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ORIGINAL PAPER

Paenibacillus agricola sp. nov., isolated from agricultural soil

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Abstract

A white-coloured, rod-shaped, motile, aerobic, and Gram-stain-positive bacterial strain S3N08^T was isolated from agricultural soil. The strain grew at temperature 10–40 °C, at 0–1.0% (w/v) NaCl concentration, and at pH 6.5–8.0. Catalase was negative and oxidase was positive. The phylogenetic analysis inferred that the strain S3N08^T belonged to the genus *Paenibacillus*, with the closest relative being *Paenibacillus periandrae* PM10^T (95.6% 16S rRNA gene sequence similarity). The only menaquinone was MK-7 and the major polar lipids were phosphatidylmonomethylethanolamine, phosphatidylglycerol, and phosphatidylethanolamine. The predominant fatty acids were antiso-$C_{15:0}$, $C_{16:0}$, and iso-$C_{15:0}$. The DNA G + C content was 45.1%. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between strain S3N08^T and with closest members were < 72.0% and < 19.0%, respectively. Altogether, the phylogenetic, genomics, phenotypic, and chemotaxonomic evidence illustrated in this study suggested that strain S3N08^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus agricola* sp. nov. is proposed. The type strain is S3N08^T (= KACC 19666 ^{T} = NBRC 113430 ^{T}).

Keywords *Paenibacillus agricola* sp. nov. · Agricultural soil · *Paenibacillaceae* · Taxonomy · Phylogeny

Abbreviations

KACC	Korean agricultural culture collection
KCTC	Korean collection for type cultures
LMG	Laboratorium voor microbiologie
DSM	Deutsche Sammlung von Mikroorganismen
NBRC	NITE biological resource center
ANI	Average nucleotide identity
dDDH	Digital DNA-DNA hybridization

Introduction

The genus *Paenibacillus*, belonging to the phylum Bacillota, was described by Ash et al. in 1993, with the type species *Paenibacillus polymyxa* (Ash et al. 1993; Tindall 2000). At the time of preparing the manuscript, this genus accommodates a total of 293 species with a validly published and correct names (https://lpsn.dsmz.de/genus/paenibacillus). The members of *Paenibacillus* have been reported from diverse sources including rhizosphere, phyllosphere, hot spring, root nodules, freshwater wetland, necrotic wound, soil, water, food, faeces, and insects (Baik et al. 2011; Glaeser et al. 2013; Menendez et al. 2016; Kämpfer et al. 2022; Wang et al. 2022). Recently, *Paenibacillus rhizolycopersici* (Thin et al. 2023) and *Paenibacillus sabuli* (Gao et al. 2022) have been retrieved from tomato plant and sea environment. Based on polyphasic approach, this study aimed to characterize and assign the taxonomic position of strain S3N08^T, which was isolated from agricultural soil and proposed the name as *Paenibacillus agricola* sp. nov.

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA sequence and whole genome shotgun sequence of strain S3N08^T are MH159222 and JAAOIW000000000, respectively.

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Materials and methods

Isolation of strain

Strain S3N08^T was isolated from an agricultural soil (rice paddy field) sample collected from Seongju-gun, Republic of Korea (GPS coordinates: 35°53′56.8″N 128°14′04.9″E) using the standard dilution plating technique on R2A medium (MB Cell, South Korea). After plating, all the plates were incubated at 28 °C for 7 days. A white-pigmented colonies of bacterial strain, S3N08^T was obtained after repeated streaking in R2A agar. The pure colonies of strain S3N08^T were then stored at -80 °C as a suspension in R2A broth with 20% (w/v) glycerol for long-term preservation. The strain S3N08^T was deposited in the Korean Agricultural Culture Collection and NITE Biological Resource Center.

16S rRNA gene sequence and phylogenetic analysis

Genomic DNA from strain S3N08^T was extracted using commercial DNA extraction kit (InstaGene Matrix, Bio-Rad, USA). The 16S rRNA gene was amplified using forward (27F) and reverse (1492R) primers (Frank et al. 2008). The sequencing and analysis of amplified 16S rRNA gene was performed as described previously (Chaudhary et al. 2017). The nearest phylogenetic members were concluded by evaluating 16S rRNA gene sequence in the the EzBioCloud server (Yoon et al. 2017b). The 16S rRNA gene sequences of all the phylogenetically affiliated species were downloaded from EzBioCloud database and aligned with SINA (v1.2.11) according to the SILVA seed alignment (https://www.arb-silva.de) (Pruesse et al. 2012). The generation of phylogenetic trees was accomplished by maximum–likelihood (ML) (Felsenstein 1981) and neighbour–joining (NJ) (Saitou and Nei 1987) algorithms using MEGA X software (Kumar et al. 2018). The topology of the phylogenetic trees was evaluated by the bootstrap resampling method with 1000 replicates (Felsenstein, 1985). Evolutionary distances were determined by Kimura's two-parameter model (Kimura 1980).

Genome analysis

The genome of strain S3N08^T was sequenced by Illumina MiSeq platform and assembled by SPAdes ver. 3.14.1 (Macrogen, South Korea). The quality control and contamination of the sequenced genome were investigated using ContEst16S algorithm (Lee et al. 2017). The annotation of the assembled genome sequence was performed by Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al. 2016) and Rapid Annotation Subsystem technology (RAST server) (Aziz et al. 2008). The putative secondary metabolites were detected with the program antiSMASH 5.0 (Blin et al. 2019). The whole genome similarities between strain S3N08^T and the closest members (*Paenibacillus periandrae* PM10^T, *Paenibacillus vulneris* CCUG 53270^T, *Paenibacillus rigui* JCM 16352^T, *Paenibacillus phytorum* LMG 31458^T, and *Paenibacillus alginolyticus* DSM 5050^T) were assessed by average nucleotide identity (ANI) tool using OrthoANIu algorithm (Yoon et al. 2017a) and Genome-to-Genome Distance Calculator (Meier-Kolthoff et al. 2013). The phylogenomic tree was generated on the Type (Strain) Genome Server (Meier-Kolthoff and Göker 2019) using FastME 2.1.6.1 tools (Lefort et al. 2015).

Morphological, physiological, and biochemical analysis

For morphological studies, colony properties were determined by observing the colonies of strain S3N08^T grown on R2A agar for 7 days at 28 °C. Cellular structure and flagella was visualized using transmission electron microscopy (TEM; Talos L120C; FEI). Gram reaction was performed using Color Gram 2 kit (bioMérieux). Endospore formation was observed by phase-contrast microscopy using BX53-DIC microscope (Olympus) after staining with 0.5% (w/v) malachite green (Oktari et al. 2017). Motility was evaluated in sulphide indole motility medium (SIM; Oxoid). Catalase and oxidase tests were conducted using ID Color Catalase and Oxidase Reagents (bioMérieux), respectively. The anaerobic growth was analysed by cultivating strain S3N08^T on R2A agar for 15 days at 28 °C in an anaerobic jar with an anaerobe atmosphere generation bag. The growth at various temperatures (4, 10, 15, 20, 25, 28, 35, 37, 40, and 45 °C) was assessed on R2A agar after 10 days incubation. NaCl tolerance was investigated in R2A broth formulated with different NaCl content (0–1.5%, w/v, at 0.5% intervals). Growth at various pH 0.5–10.0 (at intervals of 0.5 pH unit) was studied in R2A broth. The pH of the medium was adjusted prior autoclaving using suitable buffers (Breznak and Costilow 2007). Casein, DNA, and starch hydrolysis tests were conducted as described (Smibert 1994). Other various biochemical studies were executed using commercial kits API ZYM, API 20NE, and API 20E (bioMérieux).

Chemotaxonomic characterization

The cellular fatty acids profile was determined after cultivating target and reference strains on R2A agar at 28 °C. Fatty acids were extracted from biomass of all strains harvested at late log phase. The extraction, analysis, and identification of fatty acids were accomplished by MIDI protocol (Sasser 1990). The peptidoglycan was analyzed as described previously (Staneck and Roberts 1974). Both polar lipids and

quinones were analysed using freeze-dried cells (Collins and Jones 1981; Minnikin et al. 1984).

Results and discussion

The nearly full-length of 16S rRNA gene of strain S3N08^T was 1481 bp (NCBI nucleotide accession number MH159222). The 16S rRNA gene sequence similarity data showed that the strain S3N08^T was affiliated to the genus *Paenibacillus*, with phylogenetically closest members being *P. periandrae* PM10^T (95.6%), *P. vulneris* CCUG 53270^T (94.5%), *P. rigui* JCM 16352^T (94.1%), *P. phytorum* LMG 31458^T (93.1%), and *P. alginolyticus* DSM 5050^T (92.3%). The 16S rRNA gene sequence similarities of strain S3N08^T with all closest species were below the cut-off values of < 98.7% used for species delineation (Stackebrandt 2006; Yarza et al. 2008). Furthermore, the phylogenetic trees (ML and NJ) found that strain S3N08^T formed a clade with *P. periandrae* PM10^T with high bootstrap values (Figs. 1 and S1). Overall, the 16S rRNA gene sequence and phylogenetic results supported the assignment of strain S3N08^T as a novel species in the genus *Paenibacillus*.

The quality control and contamination test of genome sequence data assured that the generated genome sequence was valid to the strain S3N08^T. The genome size of strain S3N08^T was 8,375,108 bp. The entire genome sequence was assembled in 76 contigs with N50 value of 319,977 bp and genome coverage of 122.6x (Table S1). The annotation of genome executed in the RAST program revealed 316 subsystem features (Fig. S2). The annotated genome data showed various functional genes and proteins related to synthesis of plant growth promoting factors (Table S2). The genome of S3N08^T comprised tryptophan synthase enzymes for auxin biosynthesis (GenBank accession numbers: NHN28712.1 and NHN28713.1) (Kriechbaumer and Glawischnig 2005), ammonium transporter for ammonia assimilation (GenBank accession numbers: NHN29230.1 and NHN31246.1), and

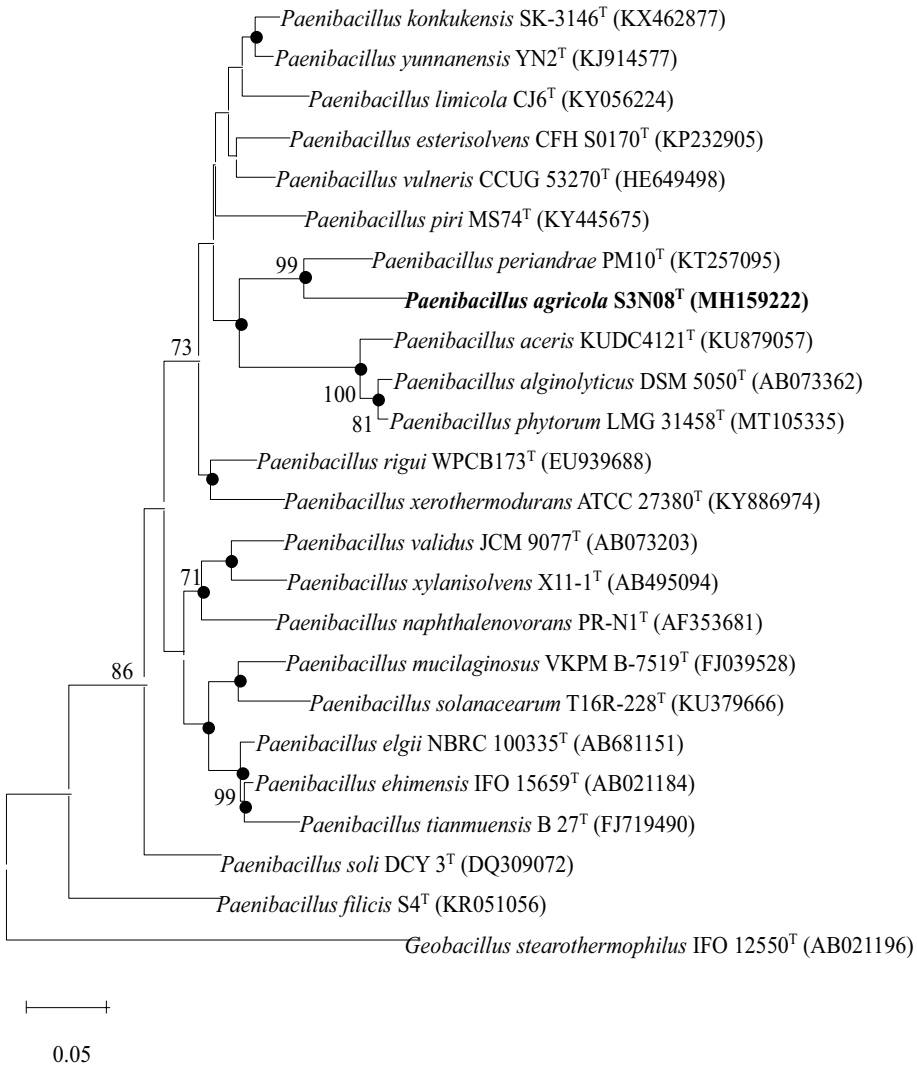

Fig. 1 Maximum likelihood tree constructed using 16S rRNA gene sequences of strain S3N08^T and closely affiliated taxa. Filled circles represent nodes recovered by both phylogenetic trees (maximum-likelihood and neighbor-joining). The numbers at the nodes indicate the percentage of 1,000 bootstrap replicates (values > 70% are only illustrated). NCBI nucleotide accession numbers are illustrated in parentheses. *Geobacillus stearothermophilus* IFO 12550^T was used as an outgroup. The scale bar represents 0.05 substitutions per nucleotide position

Table 1 Differentiating features of strain S3N08^T and phylogenetically closely related taxa

Characteristic	1	2	3	4	5*	6
Growth temperature (˚C)	10–40	15–37	15–50	15–37	15–40	15–35
Highest salt tolerance (%, w/v)	1.0	2.0	2.0	1.0	1	1
pH range	6.5–8.0	6.5–8.0	5.5–11.5	5.0–8.0	6.0–7.0	5.0–7.5
Catalase/oxidase	−/+	−/+	+/+	+/+	+/+	+/−
Nitrate reduction	−	−	−	+	+	+
Hydrolysis of						
Casein	−	+	−	−	−	−
DNA	−	−	−	−	−	−
Starch	−	w	−	+	−	−
Urea	−	+	−	+	−	−
Enzyme activity						
Alkaline phosphatase	+	+	−	−	−	−
Leucine arylamidase	−	+	+	+	w	−
Acid phosphatase	+	+	−	+	−	+
α-Galactosidase	−	−	−	+	−	+
β-Glucuronidase	−	+	–	–	−	−
α-Glucosidase	−	+	–	+	−	+
β- Glucosidase	–	+	+	+	−	+
Assimilation from						
(API 20NE and API 20E)						
D-Glucose	–	+	+	+	+	+
L-Arabinose	–	+	+	+	+	+
D-Mannose	–	+	–	+	+	−
D-Mannitol	–	–	+	+	+	+
N-acetyl- D-glucosamine	–	+	+	–	+	+
Potassium gluconate	–	–	–	+	+	−
Malic acid	–	+	+	+	+	+
D-Sucrose	–	–	–	+	ND	−
DNA G + C content (%)	45.1	52.9*	49.3*	48.3*	44.6*	47–49*

Strains: 1, S3N08^T; 2, *P. periandrae* LMG 28691^T (Menendez et al. 2016); 3, *P. vulneris* DSM 27954^T (Glaeser et al. 2013); 4, *P. rigui* KCTC 13282^T (Baik et al. 2011); 5, *P. phytorum* LMG 31458^T (Qi et al. 2021); 6, *P. alginolyticus* KACC 11445^T (Shida et al. 1997). All data were obtained in this study except the data marked with asterisk (*), which were retrieved from respective references. + positive, w weakly positive, − negative, ND data not available

iron-siderophore protein for iron acquisition (GenBank accession numbers: NHN30782.1 and NHN31881.1). These functional proteins of strain S3N08^T help to enhance the plant growth activities (Grady et al. 2016). The biosynthetic gene clusters (BGCs) assessment revealed several genes in the genome of strain S3N08^T that are responsible for putative secondary metabolites (terpene, cyclic-lactone-autoinducer, and type III polyketide synthase) (Table S3).

The DNA G + C content analyzed from genome data was 45.1%. The ANI and digital DNA–DNA hybridization (dDDH) values between strain S3N08^T and reference strains were in the ranges of 69.9–76.1% and 19.1–26.9%, respectively (Table S4). These genome relatedness data were below than the threshold values of ANI (95.0%) and dDDH (70.0%), indicating that the strain S3N08^T differs genomically from closest species of the genus *Paenibacillus* (Wayne et al. 1987; Richter and Rosselló-Móra 2009). The phylogenomic tree generated with genome sequence data assured the affiliation of strain S3N08^T with the genus *Paenibacillus*, forming clade with *P. periandrae* PM10^T, *P. phytorum* LMG 31458^T, and *P. alginolyticus* DSM 5050^T, but generating separate lineage (Fig. S3).

Cells of strain S3N08^T were Gram-stain-positive, and rod shaped with peritrichous flagella (Fig. S4). Catalase test was negative for S3N08^T and *P. periandrae* LMG 28691^T and positive for *P. vulneris* DSM 27954^T and *P. rigui* KCTC 13282^T. Oxidase test was positive for strain S3N08^T and all reference strains except *P. alginolyticus* KACC 11445^T.

Table 2 Cellular fatty acid profiles (% of totals) of S3N08^T and phylogenetically related references

Fatty acid	1	2	3	4	5*	6
Saturated						
$C_{14:0}$	2.8	2.5	1.9	2.4	1.5	1.2
$C_{16:0}$	13.2	4.8	6.9	12.0	5.9	1.7
$C_{17:0}$ cyclo	1.2	–	–	–	–	–
Unsaturated						
$C_{16:1}$ *ω7c* alcohol	TR	TR	1.1	TR	–	7.3
$C_{16:1}$ *ω11c*	TR	TR	1.2	TR	–	2.9
Branched saturated						
iso-$C_{14:0}$	2.3	5.8	3.8	3.3	4.6	4.1
iso-$C_{15:0}$	11.2	10.4	7.9	3.0	5.8	4.7
iso-$C_{16:0}$	4.2	4.8	9.9	5.7	12.6	3.4
iso-$C_{17:0}$	2.9	1.1	1.7	TR	–	1.5
anteiso-$C_{15:0}$	54.9	66.1	60.2	66.57	58.2	68.1
anteiso-$C_{17:0}$	3.1	1.5	4.2	3.0	2.5	2.1

Strains: 1, DKR-2^T; 2, *P. periandrae* LMG 28691^T; 3, *P. vulneris* DSM 27954^T; 4, *P. rigui* KCTC 13282^T; 5, *P. phytorum* LMG 31458^T (Qi et al. 2021); 6, *P. alginolyticus* KACC 11445^T. All data were obtained in this study except the data marked with asterisk (*), which were retrieved from literature. TR trace amount (< 1.0%); – not detected

Strain S3N08^T was able to grow at 10 °C and can tolerate 1.0% NaCl. Nitrate reduction was negative for S3N08^T, *P. periandrae* LMG 28691^T, *P. vulneris* DSM 27954^T and *P. alginolyticus* KACC 11445^T, but positive for *P. rigui* KCTC 13282^T and *P. phytorum* LMG 31458^T. Alkaline phosphatase and acid phosphatase were positive for strain S3N08^T, whereas most of the other enzymatic and assimilation tests were negative. Leucine arylamidase, D-glucose, L-arabinose, and malic acid were negative for strain S3N08^T, but were positive for all reference strains. Several other distinguishing phenotypic features of strain S3N08^T are presented in the species description and illustrated along with closest reference strains in Table 1. The detail enzymatic and assimilation properties obtained from API commercial test kits are provided in Table S5.

The only menaquinone detected in strain S3N08^T was menaquinone MK-7, which was consistent with other species of the genus *Paenibacillus* (Baik et al. 2011; Kämpfer et al. 2022). Phosphatidylmonomethylethanolamine (PME), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE) were major polar lipids found in strain S3N08^T. Additionally one unidentified phospholipid (PL1) was observed as minor polar lipid (Fig. S5). The polar lipids profiles were identical with other species of the genus *Paenibacillus* (Glaeser et al. 2013; Menendez et al. 2016). The predominant fatty acids of strain S3N08^T were antiso-$C_{15:0}$ (54.9%), $C_{16:0}$ (13.2%), and iso-$C_{15:0}$ (11.2%). The major fatty acids contents of strain S3N08^T were similar with closely related reference strains. However, some proportional differences were observed with minor fatty acids composition between strain S3N08^T and reference strains. Strain S3N08^T reported $C_{17:0}$ cyclo (1.2%) which was absent from reference strains (Table 2).

Taxonomic conclusion

In overall, the polyphasic taxonomic data provided in this study confirmed that the strain S3N08^T represents a novel species in the genus *Paenibacillus* for which the name *Paenibacillus agricola* sp. nov. is proposed.

Description of *Paenibacillus agricola* sp. nov.

Paenibacillus agricola sp. nov. [a.gri′co.la. L. masc. n. *ager* field, L. suff. *cola* (from L. n. *incola*) a dweller, inhabitant, L. masc. n. *agricola* field dwelling].

Cells (3.3–4.5 × 0.7–0.9 μm) are Gram-stain-positive, aerobic, rod-shaped, and motile with flagella. Colonies on R2A agar are white, convex, circular, smooth, and translucent with 1.0–1.5 mm in diameter. Cells grow at temperature 10–40 °C (optimum, 25–28 °C), at pH 6.5–8.0 (optimum, 6.5), and at 0–1.0% NaCl concentration (optimum without NaCl). Endospores are formed in a sub-terminal position. Negative for catalase and positive for oxidase tests. Aesculin is hydrolysed, but gelatine, urea, casein, DNA, and starch are not hydrolysed. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, and *β*-galactosidase. The cells assimilate D-maltose and produce acetoin. The only menaquinone is MK-7; principal polar lipids are PME, PG, and PE; and

main cellular fatty acids are antiso-$C_{15:0}$, $C_{16:0}$, and iso-$C_{15:0}$. The DNA G + C content of the type strain is 45.1%.

The type strain, S3N08^T (= KACC 19666 ^{T} = NBRC 113430 ^{T}), was isolated from an agricultural soil in Seongju-gun, Republic of Korea.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA sequence and whole genome shotgun sequence of strain S3N08^T are MH159222 and JAAOIW000000000, respectively.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-023-03578-w.

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Author contributions HL and DK designed the study. HL, DKC and OBL contributed the experimental work, data analysis, and original draft preparation. HL and DKC reviewed and finalized the manuscript. DK supervised the project.

Data availability The GenBank/EMBL/DDBJ accession number for the 16S rRNA sequence of strain S3N08^T is MH159222. The whole genome shotgun sequence of strain S3N08^T has been deposited at DDBJ/ENA/GenBank under the accession JAAOIW000000000. The version described in this paper is version JAAOIW010000000.

Declarations

Conflict of interest The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Ethical approval This study does not describe any experimental work related to human and animal.

Consent to publication All the authors have given their consent for submission and publication of this manuscript to 'Archives of Microbiology'.

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