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ORIGINAL PAPER

Paenibacillus turpanensis sp. nov., isolated from a salt lake of Turpan city in Xinjiang province, north-west China

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Abstract

Strain YIM B00363^T, a Gram-positive, aerobic, non-motile, rod-shaped, spore-forming bacterium, was isolated from saline soil samples collected from a salt lake in Xinjiang province, north-west China, and was characterized using a polyphasic approach. The optimum growth temperature was 37 °C and the optimum pH was 7.5–8.0. The major menaquinone was MK-7; anteiso-$C_{15:0}$ (53.52%), iso-$C_{15:0}$ (15.04%) and $C_{16:0}$ (12.76%) were the predominant cellular fatty acids. The diagnostic diamino acid of the cell wall peptidoglycan was meso-diaminopimelic acid. The phospholipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, unidentified phospholipids, unidentified glycolipids and unknown lipids. The DNA G + C content of the type strain was 50.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain YIM B00363^T belonged to a cluster comprising species of the genus *Paenibacillus.* The nearest relatives were *P. residui* MC-246^T and *P. senegalensis* JC66^T, with 93.2% and 92.8% gene sequence similarities, respectively. On the basis of its phenotypic characteristics and phylogenetic distinctivenes, strain YIM B00363^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus turpanensis* sp. nov. is proposed. The type strain is YIM B00363^T (= CGMCC 1.17507^T = KCTC 43184^T).

Keywords *Paenibacillus turpanensis* sp. nov. · 16S rRNA sequence analysis · Chemotaxonomy

Abbreviations

NFA	Nitrogen Fixing agar
TSA	Tryptic soy agar
DPG	Diphosphatidylglycerol
PG	Phosphatidylglycerol
PE	Phosphatidylethanolamine
PL	Unknown phospholipid
GL	Unidentified glycolipids
UL	Unknown lipids

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Li Yang and Hua-Wei Huang equally contributed to this work.

The NCBI GenBank accession number for the 16S rRNA gene sequence of strain YIM B00363^T is MT032315. The draft whole-genome sequence for YIM B00363^T has been deposited at DDBJ/ENA/GenBank under accession number WTLI00000000.

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Introduction

On the basis of the analysis of the 16S rRNA gene sequences of group 3 bacilli, the genus *Paenibacillus* was proposed by Ash et al. (1993) and then emended by Shida et al. (1997). At the time of writing, the names of more than 255 species of the genus *Paenibacillus* have been validly published (https://www.bacterio.net/paenibacillus.html). Some species had been reported to have the capacity of fixing nitrogen, such as *P. polymyxa*, *P. taohuashanense*, *P. azotofixans*, *P. sabinae*, *P. sonchi*, *P. forsythiae* and *P. sophorae* (Grau and Wilson 1962; Xie et al. 2012; Seldin et al. 1984; Ma et al. 2007; Hong et al. 2009; Ma and Chen 2008; Jin et al. 2011). Some *Paenibacillus* strains play a significant role in agriculture and industry (Seldin 2011). Previous studies have reported that microorganisms found in extreme environments can produce a variety of natural compounds and their specific mechanisms for adapting to extreme environments (Tang et al. 2002). In order to explore more multifunctional, special mechanisms and valuable microbes, we targeted halophilic microorganisms in hypersaline ecosystems. In the investigation of microbial resources of salt environment in Xinjiang province, one novel strain YIM B00363^T was isolated from the hypersaline sediment of Wuzunbulake salt lake. In this study, we report the novel strain YIM B00363^T belonging to the genus *Paenibacillus* by means of a polyphasic taxonomic study.

Methods and materials

Bacterial isolation and cultivation

Strain YIM B00363^T was isolated from saline soil samples collected from Wuzunbulake salt lake, Turpan city, Xinjiang province, by the spread plate method on Nitrogen Fixing agar (NFA) medium (0.2 g KH_2PO_4, 0.2 g $MgSO_4$, 0.2 g NaCl, 5.1 g $CaCO_3$, 10 g mannitol, 15 g agar, 1L H_2O and pH 7.0). The plates were then incubated at 37 °C for 7 days. Strain YIM B00363^T was one of the isolates that appeared on the NFA plates under aerobic condition. Single colonies were purified by transferring them onto tryptic soy agar (TSA) medium plates. It was maintained on TSA at 4 °C and as a glycerol suspension (20%, v/v) at − 80 °C.

Phylogenetic and genotypic analysis

Genomic DNA for PCR amplification was prepared using TIANamp Bacteria DNA Kit (TIANGEN BIOTECH (BEIJING) CO., LTD), according to the manufacturer's instructions. The 16S rRNA gene sequence of YIM B00363^T was amplified using the universal bacterial primers PA (5′-CAG AGTTTGATCCTGGCT-3′) and PB (5′-AGGAGGTGATCC AGCCGCA-3′), synthesized by Sangon Biotech Co. Ltd (Shanghai, PR China). The amplification programme was used as described previously (Ueda et al. 2013). The PCR products were cloned into pEASY®-T5 Zero Cloning Vector (Kit, TransGen Biolech). The sequence was determined by the Tsingke Company (Beijing, PR China). Sequence similarities to the related type strains were calculated by using the EzBiocloud (https://www.ezbiocloud.net/) (Yoon et al. 2017). Multiple alignments with sequences of the most closely relatives were carried out using the CLUSTAL X 1.8 program (Thompson et al. 1997). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou and Nei 1987), maximum parsimony (Fitch 1971) and maximum likelihood (Felsenstein 1981) tree-making algorithms using the software packages MEGA version 7.0 (Kumar et al. 2016). The stability of relationships was assessed by performing bootstrap analyses with 1000 resamplings (Felsenstein 1985).

The genome of YIM B00363^T was sequenced using a PacBio + Illumina Hiseq at Shanghai Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China). The sequenced reads were assembled using SOAPdenovo software version 2.04 (https://soap.genomics.org.cn/soapdenovo.html). The DNA G + C mol% value was obtained from the genomic sequences.

Phenotypic, physiological and biochemical characteristics

Cell morphology was examined by light microscopy (BX41; Olympus) and transmission electron microscopy (JEM-2100, JEOL) using cultivated on TSA at 37 °C for 2 days. The presence of endospores was investigated by using the phase contrast microscope (BX51M; Olympus) and transmission electron microscopy (JEM-2100, JEOL) when cells were cultivated on TSA plates at 37 °C for 10 days. Gram staining was performed by the Burke method (Nam et al. 2008) and the result was confirmed by the KOH test (Baron and Finegold 1990). Motility was analysed by the wet-mount method (Nam et al. 2008). Anaerobic growth was determined using the GasPak anaerobic system (BBL) according to the manufacturer's instructions. Growth at different temperatures (4, 10, 15, 20, 25, 30, 35, 37, 40, 45, 50 and 55 °C) and NaCl concentrations (0–10%, w/v, at 1% intervals) were examined by using TSA medium at 37 °C for 14 days. The ability of the strain to grow at different pH values (4.0–10.0, at 0.5 intervals by using the buffer system described by Tang et al. 2010). Catalase activity was determined by production of bubbles after adding 3% H_2O_2 to the tested bacteria (Tarrand and Gröschel 1982). Tests for hydrolysis of gelatin, starch, Tween 20, 40 and 80, urease activity, nitrate reduction, and

hydrogen disulphide production were determined by using traditional methods (Dong and Cai 2001). Carbon sources utilization and chemical sensitivity were determined by using BIOLOG GEN III MicroPlate. Using API ZYM systems tested enzyme activities. Acid production was detected by 50CH systems. Additional biochemical tests were performed using two Gallery systems (bioMérieux): API 20 NE and API 20 E according to the manufacturer's instructions. Antibiotic susceptibility tests were performed using discs impregnated with various antimicrobial compounds.

Chemotaxonomic characteristics

Biomass for chemical and molecular studies was obtained by cultivation in TSA without agar (pH 7.0) at 37 °C and collected when the bacteria reached their mid-exponential phase. Analyses of diaminopimelic acid in the cell wall and sugars of whole-cell hydrolysates were performed according to the procedures described by Lechevalier and Lechevalier (1970) and Tang et al. (2009). Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer's instructions. Fatty acid methyl esters were analysed by using the Microbial Identification software package (Sherlock Version 6.1; MIDI databaseTSBA6) (Sasser 1990). The respiratory quinones of YIM B00363^T were extracted from lyophilized cells (Collins et al. 1977), purified by TLC and then analysed by HPLC according to the methods of Xie and Yokota (2003). Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures described by Collins and Jones (1980) and Minnikin et al. (1979).

Results and discussion

Molecular phylogenetic analysis

An almost complete 16S rRNA gene sequence (1536 bp) of strain YIM B00363^T was generated. YIM B00363^T was most moderately related to *P. hodogayensis* SG^T (Takeda et al. 2005) and *P. ginsengarvi* Gsoil 139^T (Yoon et al. 2007) with 93.8% sequence similarity, which is far below 97%. The available data indicate that organisms having less than 97.0% 16S rRNA gene sequence similarity will not exhibit more than 60% reassociation, irrespective of the hybridization method applied (Stackebrandt and Goebel 1994; Keswani and Whitman 2001). Therefore, neither wet lab nor in-silico DNA-DNA hybridization were carried out. Meanwhile, these strains clustered in the genus *Paenibacillus* stably after phylogenetic analyses of the 16S rRNA gene sequences (Fig. 1, S1, S2). The NJ tree clustered YIM

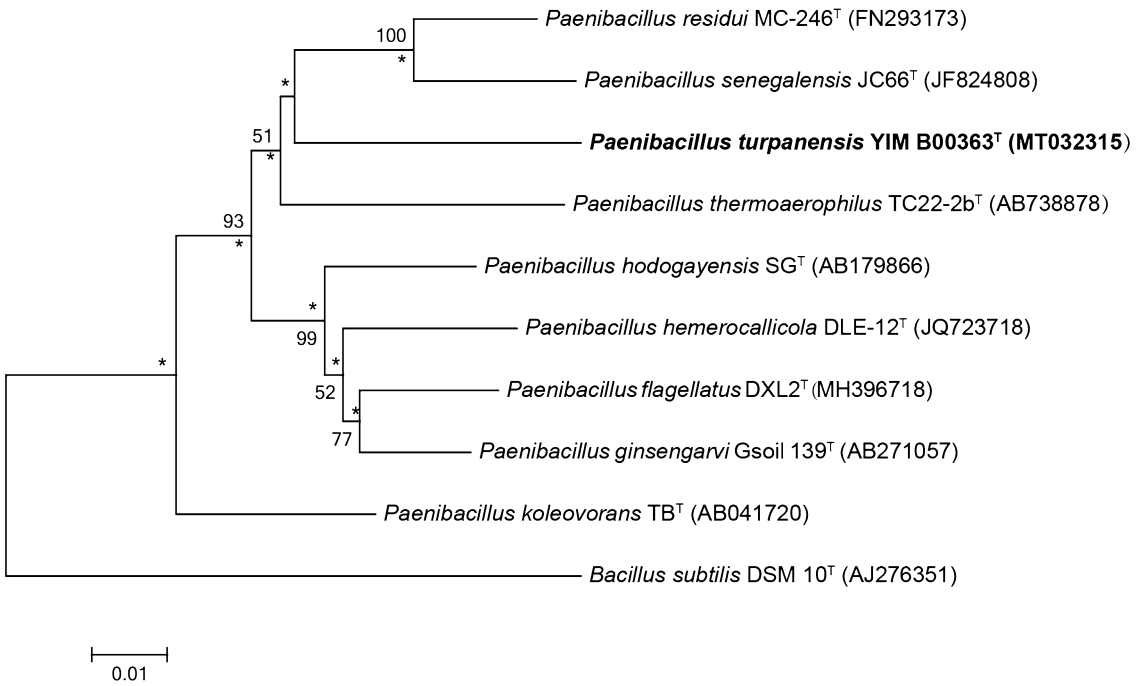

Fig. 1 Neighbour-joining tree based on 16S rRNA gene sequences showing the relationships between strain YIM B00363^T and some members of the genus *Paenibacillus*. Bootstrap values (> 50%) based on 1000 replicates are shown at the branch nodes. Asterisks indicate that the corresponding branches were also recovered in trees generated with the maximum parsimony and maximum likelihood methods. *Bacillus subtilis* DSM 10^T was used as an outgroup. Bar, 1% sequence divergence

B00363^T with *P. residui* MC-246^T (93.2%), *P. senegalensis* JC66^T (92.8%) and *P. thermoaerophilus* TC22-2b^T (92.8%) (Fig. 1). The ML and MP trees showed similar results (Figs S1 and S2). According to 16S rRNA gene sequence similarities and topology of the phylogenetic trees, the literature data of above five strains, *P. hodogayensis, P. ginsengarvi, P. residui, P. senegalensis* and *P. thermoaerophilus,* were selected for comparison in this study. The DNA G + C content of strain YIM B00363^T was 50.4 mol%. These data supported the finding that strain YIM B00363^T represents a different genomic species of the genus *Paenibacillus.*

Phenotypic, physiological and biochemical characteristics

YIM B00363^T was Gram positive, aerobic, rod shaped without peritrichous flagella (Fig S3a), swollen by ellipsoidal spores and approximately 2–3 μm long and 0.5–1.5 μm wide (Fig S3b). It could grow at 20–45 °C (optimum at 37 °C), at pH 6.0–9.5 (optimum at 7.5–8.0) and in the presence of 0–5% (w/v) NaCl (optimum without NaCl). Other physiological characteristics are given in Table 1, Table S1 and in the species description.

Chemotaxonomic characteristics

Amino acids contained *meso*-diaminopimelic acid as the cell wall diamino acid, with glucose (11.2%), galactose (10.8%), xylose (12.7%), arabinose (20%) and fucose (18.1%) as the major whole-cell sugars (> 10%). The predominant menaquinone was MK-7. The polar lipids were phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), unidentified phospholipids (PL), unidentified glycolipids (GL) and unknown lipids (UL). (Fig S4). The cellular fatty acid profile of strain YIM B00363^T contained anteiso-$C_{15:0}$ (53.52%), iso-$C_{15:0}$ (15.04%) and $C_{16:0}$ (12.76%) as major fatty acids (> 10%), and $C_{14:0}$ (5.62%), iso-$C_{16:0}$ (3.66%), anteiso-$C_{17:0}$ (2.07%), iso-$C_{14:0}$ (2.00%), iso-$C_{17:0}$ (1.95%) and $C_{12:0}$ (1.52%) as minor fatty acids. Compared with the data of other two reference strains, iso-$C_{16:0}$ was minor fatty acid of YIM B00363^T, but major fatty acid (> 10%) of others (Table 2).

All chemotaxonomic data conform to the characteristics of the genus *Paenibacillus,* which was described by Logan et al. (2009) and Tindall et al. (2010). However, there are some differences between strain YIM B00363^T and four reference strains, for example, the content of iso-$C_{16:0}$. Based on the phenotypic, genotypic, phylogenetic and chemotaxonomic data presented here, strain YIM B00363^T should belong to the genus *Paenibacillus*. Thus, it represents a novel species, for which the name *Paenibacillus turpanensis* sp. nov. is proposed.

Description of *Paenibacillus turpanensis* sp. nov.

Paenibacillus turpanensis (tur.pan.en'sis. N.L. masc. adj. *turpanensis* relating to Turpan, the name of a city in Xinjiang, China, the geographical origin of isolation of the type strain).

Paenibacillus turpanensis is Gram stain-positive, nonmotile, aerobic, rod-shaped, spore-forming bacterium. Cells are rods with a width of 0.5–1.5 μm and length of 2–3 μm. It can grow at 20–45 °C (optimum 37 °C), at pH 6–9.5 (optimum pH 7.5–8) and in the presence of 0–5% (w/v) NaCl (optimum without NaCl). It is positive for catalase activity, nitrate reduction, aesculin ferric citrate, hydrolysis of starch, and Tween 20, 40 and 80 and negative for production of H_2S, production of indole, Voges–Proskauer test, hydrolysis of gelatin, L-arginine and urea. D-glucose, L-arabinose and maltose are assimilated. For enzyme activities (API ZYM system), it is positive for alkaline phosphatase, esterase C4, esterase lipase C8, lipase C14, acid phosphatase, naphthol-*AS-BI*-phosphohydrolase, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase. For the utilization of carbon sources (API Biolog GEN III systems), the following substrates are utilized for growth: dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, Stachyose, D-raffinose, *α*-D-lactose, D-melibiose, D-salicin, *N*-acetyl-D-glucosamine, *α*-D-glucose, D-fructose, D-galactose, L-rhamnose, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, glucuronamide and acetic acid as substrates. With API 50CH, acid is produced from glycerol, L-arabinose, D-ribose, D-xylose, methyl-*βd*-xylopyranoside, D-galactose, D-glucose, fructose, D-mannose, L-rhamnose, methyl-*αd*-glucopyranoside, aesculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, D-raffinose, amidon (starch), glycogen, D-gentiobiose, D-turanose, D-tagatose and potassium 5-ketogluconate and weakly produced from L-sorbose, amygdalin, D-lyxose and L-fucose. It is sensitive to kanamycin (30 μg), gentamicin (10 μg), neomycin (30 μg), amoxicillin/clavulanic acid (10 μg), erythromycin (5 μg), carbenicillin (100 μg), chloramphenicol (30 μg), tetracycline (30 μg), novobiocin (5 μg) and streptomycin (300 μg). The whole-cell hydrolysates contain meso-diaminopimelic acid as the cell wall diamino acid. Glucose, galactose, xylose, arabinose and fucose are the major whole-cell sugars. The polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, unidentified phospholipids, unidentified glycolipids and unknown lipids. The predominant menaquinone is MK-7. Anteiso-$C_{15:0}$ (53.52%), iso-$C_{15:0}$ (15.04%) and $C_{16:0}$ (12.76%) are the major fatty acids. The DNA G + C content of the type strain is 50.4 mol%.

The type strain is YIM B00363^T (= CGMCC 1.17507^T = KCTC 43184^T), isolated from the saline soil

Table 1 Phenotypic characteristics of strain YIM B00363^T and the type strains of closely related species of the genus *Paenibacillus*

Characteristic	1	2	3	4	5
Growth temperature (C)					
Range	20–45	25–58	25–50	20–40	18–45
Optimum	37	50–55	37	30	37
Growth pH					
Range	6.0–9.5	6.0–9.0	7.0–9.0	6.2–9.2	5.0–8.5
Optimum	7.5–8.0	7.0–8.0	7.0	8.0	6.5–7.0
Growth NaCl range (%, w/v)	0–5	0–3.5	0–2	0–5	0–2
Nitrate reduction	+	−	−	−	−
Voges-Proskauer test	−	w	−	−	+
Hydrolysis of					
Aesculin	+	−	+	−	−
Tween 80	+	NG	−	−	−
Starch	+	+	−	−	−
Gelatin	−	−	−	−	−
Acid production from					
Glycerol	+	−	+	+	+
D-Arabinose	−	−	−	−	+
L-Arabinose	+	−	−	−	+
D-Ribose	+	−	+	W	+
D-Xylose	+	−	+	−	+
D-Galactose	+	−	−	−	−
Fructose	+	w	+	−	−
D-Mannose	+	w	−	−	−
L-Sorbose	w	−	−	−	−
L-Rhamnose	+	w	−	−	−
D-Mannitol	−	+	−	+	−
N-Acetylglucosamine	−	+	−	w	+
Arbutin	−	−	+	−	+
D-Lactose	+	−	−	w	−
D-Melezitose	+	−	−	−	−
D-Raffinose	+	+	−	−	+
Starch	+	+	−	−	−
Glycogen	+	+	−	−	−
Gentiobiose	+	+	+	−	−
D-Lyxose	w	−	−	−	−
D-Tagatose	+	−	−	−	−
L-Fucose	w	−	−	−	−
potassium 5-Ketogluconate	+	w	−	w	−
Assimilation of					
L-arabinose	+	−	NR	−	−
D-mannitol	−	+	−	+	+
N-Acetylglucosamine	−	+	−	+	+
Maltose	+	NR	+	+	−
Potassium gluconate	−	−	−	+	+
DNAG + Ccontent (mol%)	50.4	59.1	49	55	48.1
Predominant menaquinone	MK-7	MK-7	MK-7	MK-(H2)	MK-7

Strains: (1) YIM B00363^T; (2) *P. thermoaerophilus* TC22-2b^T (Ueda et al. 2013); (3) *P. residui* MC-246^T (Vaz-Moreira et al. 2010); (4), *P. hodogayensis* SG^T (Takeda et al. 2005; Dai et al. 2019); (5) *P. ginsengarvi* Gsoil 139^T (Yoon et al. 2007; Dai et al. 2019). The reference data for *P. senegalensis* JC66T was not cited because most traits were not recorded

+ positive, − negative, *w* weakly positive, *NG* no growth, *NR* no reported

Table 2 Cellular fatty acid profile of strain YIM B00363^T and type strains of phylogenetically related species of the genus *Paenibacillus*

Fatty acid	1	2	3	4	5
Straight-chain saturated					
$C_{10:0}$	< 1	–	–	–	–
$C_{12:0}$	1.52	1.6	–	–	–
$C_{14:0}$	5.62	3.2	< 1	1.3	< 1
$C_{15:0}$	–	3.2	2.61	–	–
$C_{16:0}$	**12.76**	**25.5**	6.83	**13.4**	8.3
$C_{17:0}$	–	< 1	1.43	1.6	< 1
$C_{18:0}$	–	1.7	–	< 1	< 1
Branched saturated					
iso-$C_{13:0}$	< 1	–	–	–	–
iso-$C_{14:0}$	2.0	1.4	< 1	4.1	6.1
iso-$C_{15:0}$	**15.04**	3.0	9.44	**19.6**	5.1
iso-$C_{16:0}$	3.66	**23.6**	**15.75**	**11.8**	**27.8**
iso-$C_{17:0}$	1.95	2.3	**11.05**	5.3	1.4
iso-$C_{18:0}$	–	< 1	< 1	–	–
anteiso-$C_{15:0}$	**53.52**	**21.5**	**36.65**	**37.1**	**44.4**
anteiso-$C_{17:0}$	2.03	**11.2**	**14.86**	4.0	4.7
Unsaturated					
$C_{16:1}\omega 7c$	–	< 1	–	< 1	< 1
$C_{18:1}\omega 9c$	< 1	–	–	–	–

Strains: (1) YIM B00363^T; (2) *P. thermoaerophilus* TC22-2b^T (Ueda et al. 2013); (3) *P. residui* MC-246^T (Vaz-Moreira et al. 2010); (4) *P. hodogayensis* SG^T (Dai et al. 2019); (5) *P. ginsengarvi* Gsoil 139^T (Dai et al. 2019)

– not detected and not reported

of Turpan city in Xinjiang province, north-west China. The GenBank accession numbers of the 16S rRNA gene and the genome sequence of YIM B00363^T are MT032315 and WTLI00000000, respectively.

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Author contributions LY and H-WH: carried out the data analysis, part of chemical classification and wrote the manuscript. YW, Y-RK and MY: performed the polyphasic taxonomy except chemical classification. YL, X-QW and G-FZ: prepared the experiments and isolated the novel strain. W-YZ and S-KT: designed the separation medium and directed the classification and takes full responsibility for the final submission. All the authors reviewed and approved the final version of the paper.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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