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The Microbiological Society of Korea



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## A005

### *Photobacterium aphysiae* sp. nov., a Lipolytic Marine Bacterium Isolated from Eggs of a Sea Hare (*Aplysia kurodai*)

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A bacterial strain, GMD509<sup>T</sup>, showing lipolytic activity was isolated from the eggs of sea hare, *Aplysia kurodai* collected at Mogiyeo of the South Sea (depth; 12 m), Korea. The strain is Gram-negative, motile, facultative anaerobic, mesophilic and weakly halophilic. Optimal growth condition of the strain GMD509<sup>T</sup> is 3.0 % (w/v) NaCl, pH 8 and 25 °C. The whole cell fatty acid profiles of the isolate include C16:1, C16:0 and C18:1 as major fatty acids and a DNA G+C content is 45 mol%. Phylogenetic analyses of 16S rDNA sequences place this bacterium in the  $\gamma$ -Proteobacteria, within the genus *Photobacterium*. 16S rDNA gene sequence of GMD509<sup>T</sup> is most similar to that of *P. frigidophilum* (97.8 %), *P. profundum* (97.5 %) and *P. indicum* (97.4 %). The DNA-DNA relatedness levels between the isolate and its closest known phylogenetic relatives, *P. frigidophilum* and *P. indicum*, are 25.3 and 13.7 %, respectively. The strain GMD509<sup>T</sup> therefore represents a new species for which we propose the name *Photobacterium aphysiae* sp. nov., and provide the type strain is GMD509<sup>T</sup> (=KCTC12383 =JCM12948).

[Supported by the Marine and Extreme Genome Research Center Program, Ministry of Maritime Affairs & Fisheries]

## A006

### Comparison of *Anabaena* and *Trichormus* (Cyanobacteria), Inferred from 16S rRNA Gene and *cpcBA*-Intergenic Spacer Sequence Analyses

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The genera *Anabaena* and *Trichormus* have been classified into the filamentous heterocystous cyanobacteria (subsection IV, family I). Traditionally, the classification of cyanobacteria has been based on morphological characters, which can vary in different environmental or growth conditions and even be lost during cultivation. Moreover, new strains were isolated to avoid identification problems caused by morphological changes during cultivation. Sequencing analysis of the 16S rRNA gene and a part of the phycocyanin operon (*cpc*), specifically the intergenic spacer (IGS) between *cpcB* and *cpcA*, was performed on 6 *Anabaena* and 8 *Trichormus* strains isolated from several rivers and reservoirs in Korea and preserved in Biological Resource Center, KRIBB. The 16S rRNA gene sequence similarities of *Anabaena variabilis* and *Trichormus variabilis* were 95.9 – 100% and 97.8 – 99.7%, respectively. Phylogenetic trees based on the 16S rRNA gene showed that *Anabaena* strains were distinguishable from *Trichormus* strains and were divided into two groups. However, phylogenetic trees based on *cpcBA*-IGS regions of 6 *Anabaena* and 8 *Trichormus* strains showed that the genera are intermixed.

## A007

### Two Novel *Paenibacillus* Species, *Paenibacillus hakamense* sp. nov. and *Paenibacillus taeanense* sp. nov. Isolated from Root of Sand Dune Plants

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Two Gram-positive, endospore forming bacteria (PHA2-4<sup>T</sup> and PBA10-1<sup>T</sup>) were isolated from two sand dune plant species, *Lathyrus japonicus* and *Glehnia littoralis* growing along the coastal areas in Tae-an, Korea, and their taxonomic status was investigated. Complete 16S rRNA gene sequence analysis indicated that both of the organisms should be placed in the genus *Paenibacillus*. Levels of 16S rRNA gene sequence similarity between existing *Paenibacillus* species and the two novel strains PHA2-4<sup>T</sup> and PBA10-1<sup>T</sup> were 92.1-97.0% and 93.6-97.5%, respectively. The major fatty acids for both strains were *anteiso*-C15:0, *n*-C16:0, and *iso*-C16:0. The DNA G+C contents of strains PHA2-4<sup>T</sup> and PBA10-1<sup>T</sup> were 43.3 and 42.9 mol%, respectively. On the basis of phenotypic and phylogenetic data, it is evident that these strains should be assigned to new species of the genus *Paenibacillus*, for which the names *Paenibacillus hakamense* sp. nov. (type strain = PHA2-4<sup>T</sup>) and *Paenibacillus taeanense* sp. nov. (type strain = PBA10-1<sup>T</sup>) are proposed.

## A008

### *Psychroserpens mesophilus* sp. nov. and *Rhodobacter incheonensis* sp. nov., Isolated from Young Marine Biofilm

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Two bacterial strains (KOPRI 13537 and KOPRI 13650) among the marine bacteria isolated from young biofilm were investigated to determine taxonomic status. Based on the 16S rRNA sequence, KOPRI 13537 and KOPRI 13650 should be placed in the genus *Rhodobacter* and genus *Psychroserpens*, respectively. Major fatty acids of KOPRI 13537 were C18:1w9tr, C16:0 and C16:1w9 and could assimilate arabinose, mannitol, maltose and citrate. Unlikely to the other members of genus *Rhodobacter* the isolate has no photosynthetic pigments. Major fatty acids of KOPRI 13650 were C18:1w9, C16:1w9 and a-C17:0. The isolate was non-motile rod form. Optimal growth was observed at 30 °C and 4-5% of NaCl concentration. Based on the phenotypic and phylogenetic data, it is proposed that KOPRI 13537 should be proposed as *Rhodobacter incheonensis* sp. nov., and KOPRI 13650 as *Psychroserpens mesophilus* sp. nov.

[Supported by NRL Program]

# Pseudorhodobacter incheonensis sp. nov., Isolated from Young Marine Biofilm

KORDI

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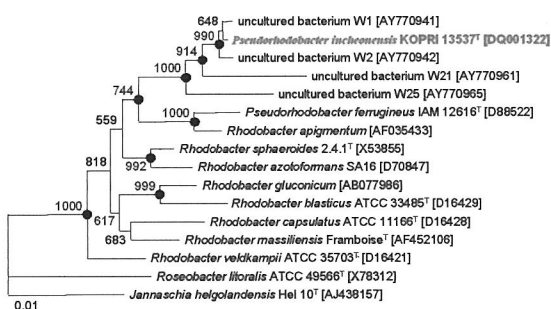
## Abstract

A marine bacterium isolated from young biofilm in Incheon harbor (KOPRI 13537<sup>T</sup>) was identified. The sequence of 16S ribosomal RNA gene of isolate showed high similarity with that of strains belonging to genus *Rhodobacter* (93.50–95.85% with validly reported strains) and *Pseudorhodobacter ferrugineus* (94.83%). Phylogenetic analysis with the strains belonging to the genus *Rhodobacter* and *P. ferrugineus* revealed that the isolate formed a distinct phyletic line with *P. ferrugineus*, invalidly reported strain *R. apigmentum* and clones from oil field within clade of the genus *Rhodobacter*. The dominant fatty acids of the isolate are 18:1ω9r, 16:0 and 16:1ω9. The major respiratory quinone is ubiquinone 10 and the DNA G+C ratio is 61.4 mol%. The isolate does not contain photosynthetic pigments. Optimal growth of the strain KOPRI 13537<sup>T</sup> requires the presence of 1% (w/v) NaCl, pH 6.5–7.0, and 26–33°C. On the basis of polyphasic taxonomical evidences, the strain KOPRI 13537<sup>T</sup> should be classified as a novel species in the genus *Pseudorhodobacter* and is here proposed as *Pseudorhodobacter incheonensis* sp. nov.

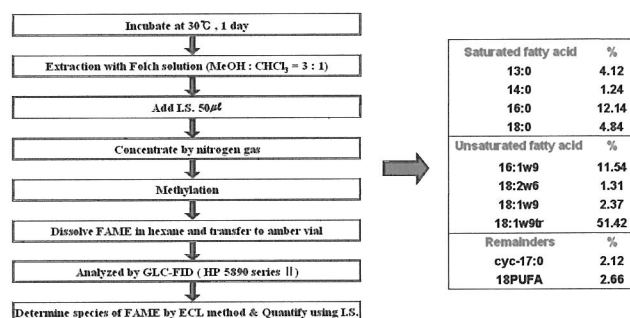
## Introduction

Genus *Rhodobacter* has been classified eco-physiologically as a member of purple nonsulfur bacterium. It contains bacteriochlorophylls and various carotenoid pigments and carrying anoxygenic photosynthesis under anaerobic condition (Madigan *et al.*, 2003). Most of the members could use sulfide as an electron donor. They showing phototrophic and are nutritionally diverse group. Hiraish and Ueda (1994) divided the genus *Rhodobacter*, the species whose habitats were seawater were transferred to the new genus *Rhodovulum*, and, as a result, the genus *Rhodobacter* involving only species whose habitats were freshwater. In recent, a strain has high similarity of 16S rRNA gene sequence with that of genus *Rhodobacter* but does not contain photosynthetic pigments, *Agrobacterium ferrugineus*, was reclassified as a novel genus *Pseudorhodobacter* (Uchino *et al.*, 2002). In the present study we also report the results of identification of a marine bacterium KOPRI 13537<sup>T</sup>, showing phylogenetically high similarity with *Rhodobacter* but does not contain photosynthetic pigments, as a novel species of the genus *Pseudorhodobacter*, *P. incheonensis* sp. nov.

## Phylogenetic position of isolated bacteria

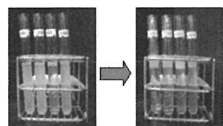


## Fatty acid analysis of strain KOPRI 13537<sup>T</sup>



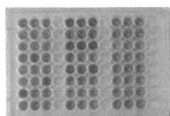
## Physiological characteristics of strain KOPRI 13537<sup>T</sup>

Cell Surface Hydrophobicity test (BATH method)

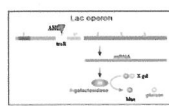


- Cell surface hydrophobicity was determined by BATH method using n-hexadecane  
 ⇒ 19% of cell surface hydrophobicity
- Attachment ability was determined by the crystal violet staining method of cells attached on polystyrene surface  
 \* Attachment ability = (OD<sub>560nm</sub> / OD<sub>560nm</sub>) ⇒ 1.197
- Soluble EPS was extracted from the 4 days old culture broth of the strains by cold-EtOH precipitation method, purified by dialysis and determine dry weight.  
 ⇒ Bound EPS 26.7 mg/L, Soluble EPS 103.7 mg/L

Attachment ability



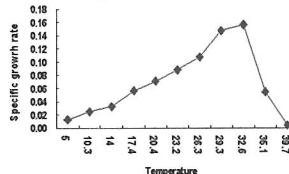
Production of quorum sensing signal molecules



Reporter Strain (*A. tumefaciens* NTL4)  
 AHL-producing bacteria

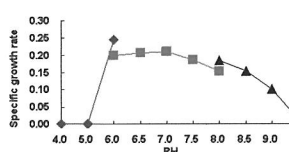
Target signal molecules: Acyl Homoserine Lactone  
 Method: cross-feeding plate bioassay  
 Reporter: Recombinated *A. tumefaciens* NTL4  
 Results: ++

Growth temperature of KOPRI 13537<sup>T</sup>



- Use temperature gradient incubator (Advantec)
- Temperature range: 5°C ~ 44°C
- Cultivation time: 1 day
- Calculate specific growth rate (OD/hr) during exponential growing phase

Growth PH of KOPRI 13537<sup>T</sup>



- Use temperature gradient incubator (Advantec)
- PH range: 4 ~ 10
- Cultivation time: 3 days
- Buffer: MIES (PH 4 ~ 6), HEPES (PH 6 ~ 8), AMPPO (PH 8 ~ 10)
- Calculate specific growth rate (OD/hr) during exponential growing phase

## Phenotypic characteristics differentiate strain KORRI 13537<sup>T</sup> from members of the genus *Pseudorhodobacter* & *Rhodobacter*.

1, KOPRI 13537<sup>T</sup>; 2, *P. ferrugineus* IAM 12616<sup>T</sup>; 3, *R. sphaeroides*; 4, *R. azotoformans* KA25<sup>T</sup>; 5, *R. veldkampii*; 6, *R. capsulatus*; 7, *R. blasticus*

Characteristics	1	2	3	4	5	6	7
Cell size (μm)	0.8-1.2	0.6-1.6 (1.0-4.0)	0.7-4.0 (2.0-2.5)	0.6-1.0 (0.9-1.5)	0.6-0.8 (0.1-1.3)	0.5-1.2 (0.2-2.5)	0.6-0.8 (0.2-2.5)
Cell shape	ovoid	Rod	Spherical, ova	Ovoid, rod	Ovoid, rod	ovoid, rod	Ovoid, rod
Motility	+	-	+	+	-	+	-
Growth temperature (°C)*	5-40 (26-33)		(30-34)	-35	(30-35)	(30-35)	(30-35)
Growth pH*	6-10 (6.5-7)		6.0-8.5 (7.0)	(7.0-7.5)	-7	6.5-7.5 (7.0)	(6.5-7.5)
Growth range of NaCl (%)	0-7 (1.0)	0-3 (1.0)	-3	0.5		<3	<3
Require NaCl	-	-	-	-	-	-	-
Growth requirements	-	-	B, T, N	B, N, T	B, T, p-ABA	T	T, B, C, B12
Growth rate	61.4	58	70.8-73.2	69.5-70.2	64.4-67.5	63.1-69.6	65.3
Insoluble quinone	Q10	Q10	Q10	Q10	Q10	Q10	Q10
Sulfate assimilated	nd	+	+	+	+	+	+
Nitrate reduction	nd	-	+	+	-	-	-
Sulfide oxidized to	nd	nd	S <sup>0</sup>	S <sup>0</sup>	Sulfate via S <sup>0</sup>	S <sup>0</sup>	-
Growth on H <sub>2</sub> CO <sub>3</sub>	nd		Slow			Excellent	+
Photosynthetic pigments	-	-	+	+	+	+	+
Utilization of							
Tartrate	nd	nd	+	-	-	-	-
Citrate	+	+	+	-	-	-	+
Mannitol	+	nd	+	+	-	-	+
Choline	-	nd	+	nd	nd	-	nd

\*Values in the parenthesis are optimum range  
 B: Biotin, N: Niacin, T: Thiamine

nd: not determined  
 p-ABA: p-aminobenzoic acid

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