Journal of Species Research 13(1):61-66, 2024

Open Access

A report of six unrecorded bacterial species isolated from soil samples in Korea

Da Som Kim, Mi Jin Jeon and Won-Jae Chi*

Species Diversity Research Division, Biodiversity Research Department, National Institute of Biological Resources, Incheon 22689, Republic of Korea

**Correspondent: wjchi76@korea.kr*

During an investigation of unrecorded prokaryotic species in Korea, six unrecorded bacterial strains were isolated from soil samples collected from Uljin-gun. Based on a similarity search using the 16S rRNA gene sequence of the isolated strains and the construction of the neighbor-joining phylogenetic tree, five strains were identified to the genus *Pseudomonas* of the family Pseudomonadaceae, while one strain was identified as a species belonging to the genus *Paenibacillus* of the family Paenibacillaceae. The details of these unreported species, including gram staining reaction, colony and cell morphology, basic biochemical characteristics, strain ID, and isolation source, are described in the description of the strains.

Keywords: 16S rRNA gene, Paenibacillus, Pseudomonas, unrecored prokaryotic specices

DOI:10.12651/JSR.2024.13.1.061

Introduction

Wetlands are unique environmental areas covered with water throughout the year and occupy an environmentally and ecologically vital position with functions such as bioremediation and carbon storage as ecotones of terrestrial and aquatic ecosystems (Mulamoottil *et al*., 1996; Jungblut *et al*., 2012; Hu *et al*., 2014). Caves are unusual environments characterized by fewer nutrients, less light, relatively low temperatures, and high humidity (Cheeptham, 2013). Recently, wetlands and caves have been recognized as essential environmental sites for isolating many previously unrecorded prokaryotic species.

Pseudomonas are gram-negative bacteria belonging to the family Pseudomonadaceae of the class Gammaproteobacteria. Many *Pseudomonas* species are well-known as animal or plant pathogens, but some are known to be suitable plant growth promotion, biocontrol, and bio-remediation agents (Weller and Cook, 1983; Sah *et al*., 2021; Vález *et al*., 2021; Ridene *et al*., 2023). Since the genome sequence of *P. aeruginoas* PAO1 was determined in 2000, more than 500 complete *Pseudomonas* genomes have been registered in the NCBI GenBank as of 2020.

The genus *Paenibacillus*, a member of the family Paenibacillaceae, was reclassified as a separate genus with the type species *Paenibacillus polymyxa* from Bacillus by Ash *et al*. (1993). Most members of the genus *Paenibacillus* are essential microorganisms for application in the agriculture, food, and medical industries (Padda *et al*., 2017; Daud *et al*., 2019). *Paenibacillus* species have been isolated from various environmental sources including soil, fresh- and saltwater, caves, air, sediment, rhizosphere, plants, food, insect larvae, and blood cultures (Baik *et al*., 2001; Berge *et al*., 2002; Scheldeman *et al*., 2004; Roux and Raout, 2004; Choi *et al*., 2008; Ashraf *et al*., 2017).

Materials and Methods

A total of 100 mg of soil sample was suspended with 100 mL of sterile water, diluted 10^{-1}–10^{-5} times in sterile water, plated on trypticase soy agar (TSA), and incubated at 30°C for three days. Colonies grown on TSA medium were transferred to a new medium and incubated at 30°C for three days to confirm that it is a pure culture.

The 16S rRNA gene sequence was searched for homology with sequences from GenBank, using the BLASTN program of the National Center for Biotechnology Information. The EzTaxon database (http://www.ezbiocloud.net/) was used for the analysis of 16S rRNA gene sequence homology with the type strain (Chun *et al*., 2007). Phylogenetic analysis was performed based on the 16S rRNA gene sequences of the type strains provided in the EzTaxon database using ClustalW, which was implemented in the

MEGA X program (Kumar *et al*., 2018).

Bacterial strains were stained using a gram stain kit (BD, USA) according to the manufacturer's instructions and observed with a light microscope. The biochemical characteristics and enzyme production of the strains were tested using API 20NE and API ZYM strips (Biomérieux, France) according to the manufacturer's instructions. In addition, the cells were stained with 1% phosphotungstic acid and observed using a transmission electron microscope (JEM1010, JEOL, Japan) to determine cellular morphology, including cell shape and size, and the presence of flagella.

Results and Discussion

Based on a 16S rRNA gene sequence similarity search of 134 bacterial strains isolated from soil samples of an alpine wetland on Mt. Sinbul, two unreported bacterial species in Korea were identified. Four strains showing over 98.7% 16S rRNA gene sequence similarity with unrecorded bacterial species in Korea were isolated from 38 bacterial strains obtained from a soil sample collected from Seongnyugul Cave. Identification of the isolates based on 16S rRNA gene sequence similarity was confirmed using phylogenetic tree analysis. The neighbor-joining phylogenetic tree indicated a close relationship between the isolates and the type strains of each corresponding species. Taxonomic composition and identification results are presented in Table 1. Strains SBS2-5, SRC3-8, SRC1-1, SRC1-3, and SRC3-1 were assigned to the family Pseudomonadaceae, whereas strain SBS9-6 was assigned to the family Paenibacillaceae.

The detailed morphological and physiological characteristics are elucidated in the strain descriptions.

Description of *Pseudomonas viciae* SBS2-5

The cells of *Pseudomonas viciae* SBS2-5 are aerobic, gram-negative, flagellated, and rod shaped. Colonies are circular, convex, and smooth after incubation for 24 h on TSA at 30°C. The cells are positive for nitrate reduction, arginine dihydrolase, and gelatin hydrolysis; but negative for indole production, glucose fermentation, urease, esculin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, gluconate, capric acid, malic acid, and trisodium citrate are utilized as sole carbon sources; but not L-arabinose, D-maltose, adipic acid, or phenylacetic acid. Strain SBS2-5 (NIBRBAC000510249) was isolated from a soil sample of an alpine wetland on Mt. Sinbul, Uljin-gun, Gyeongsanbuk-do, Korea. The GenBank accession number for the 16S rRNA gene sequence of SBS2-5 is OR230 093.

Table 1. Summary of strains isolated and their taxonomic affiliations.

Order	Family	Strain ID	NIBR ID	GenBank accession number	Closest species	Similarity (%)	Source
Pseudomonadales	*Pseudomonadaceae*	SBS2-5	NIBRBAC000510249	OR230093	*Pseudomonas viciae*	99.44	Soil
	Pseudomonadaceae	SRC3-8	NIBRBAC000510251	OR230092	*Pseudomonas atagonensis*	99.22	Soil
	Pseudomonadaceae	SRC1-1	NIBRBAC000510252	OR230089	*Pseudomonas farsensis*	99.51	Soil
	Pseudomonadaceae	SRC1-3	NIBRBAC000510253	OR230090	*Pseudomonas arcuscaelestis*	99.30	Soil
	Pseudomonadaceae	SRC3-1	NIBRBAC000510254	OR230091	*Pseudomonas hutmensis*	100	Soil
Bacillales	*Paenibacillaceae*	SBS9-6	NIBRBAC000510250	OR230094	*Paenibacillus pseudetheri*	99.32	Soil

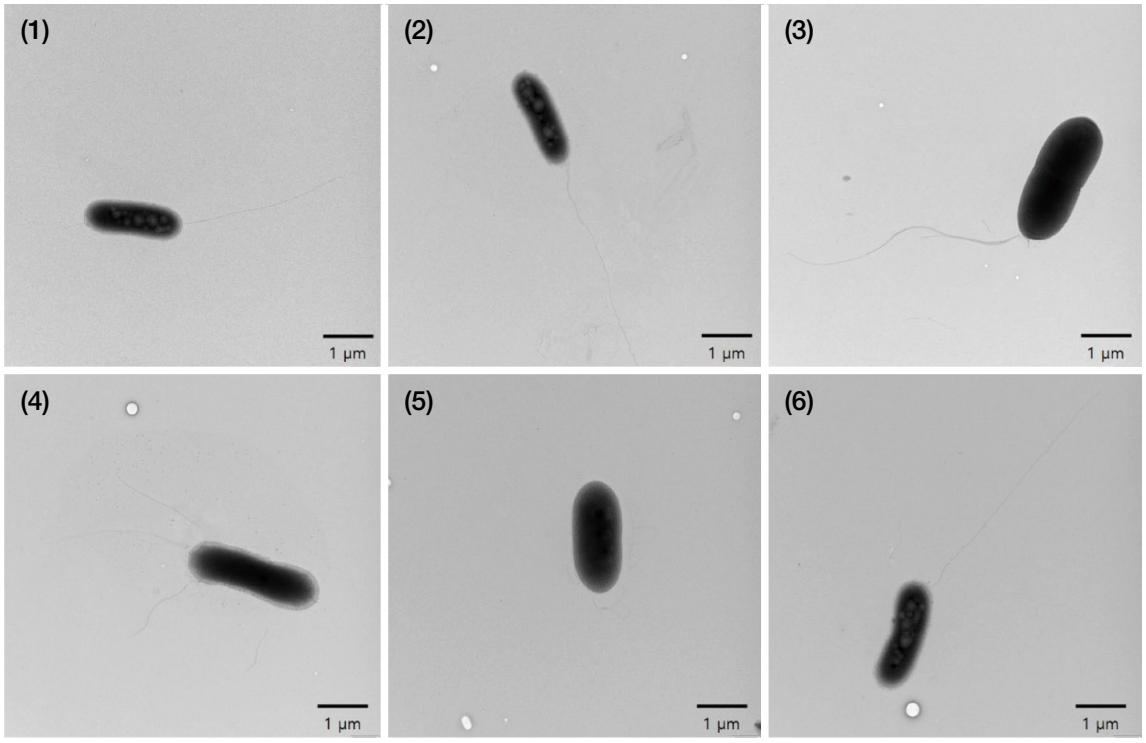

Fig. 1. Transmission electron micrographs of the strains in this study. Strains: 1, SBS2-5; 2, SRC3-8; 3, SRC1-1; 4, SRC1-3; 5, SRC3-1; 6, SBS9-6.

Description of *Pseudomonas atagonensis* SRC3-8

The cells of *Pseudomonas atagonensis* SRC3-8 are aerobic, gram-negative, flagellated, and rod shaped. Colonies are circular, convex, moist, and creamy white after incubation for 24 h on TSA at 30°C.

The cells are positive for nitrate reduction, arginine dihydrolase, and gelatin hydrolysis; but negative for indole production, glucose fermentation, urease, esculin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, gluconate, capric acid, malic acid, and trisodium citrate are utilized as sole carbon sources; but not D-maltose, adipic acid, or phenylacetic acid. Strain SRC3-8 (NIBRBAC000510251) was isolated from a soil sample collected from the Seongnyugul Cave, Uljin-gun, Gyeongsanbuk-do, Korea. The GenBank accession number for the 16S rRNA gene sequence of strain SRC3-8 is OR230092.

Description of *Pseudomonas farsensis* SRC1-1

The cells of *Pseudomonas farsensis* SRC1-1 are aerobic, gram-negative, flagellated, and rod shaped. Colonies are circular, convex, smooth, and cream-colored after incubation for 24 h on TSA at 30°C. The cells are positive for nitrate reduction and arginine dihydrolase; but negative for indole production, glucose fermentation, urease, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, D-mannose, gluconate, capric acid, malic acid, trisodium citrate, and phenylacetic acid were utilized as sole carbon sources; but not L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, and adipic acid. Strain SRC1-1 (NIBRBAC000510252) was isolated from a soil sample collected from the Seongnyugul Cave, Uljin-gun, Gyeongsanbuk-do, Korea. The GenBank accession number for the 16S rRNA gene sequence of strain SRC1-1 is OR230089.

Description of *Pseudomonas arcuscaelestis* SRC1-3

The cells of *Pseudomonas arcuscaelestis* SRC1-3 are aerobic, gram-negative, flagellated, and rod shaped. Colonies are circular, convex, smooth, and cream-colored after incubation for 24 h on TSA at 30°C. The cells are positive for nitrate reduction, arginine dihydrolase, and gelatin hydrolysis; but negative for indole production, glucose fermentation, urease, esculin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, gluconate, capric acid, malic acid, and trisodium citrate, are utilized as sole carbon sources; but not D-mannose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, adipic acid, and phenylacetic acid. Strain SRC1-3 (NIBRBAC000510253) was isolated from soil samples collected from the Seongnyugul Cave, Uljin-gun, Gyeongsanbuk-do, Korea. The GenBank accession number for the 16S rRNA gene seq-

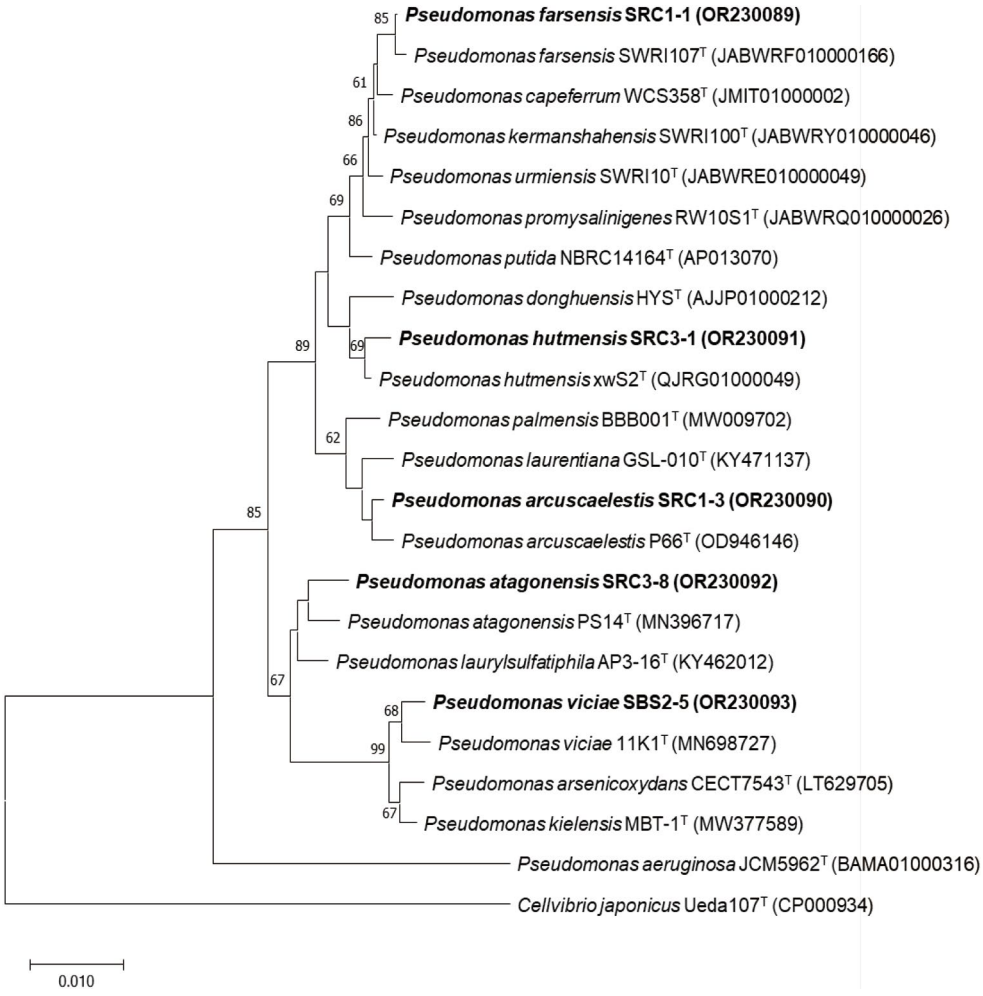

Fig. 2. Neighbor-joining phylogentic tree based on 16S rRNA gene sequences of the isolates in this study and their relatives of the genus *Pseudomonas*. Strain: SBS2-5, SRC3-8, SRC1-1, SRC1-3, SRC3-1. Bootstrap values (>50%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 substitutions per nucleotide position.

uence of strain SRC1-3 is OR230090.

Description of *Pseudomonas hutmensis* SRC3-1

The cells of *Pseudomonas hutmensis* SRC3-1 are aerobic, gram-negative, flagellated, and rod shaped. Colonies are circular, convex, smooth, moist, and creamy white after incubation for 24 h on TSA at 30°C. The cells are positive for nitrate reduction and arginine dihydrolase; but negative for indole production, glucose fermentation, urease, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, gluconate, capric acid, malic acid, trisodium citrate, and phenylacetic acid are utilized as sole carbon sources; but not D-mannose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, or adipic acid. Strain SRC3-1 (NIBRBAC000510254) was isolated from a soil sample collected from the Seongnyugul Cave, Uljin-gun, Gyeongsanbuk-do, Korea. The GenBank accession number for the 16S rRNA gene sequence of strain SRC3-1 is OR230091.

Description of *Paenibacillus pseudetheri* SBS9-6

The cells of *Paenibacillus pseudetheri* SBS9-6 are aerobic, gram-positive, flagellated, and rod shaped. Colonies are creamy, smooth with irregular margins, and cream-colored after incubation for 24 h on TSA at 30°C. The cells are positive for nitrate reduction, esculin hydrolysis, and β-galactosidase; but negative for indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis in API 20NE. D-Glucose, *N*-acetyl-glucosamine, and D-maltose are utilized as sole carbon sources; but not D-mannose, D-mannitol, gluconate, capric acid, malic acid, trisodium citrate, L-arabinose, adipic acid, and phenylacetic acid. Strain SBS9-6 (NIBR BAC000510250) was isolated from a soil sample col-

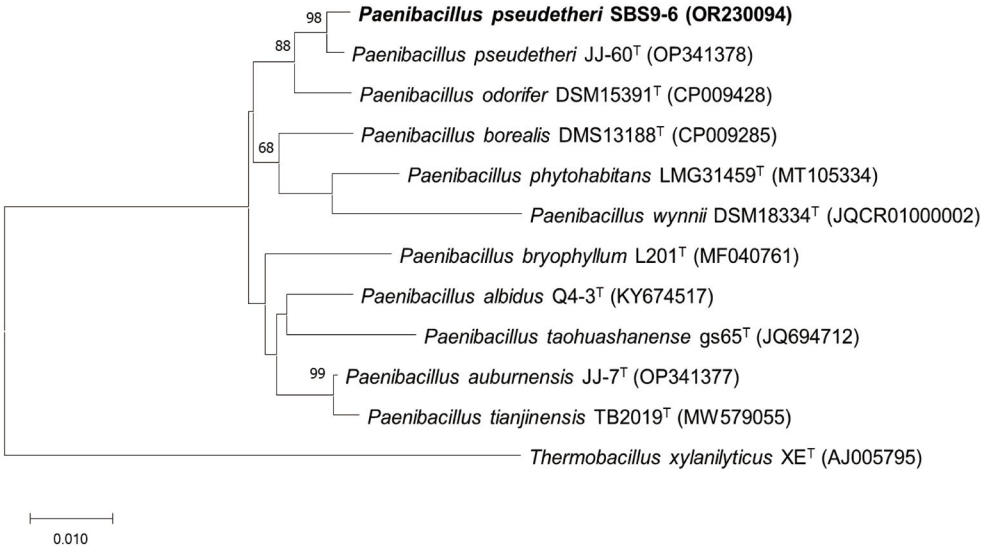

Fig. 3. Neighbor-joining phylogentic tree based on 16S rRNA gene sequences of the isolate in this study and their relatives of the genus *Paenibacillus*. Strain: SBS9-6. Bootstrap values (>50%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 substitutions per nucleotide position.

lected from an alpine wetland on Mt. Sinbul, Uljin-gun, Gyeongsanbuk-do, Korea. The GenBank accession number for the 16S rRNA gene sequence of strain SBS9-6 is OR230094.

Conflicts of Interest

The author of this paper has no affiliation with any interests and is solely responsible for the paper.

Acknowledgements

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202304106).

References

Ash, C., F.G. Priest and M.D. Collins. 1993. Molecular identification of rRNA group bacilli 3 using a PCR probe test. Antonie Van Leeuwenhoek 64:253-260.

Ashraf, S., M.R. Soudi, M.A. Amoozegar, M.M. Nikou and C. Spröer. 2017. *Paenibacillus xanthanilyticus* sp. nov. a xanthan-degrading bacterium isolated from soil. Int J Syst Evol Microbiol. 68:76-80.

Baik, K.S., H.N. Choe, S.C. Park, E.M. Kim and C.N. Seong. 2001. *Paenibacillus wooponensis* sp. nov., isolated from wetland freshwater. Int J Syst Evol Microbiol. 61:2763-2768.

Berge, O., M.H. Guinebretière, W. Achouak, P. Normand and T. Heulin. 2002. *Paenibacillus graminis* sp. nov. and *Paenibacillus odorifer* sp. nov., isolated from plant roots, soil and food. Int J Syst Evol Microbiol. 52:607-616.

Cheeptham, N. 2013. Advances and challenges in studying cave microbial diversity. In: N. Cheeptham (ed.), Cave Microbiomes: A Novel Resource for Drug Discovery. Springer Briefs in Microbiology, vol. 1, NY: Springer, New York.

Choi, J.H., W.T. Im, J.S. Yoo, S.M. Lee, D.S. Moon, H.J. Kim, H.J. Kim, S.K. Rhee and D.H. Roh. 2008. *Paenibacillus donghaensis* sp. nov., a xylan-degrading and nitrogen-fixing bacterium isolated from East Sea sediment. J Microbiol Biotechnol. 18:189-193.

Chun, J., J.H. Lee, Y. Jung, K. Kim, S. Kim, B.K. Kim and Y.W. Lim. 2007; EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int J Syst Evol Microbiol. 57:2259-2261.

Daud, N.S., A.R.J. Mohd Din, M.A. Rosli, Z.M. Azam, N.Z. Othman and M.R. Sarmidi. 2019. *Paenibacillus polymyxa* bioactive compounds for agricultural and biotechnological applications. Biocatal Agric Biotechnol. 18:101092.

Hu, B.L., L.D. Shen, X. Lian, Q. Zhu, S. Liu, Q. Huang, Z.F. He, S. Geng, D.Q. Cheng, L.P. Lou, X.Y. Xu, P. Zheng and Y.F. He. 2014. Evidence for nitrite-dependent anaerobic methane oxidation as a previously overlooked microbial methane sink in wetlands. PNAS 111:4495-4500.

Jungblut, A.D., S.A. Wood, I. Hawes, J. Webster-Brown and C. Harris. 2012. The pyramid trough wetland: environmental and biological diversity in a newly created Antarctic protected area. FEMS Microbiol Ecol. 82:356-366.

Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 35:1547-1549.

Mulamoottil, M., B.G. Warner and E.A. McBean. 1996. Wetlands: Environmental gradients, boundaries and buffers. Boca Raton: CRC Press.

Padda, K.P., A. Puri and C.P. Chanway. 2017. *Paenibacillus polymyxa*: a prominent biofertilizer and biocontrol agent for sustainable agriculture. In: V. Meena, P. Mishra, J. Bisht and A. Pattanayak (eds.), Agriculturally important microbes for sustainable agriculture. Springer, Singapore. pp. 165-191.

Ridene, S., N. Werfelli, A. Mansouri, A. Landoulsi and C. Abbes. 2023. Bioremediation potential of consortium *Pseudomonas* Stutzeri LBR and Cupriavidus Metallidurans LBJ in soil polluted by lead. PLoS ONE 18:e0284120.

Roux, V. and D. Raoult. 2004. *Paenibacillus massiliensis* sp. nov., *Paenibacillus sanguinis* sp. nov. and *Paenibacillus timonensis* isolated from blood cultures. Int J Syst Evol Microbiol. 54:1049-1054.

Sah, S., S. Krishnani and R. Singh. 2021. *Pseudomonas* mediated nutritional and growth promotional activities for sustainable food security. Curr Res Microbial Sci. 2:100084.

Scheldeman, P., K. Goossens, M. Rodriguez-Diaz, A. Pil, J. Goris, L. Herman, P.D. Vos, N.A. Logan and M. Heyndrick. 2004. *Paenibacillus lactis* sp. nov., isolated from raw and heat-treated milk. Int J Syst Evol Microbiol. 54:885-891.

Vález, J.M.B., J.G. Martínez, J.T. Ospina and S.O. Agudelo. 2021. Bioremediation potential of *Pseudomonas* genus isolates from residual water, capable of tolerating lead through mechanisms of exopolysaccharide production and biosorption. Biotechnol Rep. 32:e00685.

Weller, D.M. and R.J. Cook. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463-469.

Submitted: October 11, 2023
Revised: November 20, 2023
Accepted: November 27, 2023