cytochrome oxidase activity is positive, but nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not assimilated. Strain LIN8 (=NIBRBA0000114804) was isolated from freshwater. The 16S rRNA gene sequence accession number is KP182161.

Description of *Limnohabitans parvus* 63ED25-2

Cells are Gram negative and cocci shaped. Grows on R2A at 25°C. Colonies are light brown colored, and circular, smooth with entire margin. Based on API20NE, cytochrome oxidase activity is positive, but nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not assimilated. Strain 63ED25-2 (= NIBRBA0000114801) was isolated from freshwater. The 16S rRNA gene sequence accession number is KP182158.

Description of *Rhodoferrax saidenbachensis* MC2F19

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are white colored, and circular, raised with entire margin. Based on API20NE, cytochrome oxidase, nitrate reduction and urease activities are positive, but indole production, glucose fermentation, arginine dihydrolase, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not assimilated. Strain MC2F19 (= NIBRBA0000114774) was isolated from freshwater. The 16S rRNA gene sequence accession number is KP196823.

Oxalobacteraceae**Description of *Herbaspirillum aquaticum* LR-14**

Cells are Gram negative and rod shaped. Grows on TSA at 30°C. Colonies are white colored, and undulate, smooth. Based on API20NE, cytochrome oxidase, β -glucosidase, protease and β -galactosidase activities are positive, but nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are assimilated, but not D-maltose. Strain LR-14 (= NIBRBA0000114808) was isolated from freshwater pond. The 16S rRNA gene sequence accession number is KP182165.

Description of *Undibacterium pigrum* WA5

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are white colored, and circular, raised with entire margin. Based on API20NE, cytochrome oxidase and nitrate reduction are positive, but

indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not assimilated. Strain WA5 (= NIBRBA0000114770) was isolated from freshwater. The 16S rRNA gene sequence accession number is KP196819.

Unclassified *Burkholderiales***Description of *Methylibium petroleiphilum* HMF2787**

Cells are Gram negative and rod shaped. Grows on R2A at 30°C. Colonies are cream colored, and circular, convex with entire margin. Diffusible pigment is not produced. Based on API20NE, cytochrome oxidase, nitrate reduction and arginine dihydrolase activities are positive, but indole production, glucose fermentation, urease, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose, D-mannose, potassium gluconate, capric acid, malic acid and trisodium citrate are assimilated, but not L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain HMF2787 (= NIBRBA0000115004) was isolated from sediment. The 16S rRNA gene sequence accession number is KP099963.

Description of *Sphaerotilus natans* subsp. *natans* HMF2472

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are beige colored, and filamentous, flat and wooly. Diffusible pigment is not produced. Based on API20NE, cytochrome oxidase, urease, β -glucosidase, protease and β -galactosidase activities are positive, but nitrate reduction, indole production, glucose fermentation and arginine dihydrolase activities are negative. D-Glucose, L-arabinose, D-mannose and *N*-acetyl-glucosamine are assimilated, but not D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain HMF2472 (= NIBRBA0000114996) was isolated from freshwater. The 16S rRNA gene sequence accession number is KP099955.

Neisseriaceae**Description of *Iodobacter arcticus* 61DPR38**

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are white to violet colored, and circular, digging and viscid. Based on API20NE, cytochrome oxidase, nitrate reduction and protease activities are positive, but indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase and β -

galactosidase activities are negative. D-Glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose and adipic acid are assimilated, but not L-arabinose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain 61DPR38 (=NIBR BA0000114797) was isolated from freshwater. The 16S rRNA gene sequence accession number is KP182154.

Epsilonproteobacteria

Description of *Arcobacter butzleri* 63ED13

Cells are Gram negative and rod shaped. Grows on R2A at 25°C. Colonies are light brown colored, and circular, entire and smooth. Diffusible pigment is not produced. Based on API20NE, cytochrome oxidase and nitrate reduction are positive, but indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase activities are negative. D-Glucose and malic acid are assimilated, but not L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain 63ED13 (=NIBRBA0000114800) was isolated from freshwater. The 16S rRNA gene sequence accession number is KP182157.

ACKNOWLEDGEMENTS

This study was supported by the research grant, "Survey of Korean Indigenous Species" sponsored by the National Institute of Biological Resources (NIBR) of the Ministry of Environment, Korea.

REFERENCES

- Callaghan, A.V., M. Tierney, C.D. Phelps and L.Y. Young. 2009. Anaerobic biodegradation of n-hexadecane by a nitrate-reducing consortium. *Applied and Environmental Microbiology* 75(5):1339-1344.
- Choi, A., J.-W. Bae, C.-J. Cha, J. Chun, W.-T. Im, K.Y. Jahng, C.O. Jeon, K. Joh, S.B. Kim, C.N. Seong, J.-H. Yoon and J.-C. Cho. 2015. A report of 39 unrecorded bacterial species in Korea, belonging to the Betaproteobacteria and Gammaproteobacteria. *Journal of Species Research* 4(2):109-126.
- de Cárcer, D.A., M. Martín, U. Karlson and R. Rivilla. 2007. Changes in bacterial populations and in biphenyl dioxygenase gene diversity in a polychlorinated biphenyl-polluted soil after introduction of willow trees for rhizoremediation. *Applied and Environmental Microbiology* 73(19):6224-6232.
- Euzéby, J.P. 2016. List of Prokaryotic Names with Standing in Nomenclature, as of February 2016 (www.bacterio.net).
- Garrity, G.M., J.A. Bell and T. Lilburn. 2005a. Class II. *Betaproteobacteria* class. nov. In: D.J. Brenner, N.R. Krieg, J.T. Staley and G.M. Garrity (eds.), *Bergey's Manual of Systematic Bacteriology*, second edition, vol. 2 (The *Proteobacteria*), part C (The *Alpha*-, *Beta*-, *Delta*-, and *Epsilonproteobacteria*), Springer, New York, 2005, p. 575.
- Garrity, G.M., J.A. Bell and T. Lilburn. 2005b. Class V. *Epsilonproteobacteria* class. nov. In: D.J. Brenner, N.R. Krieg, J.T. Staley and G.M. Garrity (eds.), *Bergey's Manual of Systematic Bacteriology*, second edition, vol. 2 (The *Proteobacteria*), part C (The *Alpha*-, *Beta*-, *Delta*-, and *Epsilonproteobacteria*), Springer, New York, 2005, p. 1145.
- Gruwell, M.E., N.B. Hardy, P.J. Gullan and K. Dittmar. 2010. Evolutionary relationships among primary endosymbionts of the mealybug subfamily Phenacoccinae (Hemiptera: Coccoidea: Pseudococcidae). *Applied and Environmental Microbiology* 76(22):7521-7525.
- Heinzel, E., S. Hedrich, E. Janneck, F. Glombitza, J. Seifert and M. Schlömann. 2009. Bacterial diversity in a mine water treatment plant. *Applied and Environmental Microbiology* 75(3):858-861.
- Kim, O.S., Y.J. Cho, K. Lee, S.H. Yoon, M. Kim, H. Na, S.C. Park, Y.S. Jeon, J.H. Lee, H. Yi, S. Won and J. Chun. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *International Journal of Systematic and Evolutionary Microbiology* 62(3):716-721.
- Lund, M.B., M. Holmstrup, B.A. Lomstein, C. Damgaard and A. Schramm. 2010. Beneficial effect of *Verminephrobacter* nephridial symbionts on the fitness of the earthworm *Aporrectodea tuberculata*. *Applied and Environmental Microbiology* 76(14):4738-4743.
- Shin, Y.M., T.-U. Kim, A. Choi, J. Chun, S. Lee, H. Kim, H. Yi, J.H. Jo, J.-C. Cho, K. Jahng, K. Kim, K. Joh, J. Chun, H.H. Lee and S.B. Kim. 2011. Species diversity of Betaproteobacteria in the Sumnunmulbengdui wetland area of Jeju Island and distribution of novel taxa. *Korean Journal of Environmental Biology* 29(3):154-161.
- Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30(12):2725-2729.

Submitted: March 3, 2016

Revised: June 27, 2016

Accepted: February 7, 2017