

Description of ten unrecorded bacterial species isolated from *Ostrea denselamellosa* and *Eriocheir japonica* from the Seomjin River

Ahyoung Choi*, Ji-Hey Han, Eui-Jin Kim, Ja Young Cho and Sun-I Hwang

Microbial Research Department, Nakdonggang National Institute of Biological Resources, Sangju 37242, Republic of Korea

*Corresponding author

Ahyoung Choi
Tel. 054-530-0863
E-mail. aychoi@nnibr.re.kr

Received: 23 October 2019

Revised: 25 November 2019

Revision accepted: 26 November 2019

Abstract: *Ostrea denselamellosa* and *Eriocheir japonica* samples were collected from the Seomjin River in 2019 as part of the "Research of Host-Associated Bacteria" research program. Almost 200 bacterial strains were isolated from the *O. denselamellosa* and *E. japonica* samples and subsequently identified by 16S rRNA gene sequencing. Among the bacterial isolates, ten strains possessed greater than 98.7% sequence similarity with published bacterial species that had not previously been recorded in Korea. These species were phylogenetically diverse, belonging to three phyla, four classes, seven orders, and eight genera. At the genus and class level, the previously unrecorded species belonged to *Pseudoalteromonas*, *Aliivibrio*, *Rheinheimera*, *Leucothrix*, and *Shewanella* of the class *Gamma-proteobacteria*, *Olleya* of the class *Flavobacteriia*, *Algoriphagus* of the class *Cytophagia*, and *Lactococcus* of the class *Bacilli*. The previously unrecorded species were further characterized by examining their Gram staining, colony and cell morphology, biochemical properties, and phylogenetic positions.

Keywords: Seomjin River, unrecorded bacterial species, *Ostrea denselamellosa*, *Eriocheir japonica*

INTRODUCTION

Estuaries are transition zones between river and marine environments. They possess a mixture of organisms that live in freshwater, seawater, and brackish water. From an ecological point of view, estuaries gain abundant nutrients from land, allowing phytoplankton and salt marsh organisms to reproduce well and have high basic productivity (Fisher *et al.* 1982). Estuaries also act as filtration devices by filtering out suspended substances before they can flow into the ocean (Day *et al.* 1989). This alters osmotic and thermal conditions, which in turn affects the basic metabolism of living organisms as well as affecting their movement, growth, and reproduction. Organisms must therefore move to environmentally favorable conditions

(Barlow 1955; Lance 1963). Additionally, there has been no construction on the banks of the Seomjin River estuary, which has allowed for the natural mixing of seawater and freshwater, resulting in a natural watershed. Seasonal changes in the salinity of the estuary also cause it to possess unique hydrodynamic characteristics.

Recent studies have focused on the symbiotic bacteria in the organs of aquatic animals. Symbiotic bacteria have been reported to be involved in nutrition and immunity of host organisms while maintaining a permanent or temporary symbiotic relationship inside or outside aquatic animals (Trabal *et al.* 2012; Zhang *et al.* 2016). In addition, the importance of microbiota is that the host can use the metabolites produced by Symbiotic bacteria (Ringø *et al.* 2016). Aquatic animals may have evolved further through

adaptive immunity with microflora. We describe unrecorded symbiotic microorganisms isolated from *Ostrea denselamellosa* and *Eriocheir japonica* collected from Seomjin River.

The wild mollusk, *Ostrea denselamellosa*, lives in the Seomjin River. In Korea the *O. denselamellosa* occurs on rocks and gravel on sandy-mud or muddy bottom at depths of 3–10 m (Min *et al.* 2004). *O. denselamellosa* is viviparous, depositing its larvae directly into the water column (Yang *et al.* 1999). *O. denselamellosa* will be uniformly sized and shaped in habitats with uncrowded spaces, with little or no interference from other oysters. Thus *O. denselamellosa* of soft substrates becomes a solitary oyster species (Noseworthy *et al.* 2016). These oysters, known as cherry blossom oysters, can be harvested beginning in January; however, the best tasting oysters are harvested between March and April. They are ten times larger than average oysters, and their rich flavor makes them a specialty of the Seomjin River.

The crustacean, *Eriocheir japonica*, can be found in many freshwater regions, including the coastal waters of Korea. *E. japonica* hatches in an estuarine watershed with a high salt concentration and moves to freshwater where it grows gradually (Kobayashi 2011). It continues to grow for 2–3 years before maturing into an adult and then it returns to the estuary for further maturation and spawning. Seaweed used to be easily visible and abundant in island rivers and mainland river basins. However, the installation of artificial structures in rivers altered their habitat, and the prevalence of seaweed has been greatly reduced. Fortunately, *E. japonica* can still be found in the Seomjin River basin, giving us the opportunity to conduct this research.

As a part of the research program ‘Research of host associated bacteria’, 200 bacterial strains were isolated from *O. denselamellosa* and *E. japonica* samples collected from the Seomjin River in 2019. Using phylogenetic analyses based on 16S rRNA gene sequencing, ten bacterial species were identified that had not been previously recorded in Korea. Here, we report the phylogenetic information and phenotypic characteristics of these bacterial species.

MATERIALS AND METHODS

Ostrea denselamellosa and *Eriocheir japonica* samples were collected from Seomjin River in March 2019, using standard dilution plating method on marine agar (MA), 1/10-diluted MA, nutrient agar (NA), tryptic soy agar

(TSA), Reasoner’s 2A (R2A) or R2A with sea water (M-R2A) media and subsequently incubated at 20–25°C for 3–7 days. All strains were purified as single colonies after serial dilution spreading and the pure cultures were stored as 20% glycerol suspension at –80°C and as lyophilized ampoules. The designation of strains, source of isolation, culture medium, and incubation conditions are summarized in Table 1.

Colony morphology of bacterial strains was observed on agar plates with a magnifying glass after the cells were cultivated to their stationary phase. Cellular morphology and cell size were examined by transmission electron microscopy. Gram staining was performed using a Gram-staining kit (bioMérieux, France). Biochemical characteristics were tested by using API 20NE galleries (bioMérieux, France) according to the manufacturer’s instructions.

Bacterial DNA extraction, PCR amplification, and 16S rRNA gene sequencing were performed using standard procedures. For the phylogenetic analysis, amplification of the 16S rRNA gene from strains were performed using a standard PCR method with a PCR pre-mix and two universal bacteria-specific primers (Bac 8F (5'-AGAGTTT-GATCCTGGCTCAG-3') and Bac 1492R (5'-GGY-TACCTTGTACGACTT-3')) (Lane 1991). The 16S rRNA gene sequences were compared with other bacterial strains with validly published names using the EzTaxon-e server (Kim *et al.* 2012). The cutoff value of 98.7% sequence similarity was applied for identification. Bacterial strains showing 98.7% or higher sequence similarity with known bacterial species never reported in Korea were selected as unreported bacterial species. For phylogenetic analyses, multiple sequence alignments between the 16S rRNA gene sequences of the isolates and those of the reference type strains were carried out using ClustalW and manually checked with EzEditor (Jeon *et al.* 2014). Based on the sequences aligned, phylogenetic trees were generated by using a neighbor-joining method (Saitou and Nei 1987) with the Kimura 2-parameter model (Kimura 1980) implemented in MEGA 7.0 software (Kumar *et al.* 2016). The robustness of the inferred phylogenetic trees was evaluated by bootstrap analyses based on 1,000 random re-samplings (Felsenstein 1985).

RESULTS AND DISCUSSION

A total of ten bacterial strains were isolated from *Ostrea denselamellosa* and *Eriocheir japonica* in Seomjin River. The

Table 1. Summary of bacterial strains isolated from *Ostrea denselamellosa* and *Eriocher japonica* and their taxonomic affiliations

Class	Order	Family	Strain ID	NNIBR ID	Most closely related species	Similarity (%)	Isolation sources	Medium	Incubation conditions
Gamma-proteobacteria	Alteromonadales	Pseudoalteromonadaceae	SJOD-M-26	NNIBR2019642BA2	<i>Pseudoalteromonas neustonica</i>	99.4	<i>Ostrea denselamellosa</i>	MA	25°C, 4 d
			SJOD-M-14	NNIBR2019642BA6	<i>Pseudoalteromonas ulvae</i>	99.4	<i>Ostrea denselamellosa</i>	MA	25°C, 2 d
			SJOD-R-25	NNIBR2019642BA7	<i>Rheinheimera pacifica</i>	98.9	<i>Ostrea denselamellosa</i>	R2A	25°C, 4 d
Gamma-proteobacteria	Vibrionales	Shewanellaceae	SJEJ-MR-7	NNIBR2019642BA9	<i>Shewanella pacifica</i>	99.2	<i>Eriocher japonica</i>	M-R2A	25°C, 4 d
			SJEJ-M-17	NNIBR2019642BA5	<i>Aliivibrio sifiae</i>	99.4	<i>Eriocher japonica</i>	MA	25°C, 4 d
Flavobacteria	Flavobacteriales	Flavobacteriaceae	SJEJ-MR-1	NNIBR2019642BA8	<i>Leucothrix arctica</i>	98.8	<i>Eriocher japonica</i>	M-R2A	25°C, 4 d
			SJOD-MR-31	NNIBR2019642BA10	<i>Olleya maritima</i>	99.9	<i>Ostrea denselamellosa</i>	MA	25°C, 4 d
			SJOD-M-32	NNIBR2019642BA1	<i>Olleya algicola</i>	100.0	<i>Ostrea denselamellosa</i>	MA	25°C, 4 d
Cytophagia	Cytophagales	Cyclobacteriaceae	SJOD-M-3	NNIBR2019642BA3	<i>Algoriphagus marinus</i>	99.3	<i>Ostrea denselamellosa</i>	M-R2A	25°C, 7 d
Bacilli	Lactobacillales	Streptococcaceae	SJOD-N-4	NNIBR2019642BA4	<i>Lactococcus raffinolactis</i>	99.9	<i>Ostrea denselamellosa</i>	NA	25°C, 7 d

strains represented ten unrecorded species in Korea belonging to three phyla, four classes, seven orders, and eight genera. The taxonomic composition and identification results of these species are summarized in Table 1. At the genus level, these unreported species belonged to the genera *Pseudoalteromonas* (two species), *Aliivibrio*, *Rheinheimera*, *Leucothrix*, and *Shewanella* of the class *Gamma-proteobacteria*, the genera *Olleya* (two species) of the class *Flavobacteriia*, the genera *Algoriphagus* of the class *Cytophagia* and the genera *Lactococcus* of the class *Bacilli*. Phylogenetic tree of bacterial strains assigned to the class *Gamma-proteobacteria* is shown in Fig. 1 and the three of classes *Flavobacteriia*, *Cytophagia* and *Bacilli* is shown in Fig. 2. As expected from the high 16S rRNA gene sequence similarities of the ten strains with their closest relatives, each strain formed a robust phylogenetic clade with the most closely related species (Figs. 1, 2). The ten unrecorded bacterial species were Gram-staining-negative or positive, rod- or coccoid-rod or coccobacilli-shaped bacteria (Fig. 3). Other detailed morphological and physiological characteristics are given in the strain descriptions. In this study a total of ten bacterial species were identified as unrecorded bacterial species that have not previously been reported from Korean ecosystems. Although ten unrecorded bacterial species had some physiological characteristics different from the previously known species, but their morphology and phylogenetic analysis showed that they were the same species. Therefore, here we describe the characteristics of these unreported bacterial species.

1. Description of *Pseudoalteromonas neustonica* SJOD-M-26

Cells are Gram-stain-negative, non-flagellated, non-pigmented and rod-shaped. Colonies grown on MA are circular, convex, entire and orange-ivory colored after incubation for 4 days at 25°C. Positive for glucose fermentation, esculin hydrolysis and gelatin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase, urease and β-galactosidase. D-Glucose, D-mannose, N-acetyl-glucosamine, D-maltose, malic acid and trisodium citrate are utilized. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain SJOD-M-26 displays the highest 16S rRNA gene sequence similarity with *Pseudoalteromonas neustonica* PAMC 28425^T (99.4%). Strain SJOD-M-26 (= NNIBR2019642BA2) was isolated from an *Ostrea denselamellosa* sample of Seomjin River. The

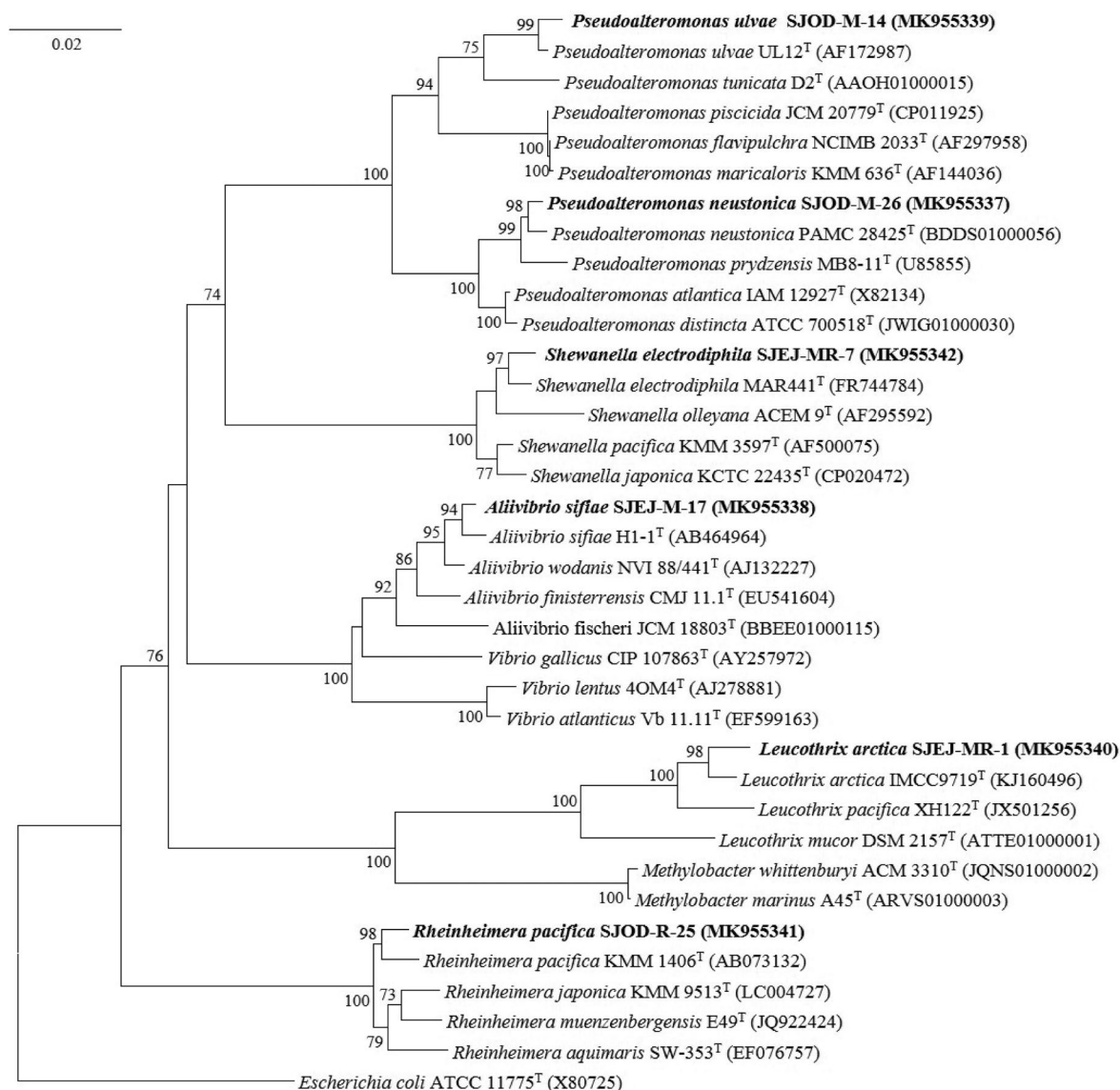


Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their relatives in the class *Gamma-proteobacteria*. Bootstrap values (> 70%) are shown at the nodes. Bar, 0.02 substitutions per nucleotide position.

GenBank accession number of 16S rRNA gene sequence is MK955337.

2. Description of *Pseudoalteromonas ulvae* SJOD-M-14

Cells are Gram-stain-negative, non-flagellated, non-pigmented and rod-shaped. Colonies grown on MA are circular, convex, smooth and cream colored after incubation for 2 days at 25°C. Positive for glucose esculin hydrolysis

and gelatin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and β -galactosidase. D-Glucose, D-mannose, D-maltose, adipic acid and trisodium citrate are utilized. Does not utilize L-arabinose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, capric acid, malic acid and phenylacetic acid. Strain SJOD-M-14 displays the highest 16S rRNA gene sequence similarity with *Pseudoalteromonas ulvae* UL12^T (99.4%). Strain SJOD-M-14 (= NNIBR2019642BA6) was isolated from

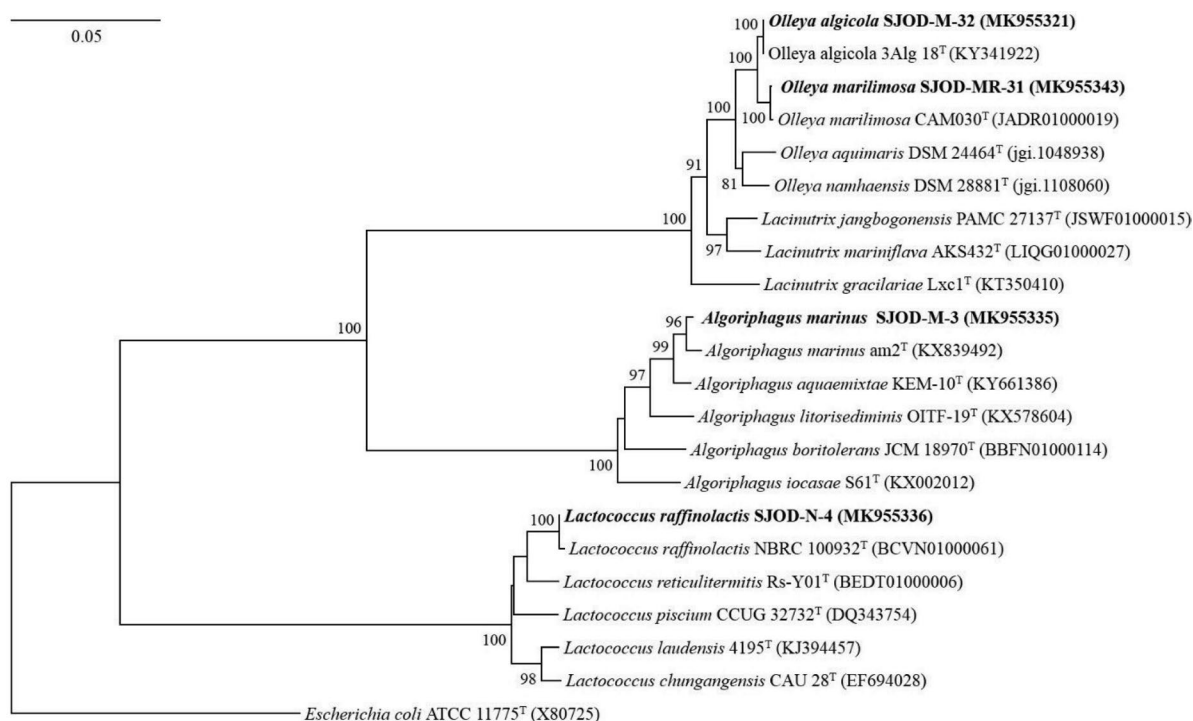


Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their relatives in the classes *Flavobacteriia*, *Cytophagia* and *Bacilli*. Bootstrap values (> 70%) are shown at the nodes. Bar, 0.05 substitutions per nucleotide position.

an *Ostrea denselamellosa* sample of Seomjin River. The GenBank accession number of 16S rRNA gene sequence is MK955339.

3. Description of *Rheinheimera pacifica* SJOD-R-25

Cells are Gram-stain-negative, non-pigmented and rod-shaped. Colonies grown on R2A are shining, convex, smooth and ivory colored after incubation for 4 days at 25°C. Positive for esculin hydrolysis, gelatin hydrolysis and β -galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose and D-maltose are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain SJOD-R-25 displays the highest 16S rRNA gene sequence similarity with *Rheinheimera pacifica* KMM 1406^T (99.9%). Strain SJOD-R-25 (= NNIBR2019642BA7) was isolated from an *Ostrea denselamellosa* sample of Seomjin River. The GenBank accession number of 16S rRNA gene sequence is MK955341.

4. Description of *Shewanella pacifica* SJEJ-MR-7

Cells are Gram-stain-negative, non-pigmented and rod-shaped. Colonies grown on R2A with seawater are circular, convex, smooth and slightly pink colored after incubation for 4 days at 25°C. Positive for nitrate reduction, esculin hydrolysis, gelatin hydrolysis and β -galactosidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase and urease. Potassium gluconate and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain SJEJ-MR-7 displays the highest 16S rRNA gene sequence similarity with *Shewanella pacifica* KMM 3597^T (99.3%). Strain SJEJ-MR-7 (= NNIBR2019642BA9) was isolated from an *Eriocheir japonica* sample of Seomjin River. The GenBank accession number of 16S rRNA gene sequence is MK955342.

5. Description of *Aliivibrio sifiae* SJEJ-M-17

Cells are Gram-stain-negative, non-pigmented and

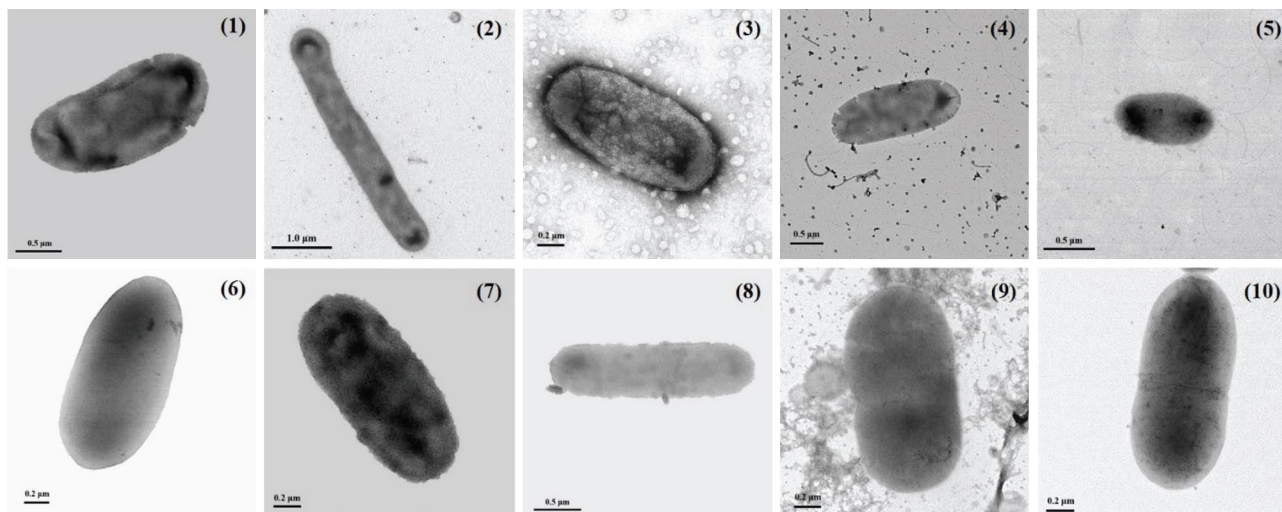


Fig. 3. Transmission electron micrographs of cells of the strains isolated in this study. Strains: 1, SJOD-M-26; 2, SJOD-M-14; 3, SJOD-R-25; 4, SJEJ-MR-7; 5, SJEJ-M-17; 6, SJEJ-MR-1; 7, SJOD-MR-31; 8, SJOD-M-32; 9, SJOD-M-3; 10, SJOD-N-4.

coccoid-rod shaped. Colonies grown on MA are circular, smooth and yellow colored after incubation for 4 days at 25°C. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis and β -galactosidase in API 20NE, but negative for indole production, arginine dihydrolase and urease. L-Arabinose, D-mannose, D-maltose, potassium gluconate, malic acid and trisodium citrate are utilized. Does not utilize D-glucose, D-mannitol, N-acetyl-glucosamine, capric acid, adipic acid and phenylacetic acid. Strain SJEJ-M-17 displays the highest 16S rRNA gene sequence similarity with *Aliivibrio sifiae* HI-1^T (99.4%). Strain SJEJ-M-17 (= NNIBR2019642BA5) was isolated from an *Eriocheir japonica* sample of Seomjin River. The GenBank accession number of 16S rRNA gene sequence is MK955338.

6. Description of *Leucothrix arctica* SJEJ-MR-1

Cells are Gram-stain-negative, non-pigmented and rod-shaped. Colonies grown on R2A with seawater are circular, convex, smooth and yellow colored after incubation for 4 days at 25°C. Positive for esculin hydrolysis and β -galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis. D-Glucose and potassium gluconate are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain SJEJ-MR-1 displays the high-

est 16S rRNA gene sequence similarity with *Leucothrix arctica* IMCC9719^T (98.8%). Strain SJEJ-MR-1 (= NNIBR2019642BA8) was isolated from an *Eriocheir japonica* sample of Seomjin River. The GenBank accession number of 16S rRNA gene sequence is MK955340.

7. Description of *Olleya marilimosa* SJOD-MR-31

Cells are Gram-stain-negative, non-flagellated and rod-shaped. Colonies grown on R2A with seawater are circular, convex and orange-yellow colored after incubation for 4 days at 25°C. Positive for arginine dihydrolase and esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, urease, gelatin hydrolysis and β -galactosidase. D-Mannose, D-mannitol, adipic acid, malic acid and trisodium citrate are utilized. Does not utilize D-glucose, L-arabinose, N-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid and phenylacetic. Strain SJOD-MR-31 displays the highest 16S rRNA gene sequence similarity with *Olleya marilimosa* CAM030^T (99.9%). Strain SJOD-MR-31 (= NNIBR2019642BA10) was isolated from an *Ostrea denselamellosa* sample of Seomjin River. The GenBank accession number of 16S rRNA gene sequence is MK955343.

8. Description of *Olleya algicola* SJOD-M-32

Cells are Gram-stain-negative, non-flagellated and rod-

shaped. Colonies grown on MA are circular, convex, smooth and yellow colored after incubation for 4 days at 25°C. Positive for arginine dihydrolase, esculin hydrolysis, gelatin hydrolysis and β -galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation and urease. D-Glucose, D-mannose, D-maltose, potassium gluconate, adipic acid and trisodium citrate are utilized. Does not utilize L-arabinose, D-mannitol, N-acetyl-glucosamine, capric acid, malic acid and phenylacetic. Strain SJOD-M-32 displays the highest 16S rRNA gene sequence similarity with *Olleya algicola* 3Alg 18^T (100.0%). Strain SJOD-M-32 (= NNIBR2019642BA1) was isolated from an *Ostrea denselamellosa* sample of Seomjin River. The GenBank accession number of 16S rRNA gene sequence is MK955321.

9. Description of *Algoriphagus marinus* SJOD-M-3

Cells are Gram-stain-negative, non-flagellated and coccobacilli-shaped. Colonies grown on MA are circular, convex, smooth and orange colored after incubation for 7 days at 25°C. Positive for esculin hydrolysis and β -galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis. D-Glucose, D-mannitol, N-acetyl-glucosamine and D-maltose are utilized. Does not utilize L-arabinose, D-mannose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic. Strain SJOD-M-3 displays the highest 16S rRNA gene sequence similarity with *Algoriphagus marinus* am2^T (99.9%). Strain SJOD-M-3 (= NNIBR2019642BA3) was isolated from an *Ostrea denselamellosa* sample of Seomjin River. The GenBank accession number of 16S rRNA gene sequence is MK955335.

10. Description of *Lactococcus raffinolactis* SJOD-N-4

Cells are Gram-stain-negative, non-flagellated and rod-shaped. Colonies grown on NA are circular, entire, smooth, opaque and ivory colored after incubation for 7 days at 25°C. Positive for esculin hydrolysis and β -galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis. D-mannose and D-mannitol are utilized. Does not utilize D-glucose, L-arabinose, N-acetyl-glucosamine, D-maltose, potassium gluconate,

capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic. Strain SJOD-N-4 displays the highest 16S rRNA gene sequence similarity with *Lactococcus raffinolactis* NBRC 100932^T (99.9%). Strain SJOD-M-3 (= NNIBR2019642BA4) was isolated from an *Ostrea denselamellosa* sample of Seomjin River. The GenBank accession number of 16S rRNA gene sequence is MK955336.

ACKNOWLEDGEMENT

This study was supported by a grant from the Nakdong-gang National Institute of Biological Resources (NNIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NNIBR201901109).

REFERENCES

- Barlow JP. 1955. Physical and biological processes determining the distribution of zooplankton in a tidal estuary. *Biol. Bull.* 109:211–225.
- Day JW Jr., CAS Hall, WM Kemp and A Yáñez Arancibia. 2018. *Estuarine Ecology*. John Wiley, New York. p. 558.
- Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791.
- Fisher TR, PR Carlson and RT Barber. 1982. Carbon and nitrogen primary productivity in three North Carolina estuaries. *Estuar. Coast. Shelf Sci.* 15:621–644.
- Jeon YS, K Lee, SC Park, BS Kim, YJ Cho, SM Ha and J Chun. 2014. EzEditor: a versatile sequence alignment editor for both rRNA- and protein-coding genes. *Int. J. Syst. Evol. Microbiol.* 64:689–691.
- Kim OS, YJ Cho, K Lee, SH Yoon, M Kim, H Na, SC Park, YS Jeon, JH Lee, HYi, S Won and J Chun. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* 62:716–721.
- Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111–120.
- Kobayashi S. 2011. Growth patterns of the Japanese mitten crab *Eriocheir japonica* (De Haan) in its river phase in Fukuoka Prefecture, Japan. *J. Crustac. Biol.* 31:653–659.
- Kumar S, G Stecher and K Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33:1870–1874.
- Lance J. 1963. The salinity tolerance of some estuarine plankton

- copepods. *Limnol. Oceanogr.* 8:440–449.
- Lane DJ. 1991. 16S/23S rRNA sequencing. p. 115. In *Nucleic Acid Techniques in Bacterial Systematics*. John Wiley and Sons, New York.
- Min DK, JS Lee, DB Koh and JG Je. 2004. Mollusks in Korea. Hanguk Graphics, Busan. pp. 410–415.
- Noseworthy RG, HJ Lee, SD Choi and KS Choi. Unique substrate preference of *Ostrea denselamellosa* Lischke, 1869 (Mollusca: Ostreidae) at Haechang Bay, on the south coast of Korea. *Korean J. Parasitol.* 32:31–36.
- Ringø E, Z. Zhou, JLG Vecino, S Wadsworth, J Romero, Å Krogdahl, RE Olsen, A Dimitroglou, A Foey, S Davies, M Owen, HL Lauzon, LL Martinsen, P de Schryver, P Bossier, S Sperstad and DL Merrifield. 2016. Effects of dietary components on the gut microbiota of aquatic animals: a never-ending story? *Aquac. Nutr.* 22:219–282.
- Saitou N and M Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406–425.
- Trabal N, JM Mazón-Suástegui, R Vázquez-Juárez, F Asencio-Valle, E Morales-Bojórquez and J Romero. 2012. Molecular analysis of bacterial microbiota associated with oysters (*Crassostrea gigas* and *Crassostrea corteziensis*) in different growth phases at two cultivation sites. *Microb. Ecol.* 64:555–569.
- Yang MH, CH Han, HS Kim and SD Choi. 1999. Environmental characteristics of natural conditions of the flat oyster, *Ostrea denselamellosa*, in Haechang Bay, Korea. *Korean J. Parasitol.* 15:105–113.