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ORIGINAL PAPER

Roseicella aquatilis sp. nov., isolated from freshwater lake

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Abstract
A novel Gram-stain-negative, non-motile, ellipsoidal-shaped, red-pigmented, facultatively aerobic strain designated NE82^T was isolated from mud sample from Jiugongli Lake in Inner Mongolia Autonomous Region, China. Optimal growth occurred at 28–33 °C (range 15–42 °C) and pH 7.0–7.5 (range 5.5–8.5) with 0% (w/v) NaCl (range 0–1.0%). Cells of strain NE82^T were 0.4–0.9 µm in diameter, catalase-positive and oxidase-negative. Q-10 was the sole respiratory quinone and the major cellular fatty acids (> 10%) in strain NE82^T were summed feature 8 ($C_{18:1}$ $\omega 7c$ and $C_{18:1}$ $\omega 6c$). The polar lipids of strain NE82^T were phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, an unidentified aminophospholipid and four unidentified phospholipids. The G+C content of the genomic DNA was 72.0 mol%. Based on the 16S rRNA gene sequence, strain NE82^T showed the highest similarity (97.2%) to *Roseicella frigidaeris* DB1506^T within the family *Acetobacteraceae*, thus representing a novel species of the genus *Roseicella*, for which the name *Roseicella aquatilis* sp. nov. is proposed. The type strain is NE82^T (= KCTC 62412^T = MCCC 1H00292^T).

Keywords *Roseicella aquatilis* sp. nov. · 16S rRNA gene · Phylogenetic analysis

Introduction

At the time of writing, there is only one species with validly published name in the genus *Roseicella*, which is *R. frigidaeris* (Khan et al. 2019). The genus belongs to the family *Acetobacteraceae* in the order *Rhodospirillales*. In this paper, a novel catalase-positive, red-pigmented, facultatively aerobic strain, NE82^T, was characterized. Based on phenotypic, chemotaxonomic and phylogenetic analyses, strain NE82^T was classified into the genus *Roseicella*, with the name *Roseicella aquatilis* sp. nov..

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Materials and methods

Bacterial isolation and cultivation

For the study of bacterial diversity from the fresh lake, a sample was obtained from mud in Jiugongli Lake in Inner Mongolia Autonomous Region, China (106° 49.721′ E, 40° 32.476′ N). The sample was serially diluted to 10^{-4} with sterile distilled water, and 0.1 ml aliquots of each dilution were spread onto R2A agar (Difco). After the incubation at 30 °C for 5 days, a red-pigmented colony was obtained and designated as NE82^T, which was stored at − 80 °C in sterile 15.0% (v/v) glycerol supplemented with 1.0% (v/v) NaCl. The type strains *R. frigidaeris* JCM 32945^T, *Paracraurococcus ruber* JCM 9931^T and *Dankookia rubra* JCM 30602^T, obtained from the Japan Collection of Microorganisms (JCM), were the most closely related type strains to strain NE82^T and used for comparative physiological and chemotaxonomic characterizations. All closely related type strains were cultured under the same conditions as strain NE82^T.

16S rRNA gene sequence and phylogenetic analysis

The 16S rRNA gene was amplified by PCR using two universal primers 27F and 1492R (Weisburg et al. 1991). The

amplification products purified ligated to the vector pGM-T (Tiangen). Sequencing reactions were carried out using an ABI BigDye 3.1 Sequencing Kit (Applied Biosystems, Waltham, MA, USA) and an automated DNA sequencer (model ABI 3730; Applied Biosystems). Similar sequences of the nearly complete 16S rRNA gene sequence (1433 bp, MG385132.1) of strain NE82^T were searched for using the BLAST algorithm. Identification of phylogenetic relationships and calculation of pairwise 16S rRNA gene sequence similarities used the NCBI BLASTN (https://blast.ncbi.nlm.nih.gov/) as well as the EzTaxon server (Kim et al. 2012). Sequences were aligned using the alignment program, CLUSTAL_X (version 1.81) (Thompson et al. 1997). Phylogenetic trees were reconstructed based on the phylogenetic analysis using the neighbour-joining (Saitou and Nei 1987), the maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971) methods implemented in MEGA (version 7.0) (Kumar et al. 2016). Bootstrap values were determined based on 1000 replicates for each of the three methods. In addition, full-length 16S rRNA gene sequence extracted from the genome assembly was compared with the 16S rRNA gene sequence obtained by Sanger method.

Genomic analysis

The genomes of strain NE82^T, *P. ruber* JCM 9931^T and *D. rubra* JCM 30602^T were sequenced by Beijing Novogene Biotechnology Co., Ltd (Beijing, China) using Illumina HiSeq. The genome of *R. frigidaeris* JCM 32945^T was obtained from NCBI (QLIX00000000). The sequencing depth of coverage was 100×. The gene content was annotated by the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al. 2016; Haft et al. 2018) and the genes included in the metabolic pathways were analyzed by KEGG Database (Kanehisa et al. 2016). The integrity of 16S rRNA gene was checked by ContEst16S (Lee et al. 2017). To determine if the strain NE82^T was a new species, the average nucleotide identity (ANI) was calculated by Web service (https://www.ezbiocloud.net/tools/ani) (Rodriguezr and Konstantinidis 2016) between strain NE82^T and the closely type strains. Besides, also the digital DNA-DNA hybridisation (dDDH) was calculated by GGDC (http://ggdc.dsmz.de/ggdc.php/) (Meier-Kolthoff et al. 2013).

Morphological, physiological and biochemical analyses

Cells of strain NE82^T grew on R2A at 30 °C for 4 days were used for morphological and physiological tests. Cell morphology and size were examined by transmission electron microscopy (JEM-1200EX), and light microscopic examinations were performed using an E600 Nikon light microscope (Tokyo, Japan) to supplement. Gram reactions of strain NE82^T were assessed as described by Smibert and Krieg (1994) and the examination of motility was carried out according to the hanging-drop method (Bernardet et al. 2002). The temperature range for growth of strain NE82^T was evaluated at 0, 4, 15, 25, 28, 30, 33, 37, 40, 42 and 45 °C on R2A agar and results were recorded every 12 h. The effects of NaCl concentration on growth was examined on R2A medium supplemented with different concentrations of NaCl (0%, 0.5%, and 1–10% in 1% increments, w/v). The pH range for growth was determined using modified R2A broth at pH 5.5–10.0 (in 0.5 unit intervals). The different buffers [MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5) and CAPSO (pH 9.0, 9.5 and 10.0) (Sangon)] were added to different levels at concentrations of 20 mM, and the pH of the medium was adjusted by adding 1 M HCl or NaOH before autoclaving. Then ranges of pH were investigated on 96-well microplates by measuring the OD_{600}.

Growth under anaerobic conditions was determined after cultivation in an anaerobic chamber on modified R2A, with or without 1% (w/v) KNO_3 for at least 2 weeks at 30 °C. The modified R2A in test tubes supplemented with 1% (v/v) nitrate was used for test of the reduction of nitrate. The inoculated and uninoculated test tubes were all placed in aerobic and anaerobic conditions at 30 °C for 7 days.

Oxidase activity was tested using the bioMerieux Oxidase Reagent kit according to the manufacturer's instructions, and catalase activity was detected by measuring the production of oxygen bubbles in a 3% (v/v) aqueous hydrogen peroxide solution. The hydrolysis tests of starch, lipids, cellulose and alginate, starch, lipids, Tweens 20, 40, 60 and 80 were determined as described by Smibert and Krieg (1994). The pigments of strain NE82^T were extracted with 3 ml acetone/methanol (7:2, v/v) per gram of wet pellet and the absorption spectra were determined at 300–800 nm with a Hitachi U-2910 spectrophotometer. Additionally, pigments were also extracted from cell pellets as described in the article (Cha et al. 2011). Susceptibility to antibiotics was investigated on R2A agar at 30 °C for 7 days using filter-paper discs containing various antibiotics as described previously (Du et al. 2014) and according to procedures outlined by the Clinical and Laboratory Standards Institute (CLSI 2018). Additional physiological and biochemical characteristics were assessed using the API 20E, API ZYM, and API 50CHB strips (bioMérieux, Marcy-l'Étoile, France) and the Biolog GEN III System according to the manufacturers' recommendations, with the exception that the NaCl concentration was adjusted to 3% (w/v).

Chemotaxonomic properties

For the determination of fatty acids, cells of strain NE82^T and closely related type strains were cultured on the R2A

agar at 30 °C and harvested after 4 days for growth. According to the standard protocol of MIDI (Sherlock Microbial Identification System, version 4.5), fatty acids were extracted, then methylated and analysed by an Agilent 6890 N gas chromatograph. Cellular fatty acids were identified using the TSBA40 database of the microbial identification system (Sasser 1990).

For the polar lipids analysis, three strains were cultured in the liquid medium at 30 °C and harvested after 4 days. Polar lipids were extracted from cells and separated via two-dimensional silica gel thin-layer chromatography (TLC). The total lipid materials were detected using molybdatophosphoric acid, and the functional groups were determined using spray reagents specific for particular functional groups (Tindall et al. 2007). Polar lipids were determined using 2D TLC (Minnikin et al. 1984).

To analyse respiratory quinones, strain NE82^T grew in R2A liquid medium at 30 °C for 4 days was collected and freeze-dried. The procedures were carried out according to the methods described by Minnikin et al. (1984), and quinone type was separated by HPLC (Hiraishi et al. 1996).

Results and discussion

16S rRNA gene sequence and phylogenetic analysis

The 16S rRNA gene sequence extracted from the genome assembly was 1491 bp (MG385132.2), which included the 16S rRNA gene sequence acquired from PCR and clone. Based on 16S rRNA gene sequences, *R. frigidaeris* JCM 32945^T (97.2% sequence similarity), *P. ruber* JCM 9931^T (96.4% sequence similarity) and the following *D. rubra* JCM 30602^T (95.8% sequence similarity) were the most closely related type strains to NE82^T. In the neighbor-joining phylogenetic tree (Fig. 1), the strain NE82^T formed a cluster with *R. frigidaeris* JCM 32945^T, the only one species of the genus *Roseicella*. Phylogenetic trees were also constructed using the maximum-likelihood and maximum-parsimony algorithms (Figs. S1, S2, available with the online Supplementary Information), which supported the result above.

Genomic analysis

The draft genome sequence of strain NE82^T was 5.9 Mb in length and produced 238 contigs. Contigs varied in length from 211 to 414,331 bp (N50 = 166,730 bp). The G+C content of the genomic DNA of strain NE82^T was 72.0 mol%. The draft genome of strain NE82^T contained 5532 genes, one 16S rRNA and 55 tRNAs annotated by the NCBI Prokaryotic Genome Annotation Pipeline. KEGG pathway annotation predicted that strain NE82^T could degrade aromatic hydrocarbon, such as benzoate. Besides, the result of prediction also showed that NE82^T could transform thiosulfate to sulfate via thiosulfate oxidation by SOX complex, which contributed to the sulfur cycle on Earth. Moreover, the draft genome sequence of *P. ruber* JCM 9931^T and *D. rubra* JCM 30602^T were also sequenced with the 100X sequencing depth, the lengths were 7.2 Mb and 7.8 Mb, respectively. *R. frigidaeris* JCM 32945^T produced 87 contigs with an N50 of 187,085 bp (Khan et al. 2019), *P. ruber* JCM 9931^T produced 786 contigs (203–188,134 bp) while *D. rubra* JCM 30602^T produced 458 contigs (202–363,070 bp). N50 of *P. ruber* JCM 9931^T and *D. rubra* JCM 30602^T were 22,677 bp and 79,244 bp.

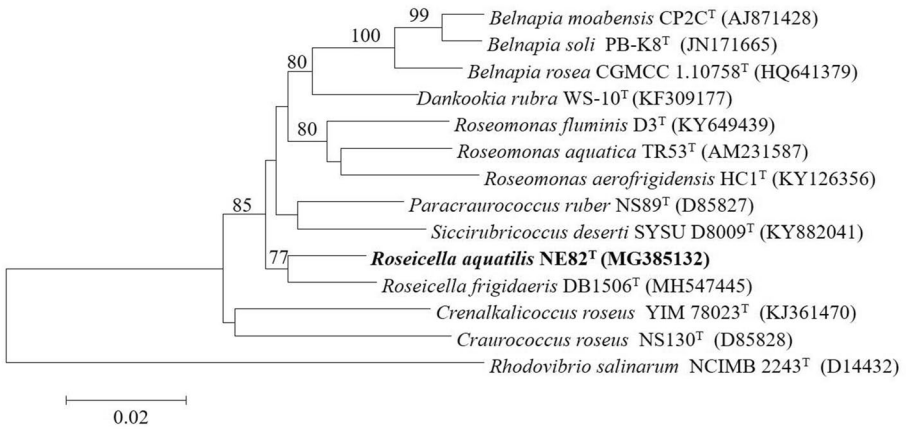

Fig. 1 Phylogenetic tree constructed with 16S rRNA gene sequence analysis using the neighbor-joining method showing the position of strain NE82^T among related taxa. The strain characterized in this study is shown in bold type. GenBank accession numbers of 16S rRNA gene sequences are given in parentheses. Numbers at nodes are bootstrap values (> 70%) based on neighbor-joining analysis of 1000 resampled datasets. *Rhodovibrio salinarum* NCIMB 2243^T (D14432) was used as an out group. The scale bar indicates 0.0100 substitutions per nucleotide position

The ANI values between strain NE82^T and *R. frigidaeris* JCM 32945^T, *P. ruber* JCM 9931^T, *D. rubra* JCM 30602^T were 83.3%, 84.1% and 83.4%, respectively, while the corresponding dDDH values were 27.2%, 27.6% and 26.8%, respectively. According to the proposed and generally accepted species boundary, ANI value < 95% or dDDH value < 70% means that the strain is a novel species (Rodriguezr and Konstantinidis 2016; Meier-Kolthoff et al. 2013), which proved strain NE82^T was a novel species distinguishable from the closely related type strains.

Morphological, physiological and biochemical characterizations

Cells of strain NE82^T were ellipsoidal, which were found to be Gram-stain-negative, non-motile and facultatively aerobic. Colonies were circular and measured about 1.0 mm in diameter on R2A agar. Growth of strain NE82^T was found to occur between 15 and 42 °C, pH 5.5–8.5 and in the presence of 0–1.0% (w/v) NaCl.

Strain NE82^T could not grow under anaerobic conditions, with or without 1% (w/v) KNO_3, after 2 weeks' cultivation in an anaerobic chamber on R2A at 30 °C. The test for the reduction of nitrate was positive. The hydrolysis of Tweens 20, 40, 60 were detected, but starch, casein, cellulose, alginate and Tween 80 were not hydrolysed, while the most close related strain *R. frigidaeris* JCM 32945^T could not hydrolyse Tween 20. These results were same with *P. ruber* JCM 9931^T (Saitoh et al. 1998), but displayed little difference with *D. rubra* JCM 30602^T, which could hydrolysing Tween 80 (Kim et al. 2016). Carotenoid was present in strain NE82^T, *R. frigidaeris* JCM 32945^T (Khan et al. 2019) and *P. ruber* JCM 9931^T, while *P. ruber* JCM 9931^T also contained Bacteriochlorophyll *a* (Saitoh et al. 1998). Strain NE82^T was found to be susceptible to carbenicillin (100 μg), chloramphenicol (30 μg), penicillin (10 μg), tetracycline (30 μg), ampicillin (10 μg), kanamycin (30 μg), cefotaxime sodium (30 μg), erythromycin (15 μg), streptomycin (10 μg), tobramycin (10 μg), rifampicin (5 μg), gentamicin (10 μg), but resistant to norfloxacin (30 μg), vancomycin (30 μg), lincomycin (2 μg), clindamycin (30 μg). Despite strain NE82^T showed many common traits with *R. frigidaeris* JCM 32945^T, it could be distinguished from this strain by a number of biochemical characteristics, such as the negative reaction of oxidase reaction, valine arylamidase, gelatinase and Voges–Proskauer reaction, the positive utilization of urease and citrate. Further comparative analyses of strain NE82^T and its related types strains are summarised in Table 1 and Table S1.

Chemotaxonomic properties

The predominant cellular fatty acids of strain NE82^T were summed feature 8 ($C_{18:1}$ *ω7c* and $C_{18:1}$ *ω6c*) (71%) and $C_{16:0}$ (7.9%), which also appeared in the *R. frigidaeris* JCM 32945^T, *P. ruber* JCM 9931^T and *D. rubra* JCM 30602^T. $C_{18:1}$ 2-OH was another major fatty acid in *R. frigidaeris* JCM 32945^T (10.0%). In addition, *D. rubra* JCM 30602^T had another two types of fatty acid as main fatty acids, which were summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) (18.5%) and $C_{16:0}$ (14.0%). The detailed fatty acid compositions of strain NE82^T and its closely related type strains are shown in Table S2.

The major polar lipids of strain NE82^T were phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylglycerol (PG), an unidentified aminophospholipid (APL1) and an unidentified phospholipid (PL1) (Fig. S5). While the phosphatidylcholine (PC) was not detected in *R. frigidaeris* JCM 32945^T, and there were four unidentified lipids (L1, L2, L3, L4) and six unidentified aminolipids (AL1, AL2, AL3, AL4, AL5, AL6) in *R. frigidaeris* JCM 32945^T (Khan et al. 2019). Besides, NE82^T cells had also three another unidentified phospholipids (PL2, PL3 and PL4). The detailed comparisons are listed in Table 1. The sole menaquinone was Q-10, which was same with the closely related type strains.

According to all these results of phenotypic, biochemical and physiological analyses, together with the phylogenetic differences, strain NE82^T can be assigned to the genus *Roseicella* within the family *Acetobacteraceae*, as representing a novel species, for which the name *Roseicella aquatilis* sp. nov. is proposed.

Description of *Roseicella aquatilis* sp. nov.

Roseicella aquatilis (a.qua'ti.lis. L. fem. adj. *aquatilis* living, growing or found in, or near, water, aquatic).

Cells are ellipsoidal, approximately 0.4–0.9 μm in diameter, Gram-stain-negative, non-motile and facultatively aerobic. Colonies are red-pigmented, circular and 1.0 mm in diameter after incubation at 30 °C for 4 days. Cells are able to grow at 15–42 °C, pH 5.5–8.5 and in the presence of 0–1.0% (w/v) NaCl and its optimal growth is at 28–33 °C, pH 7.0–7.5, with 0% NaCl. Cells can reduce nitrate and are catalase positive, but oxidase negative. Tweens 20, 40, 60 are hydrolysed, but starch, casein, cellulose, alginate, and Tween 80 are not hydrolysed. Cells can produce alkaline phosphatase, esterase (C4), esterase lipase (C8) (weakly), naphthol-AS-BI-phosphohydrolase, acid phosphatase (weakly) and leucine arylamidase. Positive for citrate utilization, urease. Acids are produced from L-arabinose (weakly), D-Ribose (weakly), D-xylose (weakly), L-xylose (weakly), L-rhamnose (weakly), potassium gluconate (weakly),

Table 1 Differential characteristics of strain NE82^T and the closely related type strains. Strains: 1, NE82^T; 2, *Roseicella frigidaeris* JCM 32945^T; 3, *Paracraurococcus ruber* JCM 9931^T; 4, *Dankookia rubra* JCM 30602^T

Characteristic	1	2	3	4
Cell size (μm)	0.4–0.9	0.8–1[a]	0.8–1.5[b]	2[c]
Colony color	Red	Pink-rose	Red	Red
Optimal conditions for growth				
NaCl (%, w/v)	0	0[a]	0[b]	0[c]
Temperature (°C)	28–33	30[a]	30–34[b]	28[c]
pH	7.0–7.5	7.0[a]	6.6–6.8[b]	7.0[c]
Oxidase reaction	−	+[a]	+[b]	+[c]
Hydrolysis of Tween 80	−	−	−	+
DNA G+C content (mol%)	72.0	72.5[a]	71.0[b]	72.7[c]
Enzyme activities (API ZYM and 20E)				
Arginine dihydrolase	−	−	+	−
Citrate utilization	+	−	+	+
Voges–Proskauer reaction	−	+	−	−
Gelatinase	−	+	−	−
Valine arylamidase	−	+	−	−
Urease	+	−	−	+
Arabinose	−	−	+	−
Acid production from (API 50 CHB)				
D-Arabinose	−	−	+	+
Glycerol	−	+	−	w
L-Arabinose	w	−	+	+
D-Xylose	w	−	+	w
Methyl-βD-xylopyranoside	−	+	−	−
L-Sorbose	−	+	−	−
Esculin ferric citrate	−	+	−	−
D-Tagatose	−	+	−	−
Potassium gluconate	w	+	−	−
Potassium 2-ketogluconate	−	+	−	−
Oxidation of (BIOLOG GEN III)				
α-D-Glucose	−	+	+	−
D-Salicin	−	+	−	−
D-Fucose	+	−	+	+
L-Fucose	+	−	+	+
Myo-Inositol	−	+	−	−
L-Glutamic acid	+	−	+	−
D-Fructose-6-PO_4	+	+	−	−
D-Glucose-6-PO_4	+	−	−	−
L-Serine	+	−	−	−
Mucic acid	+	−	+	+
D-Saccharic Acid	+	−	+	+
α-Hydroxy-butyric acid	−	+	+	−
Acetic acid	+	−	+	+
Citric acid	+	−	+	+
D-Galactose	−	−	+	+
Quinic acid	−	−	+	+
Bromo-Succinic Acid	−	−	+	+
ρ-Hydroxy-phenylacectic acid	+	−	−	−
Glycyl-L-Proline	−	−	−	+
D-Lactic acid methyl ester	−	−	−	+
D-Gluconic acid	−	−	+	+
Glycerol	−	−	−	+

Table 1 (continued)

Characteristic	1	2	3	4
L-Rhamnose	−	−	+	−
Polar lipids[a]	PE, PC, PG, PL, APL	PE, PG, PL, APL, L, AL[a]	ND	PC, PG, PE, AL[c]

All data were from this study, except where indicated otherwise. All strains were able to reduce nitrate, produce catalase, and hydrolyse Tweens 40, and 60, but not hydrolyse casein and starch. All strains were positive for the production of alkaline phosphatase, esterase (C4), acid phosphatase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, α-fucosidase. All strains could produce acid from D-Ribose, L-xylose and potassium 5-ketogluconate

+, positive; −, negative; w, weakly positive; ND, not determined

Data from: [a]Khan et al. (2019); [b]Saitoh et al. (1998); [c]Kim et al. (2016)

[a]PE: phosphatidylethanolamine; PC: phosphatidylcholine; PG: phosphatidylglycerol; APL: aminophospholipid; PL: phospholipid; L: lipids; AL: aminolipids

potassium 5-ketogluconate (weakly). The sole menaquinone is Q-10. The main polar lipids are phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylglycerol (PG), an aminophospholipid (APL1), an unidentified phospholipids (PL1) and the dominant fatty acids are summed feature 8 ($C_{18:1}$ $\omega 7c$ and $C_{18:1}$ $\omega 6c$). The DNA G+C content of the strain is 72.0 mol%.

The type strain, NE82^T (= KCTC 62412^T = MCCC 1H00292^T), was isolated from Jiugongli Lake in Inner Mongolia Autonomous Region, China (106° 49.721′ E, 40° 32.476′ N).

The GenBank accession numbers of strain NE82^T for the 16S rRNA gene and genome sequences are MG385132 and SKBM00000000, respectively.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-02996-6.

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Declarations

Conflict of interest The authors declare that they have conflict of interest.

Ethical statement This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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