

Paenibacillus Piscarius Sp. Nov., a Novel Nitrogen-Fixing Species Isolated from the Gut of the Armored Catfish *Parotocinclus Maculicauda*

Mariana Barbalho Farias da Silva

Universidade Federal do Rio de Janeiro

Ericka Arregue Lemos

Universidade Federal do Rio de Janeiro

Renata E. Vollú

Universidade Federal do Rio de Janeiro

Fernanda Abreu

Universidade Federal do Rio de Janeiro

Alexandre S. Rosado

King Abdullah University of Science and Technology

LUCY SELDIN (✉ lseldin@micro.ufrj.br)

Universidade Federal do Rio de Janeiro <https://orcid.org/0000-0002-4992-6395>

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Abstract

A gram-positive, nitrogen-fixing and endospore-forming strain, designated P121^T, was isolated from the gut of the armored catfish (*Parotocinclus maculicauda*) and identified as a member of the genus *Paenibacillus* based on the sequences of the 16S rRNA encoding gene, *rpoB*, *gyrB* and *nifH* genes and phenotypic analyses. The most closely related species to strain P121^T were *Paenibacillus rhizoplanae* DSM 103993^T, *Paenibacillus silagei* DSM 101953^T and *Paenibacillus borealis* DSM 13188^T, with similarity values of 98.9%, 98.3% and 97.6%, respectively, based on 16S rRNA gene sequences. Genome sequencing revealed a genome size of 7,513,698 bp, DNA G + C content of 53.9 mol% and the presence of the structural nitrogenase encoding genes (*nifK*, *nifD* and *nifH*) necessary for nitrogen fixation. Digital DNA-DNA hybridization (dDDH) experiments and average nucleotide identity (ANI) analyses between strain P121^T and the type strains of the closest species demonstrated that the highest values were below the thresholds of 70% dDDH (42.3% with *P. borealis*) and 95% ANI (84.28% with *P. silagei*) for bacterial species delineation, indicating that strain P121^T represents a distinct species. Its major cellular fatty acid was anteiso-C_{15:0} (42.4%), and the major isoprenoid quinone was MK-7. Based on physiological, genomic, biochemical and chemotaxonomic characteristics, we propose that strain P121^T represents a novel species for which the name *Paenibacillus piscarius* sp. nov. is proposed (type strain = DSM 25072 = LFB-Fiocruz 1636).

Introduction

The genus *Paenibacillus* was defined in 1993 after an extensive comparative analysis of 16S RNA gene sequences of 51 species of the genus *Bacillus* (Ash et al. 1991; 1993). At that time, the genus comprised 11 species, with *P. polymyxa* as the type species. Currently, the genus comprises 263 validated species and five subspecies (<http://lpsn.dsmz.de>) and harbors strains of industrial and agricultural importance relevant to humans, animals, plants, and the environment (Seldin 2011; Grady et al. 2016). Members of *Paenibacillus* can be found from polar regions to the tropics and from aquatic environments to the driest deserts (for review, see Grady et al. 2016). Within the genus *Paenibacillus*, different strains are considered plant growth-promoting bacteria (PGPB, Jeong et al. 2019) and/or are under evaluation for their use in biological control (Ruiu 2020). More than 20 *Paenibacillus* species encompass nitrogen-fixing strains found in different kinds of soils and in a variety of plant phyllospheres, roots and/or rhizospheres, such as wheat (Ripa et al. 2019), maize (Seldin et al. 1998; von der Weid et al. 2002), sugarcane (Seldin et al. 1984), cucumber (Hao and Chen 2017), *Sabina squamata* (Ma et al. 2007a), *Zanthoxylum simulans* (Ma et al. 2007b), *Sonchus oleraceus* (Hong et al. 2009), *Arabidopsis thaliana* (Qi et al. 2021), and many other plants.

Conversely, in a previous bioprospection study of our group carried out to search for new sources of compounds that may have economic potential (Castro et al. 2011), a novel nitrogen-fixing *Paenibacillus* strain - designated P121 - was isolated from the gut of the armored catfish *Parotocinclus maculicauda*. Fish belonging to this species are popularly known as Red Fin Dwarf Pleco and are usually found in

southern Brazil (Garavello, 1977). The search for novel microorganisms from poorly studied environments, such as those associated with *P. maculicauda*, can bring new insights into novel genes and enzymes. The cellulolytic *Paenibacillus* strain previously described in Castro et al. (2011) and the novel nitrogen-fixing bacterial strain described here are good examples of this kind of study. The presence of these *Paenibacillus* strains in the fish gut could be explained by the diet of *P. maculicauda*, which consists mainly of plant material (Hansen and Olafsen 1999).

In this study, we report a polyphasic taxonomic description of this nitrogen-fixing bacterium strain isolated from the gut of *P. maculicauda*. The phenotypic, chemotaxonomic and genotypic properties indicate that strain P121^T represents a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus piscarius* sp. nov. is proposed.

Materials And Methods

Isolation of the bacterial strain and culture conditions

The bacterial strain studied here was isolated from the gut of the armored catfish *Parotocinclus maculicauda*. Twenty fishes belonging to this species were obtained from the Mato Grosso River (Saquarema, Rio de Janeiro, Brazil) and taken to the laboratory for dissection. The food content from the digestive tract of the different fish samples was transferred to sterilized tubes, and portions of 200 mg were mixed with 1.8 ml of saline (NaCl 0.85%) and plated onto trypticase soy broth (TSB, Difco)-agar (1.2%). After 48 h of incubation at 32°C, different colonies were selected based on their morphotypes. Phylogenetic characterization of the isolates based on 16S rRNA gene sequencing surprisingly revealed that one strain (denoted P121^T) clustered within the nitrogen-fixing *Paenibacillus* group. Therefore, it was selected for further phenotypic, phylogenetic and chemotaxonomic characterization.

Bacterial DNA extraction

DNA from strain P121^T was isolated according to the method described in Seldin et al. (1998). Further purification steps were those described in Seldin and Dubnau (1985). The DNA was quantified spectrophotometrically using a Qubit[™] fluorimeter (Thermo Fisher Scientific, MA, USA).

Sequencing of the 16S rRNA-encoding gene from strain P121^T and phylogenetic analysis

The gene encoding 16S rRNA from P121^T was amplified by PCR using the pair of universal primers pA and pH and the conditions described in Massol-Deya et al. (1995) and the products sequenced using Macrogen (South Korea) facilities. The 16S rRNA sequence obtained (1,447 bp) was compared with the sequences previously deposited in the GenBank database using the BLAST-N facility

(www.ncbi.nlm.nih.gov/blast). For phylogenetic tree analysis, the sequences of closely related bacterial strains were recovered from the GenBank database and aligned to the sequence obtained in this study using the online multiple alignment program MAFFT version 7 (<https://mafft.cbrc.jp/alignment/software/>). Phylogenetic analyses were performed using the RaxML-HPC2 model in the CIPRES Science Gateway (Miller et al. 2010), with phylogenetic tree inference using a maximum likelihood/rapid bootstrapping run. The sequence generated in this study was deposited in NCBI GenBank under accession number JF892726.1.

Whole genome sequencing (WGS) and genome features

The whole genome of strain P121^T was sequenced on the Illumina Hi-seq 2500 platform as recommended by the manufacturer. An amount of approximately 5 µg/µl gDNA was used for the construction of paired-end sequencing libraries (2 × 150 bp) of 450 bp insert size. Quality analysis of the final libraries was performed using a 2100 bioanalyzer (Agilent Technologies, CA, USA). The quality of the reads in the genome assembly process was checked through FastQC (Andrews 2010) and Adapter Removal (Lindgreen 2012) software. The estimated best k-mers were selected by KmerStream (Melsted and Halldórsson 2014), followed by assembly using Edena (Hernandez et al. 2008) and SPAdes (Bankevich et al. 2012). The PSI-CD-HIT package (Fu et al. 2012) was used to obtain the final contig file.

Annotation of the genome was performed by rapid annotation using Subsystem Technology server v. 2.0 (RAST; <https://rast.nmpdr.org>) (Aziz et al. 2008) and Prokka v.1.11 (Seemann 2014). The anti-SMASH server was used to identify the secondary metabolite biosynthesis gene clusters (Blin et al. 2019). Comparative genome analysis for *Paenibacillus piscarius* P121^T, *Paenibacillus borealis* DSM 13188^T, *Paenibacillus silagei* DSM 101953^T and *Paenibacillus rhizoplanae* DSM 103963^T was performed by the OrthoVenn2 webserver (<https://orthovenn2.bioinfotoolkits.net/>) (Xu et al. 2019). The comparative genome map was represented through a BLASTN-based ring generated by BLAST Ring Image Generator (BRIG) version 0.95 (Alikhan et al. 2011). The *P. piscarius* P121^T genome was used as a reference for comparison.

Phylogenetic analyses using 16S rRNA, *rpoB*, *gyrB* and *nifH* genes

Multilocus sequence analysis (MLSA) was performed using the concatenated sequences of the 16S rRNA, *rpoB*, *gyrB* and *nifH* genes. Partial sequences of these genes (*gyrB* – 1,911 bp, *rpoB* – 3,540 bp and *nifH* – 245 bp) from different *Paenibacillus* species were obtained using their genome sequences deposited in the NCBI (<https://www.ncbi.nlm.nih.gov/>) and JGI (<https://img.jgi.doe.gov/cgi-bin/m/main.cgi>) databases and from the draft genome sequences of strain P121^T determined in this study. Sequences were compared using the BLAST program. All multiple alignments were performed using the online Multiple alignment program MAFFT version 7

(<https://mafft.cbrc.jp/alignment/software/>). Phylogenetic analyses were performed using the RaxML-HPC2 model in the CIPRES Science Gateway (Miller et al. 2010), with phylogenetic tree inference using a maximum-likelihood/rapid bootstrapping run. Phylogenetic trees based on the concatenated sequences were reconstructed by also applying the neighbor-joining (Saitou and Nei 1987) and maximum-parsimony (Fitch 1971) algorithms using Molecular Evolutionary Genetics Analysis (MEGA) software version X (Kumar et al. 2018). Bootstrap analysis (1,000 replications) was used to warrant cluster stability.

Digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI)

The ANI between strain P121^T and the most closely related *Paenibacillus* species chosen in the phylogenetic trees was calculated using JSpeciesWS software (<http://jspecies.ribohost.com/jspeciesws>) (Ritcher et al. 2016). DNA digital hybridization (dDDH) was performed using the Genome-to-Genome Distance Calculator - GGDC 2.1 (Meier-Kolthoff et al. 2013) provided by Leibniz on the DSMZ Institute website (<http://ggdc.dsmz.de/distcalc2.php>) with the recommended parameters and/or default settings.

Phenotypic and biochemical characterization

Most cultural and biochemical tests were performed by using the methodology described in Gordon et al. (1973). Either TSB or TBN (Seldin et al. 1984) liquid media were used to propagate cultures (P121^T and other *Paenibacillus* strains used in different tests for comparative purposes) for 24–48 h without shaking at 32°C. Different media were supplemented with 1.2% agar to obtain solid media. Cells were observed by Nomarski differential interference contrast on a Zeiss Axioplan microscope (Carl Zeiss, Oberkochen, Germany) to determine the size of vegetative cells. The length and width (n = 700) of P121^T cells were measured using iTEM software (iTEM Software Inc., Whiteley, UK). Gram staining was carried out by using the standard Gram reaction. Cellular motility was observed in fresh wet mounts of young (24-h) bacterial cultures in TSB. Cells were displaced on formvar-coated copper grids (Electron Microscopy Sciences, PA, USA), negatively stained with uranyl acetate 2%, and observed on an FEI Morgagni transmission electron microscope (FEI Company, Hillsboro, OR, USA) operating at 80 kV for flagella observation. For ultrathin section analysis, cells were fixed in glutaraldehyde 2.5% in sodium cacodylate buffer 0.1 M, then washed three times in the same buffer, postfixed in osmium tetroxide 1%, dehydrated in acetone series and embedded in Polybed 812. Ultrathin sectioning was performed on an EM UC6 microtome (Leica Microsystems). Samples were recovered on 300-mesh copper grids (Electron Microscopy Sciences), stained with uranyl acetate and lead citrate, and observed on an FEI Morgagni transmission electron microscope (FEI Company, Hillsboro, OR, USA) at 80 kV.

For all tests that required complex media (temperature, pH and salinity range of growth, resistance to lysozyme, hydrolysis of starch, and liquefaction of gelatin), appropriately adjusted TSB was employed. Cell growth was monitored by the increase in optical density at 600 nm. Anaerobic growth was observed

by incubating a TSB-agar-containing plate inoculated with strain P121^T in an anaerobic chamber (filled with 80% N₂, 10% CO₂ and 10% H₂) for 5 days. Cytochrome oxidase was determined by the standard paper strip Kovacs oxidase test. Catalase activity was observed by bubble formation with the addition of 3% H₂O₂ solution. The Voges-Proskauer test, formation of crystalline dextrans, utilization of citrate, reduction of nitrate to nitrite, production of indole, decomposition of casein were performed in media and conditions described in Gordon et al. (1973). Strain P121^T was also characterized by using the API 50CH kit (BioMérieux, France) as described in Seldin and Penido (1986). Data from API tests composed of 49 different carbohydrates were recorded as described previously (Rosado et al. 1998). An API 20NE (BioMérieux) containing 20 miniature biochemical tests was inoculated, and the biochemical results were converted into a numerical profile or code used to identify the bacteria as indicated by the manufacturer.

Respiratory quinones and fatty acids

The Identification Service and Dr. Brian Tindall, DSMZ, Braunschweig, Germany, carried out the analyses of respiratory quinones and fatty acids.

Acetylene reduction

To confirm the nitrogen-fixing capacity of strain P121^T, acetylene reduction to ethylene (nitrogenase activity) was tested by gas chromatography as described previously (Seldin et al. 1983; 1984), using *P. riograndensis* SBR5^T and *P. graminis* RSA19^T as positive controls.

Results And Discussion

Phylogenetic analysis of 16S rRNA gene

Different isolates from the gut of the armored catfish *Parotocinclus maculicauda* were phylogenetically characterized based on 16S rRNA gene sequence analysis. One strain (denoted P121^T) was identified as belonging to the genus *Paenibacillus*, and its closest relatives were *P. borealis* DSM 13188^T, *P. silagei* DSM 101953^T and *P. rhizoplanae* DSM 103993^T, all considered nitrogen-fixing *Paenibacillus* species (<https://lpsn.dsmz.de/genus/paenibacillus>; Elo et al. 2001; Tohno et al. 2016; Kämpfer et al. 2017). The phylogenetic similarity indicated by the 16S rRNA gene sequence data was in agreement with the levels of 16S rRNA gene sequence similarity obtained with the novel strain and *P. rhizoplanae* DSM 103993^T (98.9% similarity), *P. silagei* DSM 101953^T (98.3% similarity) and *P. borealis* DSM 13188^T (97.6% similarity).

16S rRNA gene-based phylogenetic reconstruction using the maximum-likelihood algorithm, including the sequences of the most related species obtained from the GenBank database, showed that P121^T

clustered and in a separate clade together with *P. borealis* DSM 13188^T, *P. rhizoplanae* DSM 103993^T, and *P. silagei* DSM 101953^T but in an independent branch (Fig. 1).

Genome sequence analysis

The draft genome sequence of strain P121^T was determined in this study, and the Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession number JAIEUI000000000. The version described in this paper is version JAIEUI010000000. Genome sequencing of strain P121^T resulted in a chromosome consisting of 7,513,698 bp. The G + C content was 53.9 mol%. According to the annotation, 6,955 coding sequences, 82 RNAs and 444 contigs were found in the P121^T genome. The RAST analysis revealed 317 subsystems (Fig. S1). In subsystem categories, carbohydrates had the highest feature counts (299), followed by amino acids and derivatives with 279 feature counts. As a nitrogen-fixing bacterium, genome analysis of P121^T revealed the presence of the *nifK* and *nifD* genes encoding dinitrogenase α and β subunits (Fe-Mo protein) and the *nifH* gene encoding the nitrogenase iron protein. Moreover, the *nifB* gene involved in the biosynthesis of the iron-molybdenum cofactor (FeMo-co or M-cluster) found in the dinitrogenase enzyme was found in the P121^T genome.

AntiSMASH analysis resulted in the identification of eight predicted secondary metabolite biosynthetic gene clusters (BGCs). One of the BGCs matched paeninodine, a bacteriocin from the lassopeptide class, with 100% similarity. Lassopeptides are a class of ribosomally synthesized and posttranslationally modified natural products with diverse bioactivities (Maksimov et al. 2012). Another BGC showed 25% similarity to clusters encoding the polyketide aurantinin b/c/d. Polyketides are a large family of structurally diverse natural products with varied biological and pharmacological activities, including antibacterial, antitumor, and immunosuppressant activities (Nivina et al. 2019). These secondary metabolites have already been described in different *Paenibacillus* species, such as *P. polymyxa* KF-1 (Li et al. 2016), *P. dendritiformis* C454 (Zhu et al. 2016) and *P. alvei* MP1 (Pajor et al. 2020).

The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values were determined between strain P121^T and the other three genomes of the closest related members of the genus *Paenibacillus* (*P. borealis* DSM 13188^T, *P. rhizoplanae* DSM 103993^T and *P. silagei* DSM 101953^T) and are shown in Table 1. The ANIb values between strain P121^T and *P. borealis* DSM 13188^T, *P. rhizoplanae* DSM 103993^T and *P. silagei* DSM 101953^T were 80.47%, 83.52% and 84.28%, respectively. The accepted threshold for species delimitation using ANIb is 95–96% (Richter and Rosselló-Móra 2009). The *in silico* DDH results were in all cases lower than 45%, a value lower than 70%, which is the cutoff value for species delineation (Goris et al. 2007). Both ANI and DDH results indicate that strain P121^T is a new species of the genus *Paenibacillus*.

Table 1

dDDH and ANI values between the genome of *Paenibacillus piscarius* P121^T as the query genome and that of closely related species. ANI values were calculated using JSpecies with the ANIb algorithm (average nucleotide identity based on BLAST). Numbers between parentheses after ANI values are percentages of conserved aligned DNA between two genomes; numbers between parentheses after dDDH values are the confidence intervals.

Query genome	Reference genome	Bioproject NCBI number	Size (Mb)	G + C (mol%)	dDDH (%)	ANI (%)
<i>P. piscarius</i> P121 ^T	<i>P. piscarius</i> P121 ^T	JAIEUI010000000	7.51	53.9	100	100
<i>P. piscarius</i> P121 ^T	<i>P. silagei</i> DSM 101953	JAGGLV010000001	7.68	52.5	36.1 (33.7–38.6)	84.28 (72.69)
<i>P. piscarius</i> P121 ^T	<i>P. rhizoplanae</i> DSM 103963	JAGGLX010000035.1	7.54	52.1	35.2 (32.8–37.7)	83.52 (70.12)
<i>P. piscarius</i> P121 ^T	<i>P. borealis</i> DSM 13188	PRJNA258353	8.16	51.39	42.3 (39.8–44.8)	80.47 (60.36)

Finally, the comparative genome analysis for *P. piscarius* P121^T, *P. borealis* DSM 13188^T, *P. silagei* DSM 101953^T and *P. rhizoplanae* DSM 103963^T revealed that the strains formed 6874 clusters, 5015 orthologous clusters (at least containing two species) and 1859 single-copy gene clusters (Fig. 2). *Paenibacillus piscarius* P121^T possesses 394 singletons, proteins not found in any cluster. Figure 3 shows a circular diagram illustrating the nucleotide similarity between *P. piscarius* P121^T and other *Paenibacillus* genomes represented by concentric rings. The *nif* operon region in P121^T is highlighted and shows more than 50% similarity with *P. borealis* DSM 13188^T *nif* genes.

Multilocus sequence analysis (MLSA) using the housekeeping genes 16S rRNA, *rpoB*, *gyrB* and *nifH*

Multilocus sequence analysis (MLSA) was performed using the concatenated sequences of the 16S rRNA, *rpoB*, *gyrB* and *nifH* genes. Concatenation of the 16S rRNA gene and the three housekeeping genes (*rpoB*, *gyrB* and *nifH*) of the different nitrogen-fixing *Paenibacillus* species and the outgroup resulted in a phylogenetic tree (Fig. S2 a) showing the same distribution as that from the 16S rRNA gene reconstruction. Again, strain P121^T grouped in a monophyletic group together with *P. borealis* DSM 13188^T, *P. rhizoplanae* DSM 103993^T and *P. silagei* DSM 101953^T but formed an independent branch in the tree. Similar results were also found in phylogenetic analyses performed for each gene separately (Fig. S2 b, c, d). Furthermore, the use of neighbor-joining and maximum-parsimony methods for phylogenetic reconstructions, including the sequences of the P121^T most related species, showed highly similar trees to those obtained using the maximum-likelihood algorithm (Fig. S3).

Phenotypic characteristics

Strain P121^T was Gram-positive or Gram-variable, cells were rod-shaped measuring $0.63 \pm 0.11 \mu\text{m}$ by $3.34 \pm 0.45 \mu\text{m}$, motile with flagella (Fig. 4A). The spores were ellipsoidal, distending the sporangia and located in the central to subterminal position in the cell (Fig. 4B). The colonies were yellowish, circular, convex and mucoid, 10 to 15 mm in diameter on TSB agar.

Different phenotypic tests were used to characterize strain P121^T based on the recommendations of Gordon et al. (1973) and Logan et al. (2009) and are described below in the species description. Strain P121^T was also characterized by using API tests (API 50CH and API 20NE). It produced acid in API 50CH from L-arabinose, D-xylose, β -methyl-xyloside, glucose, D-fructose, D-mannose, mannitol, N-acetylglucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, D-raffinose, starch, glycogen, β -gentibiose and turanose. A weak reaction was observed with methyl-D-mannoside, methyl-D-glucoside and xylitol. The novel strain was not able to produce acid with 22 of the other carbohydrates tested. Using API 20NE, strain P121^T was able to reduce nitrate to nitrite, produce urease, β -galactosidase and arginine dehydrolase, and assimilate glucose, arabinose, mannitol and maltose. Phenotypic characteristics that differentiate the novel isolate from the three closely related species *Paenibacillus borealis* DSM 13188^T (Elo et al. 2001), *Paenibacillus rhizoplanae* DSM 103993^T (Kämpfer et al. 2017) and *Paenibacillus silagei* DSM 101953^T (Tohno et al. 2016), also considered nitrogen-fixing *Paenibacillus* species, are presented in **Table 2**. When the phenotypic characteristics of the novel isolate were compared with those of the three closely related *Paenibacillus* species, it became clear that P121^T could not be considered to represent typical members of any one of these previously established species (**Table 2**).

Table 2. Characteristics that differentiate strain P121^T from the closest type strains of selected *Paenibacillus* species Species: **1**, strain P121^T; **2**, *P. rhizoplanae* DSM 103963^T; **3**, *P. silagei* DSM 101953^T; and **4**, *P. borealis* DSM 13188^T. +, positive reaction; -, negative reaction; nd, not determined.

Characteristics	1	2	3	4
Motility	+	-	-	+
Growth in the presence of 2% NaCl	-	+	+	+
oxidase	-	+	nd	-
Growth in pH 5.7	-	-	nd	+
Growth at 5°C	-	-	+	+
Nitrate reduction	+	nd	nd	-
Acid production from:				
glycerol	-	nd	+	+
L-arabinose	+	-	+	+
D-xylose	+	-	+	+
D-mannose	+	-	+	+
Rhamnose	-	-	+	-
Mannitol	+	-	+	-
Maltose	+	-	+	nd
Lactose	+	-	+	+
Melezitose	-	nd	-	+
D-arabitol	-	-	-	+

Chemotaxonomic characteristic

In accordance with other species of the genus *Paenibacillus*, meso-diaminopimelic acid was detected. The quinone system was composed predominantly of menaquinones MK-7, which is also in line with other species of the genus.

The fatty acids comprised mainly iso- and anteiso-branched components, and the fatty acid profile was very similar to those of the most closely related *Paenibacillus* species. The major cellular fatty acids are anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{15:0} and C_{14:0}. The detailed cellular fatty acid profiles (%) of *P. piscarius* sp. nov. P121^T and closely related *Paenibacillus* species is shown in **Table S1**.

Nitrogen fixation

The new isolate, together with the *P. graminis* and *P. riograndensis* type strains (RSA19^T and SBR5^T, respectively), effectively reduced acetylene, showing values varying from 2.08 to 4.73 nmol ethylene/mg protein/h (Table 3).

Table 3. Nitrogenase activity of strain P121^T compared with two other nitrogen-fixing *Paenibacillus* species

The results are the means \pm standard deviation of triplicates; (*) two independent experiments.

<i>Strain</i>	<i>Acetylene reduction</i> (nmol ethylene per mg protein per h)
P121 ^T	2.43 \pm 0.28/3.36 \pm 0.23 *
<i>P. riograndensis</i> SBR5 ^T	4.73 \pm 0.63
<i>P. graminis</i> RSA19 ^T	2.08 \pm 0.20

Description of *Paenibacillus piscarius* sp. nov.

Paenibacillus piscarius (pis.ca'ri.us. L. masc. adj. *piscarius*, of or pertaining to fish).

Cells are straight, motile rods ($0.63 \pm 0.11 \mu\text{m}$ in width, $3.34 \pm 0.45 \mu\text{m}$ in length) with flagella. Spores are oval to ellipsoidal and predominantly central to subterminal and distend the sporangium. Young trypticase soy broth (TSB) cultures are Gram-positive or Gram-variable. On TSB agar, colonies are 10 to 15 mm in diameter, yellowish, circular, convex and mucoid. Do not grow at 10°C or 40°C; optimum is near 25°C. Growth was not observed at pH 5.7 (optimum pH 7.5-8) or in the presence of 2% NaCl. Resistant to 0.001% lysozyme. Facultatively anaerobic. Catalase and urease are produced. Voges-Proskauer negative. Nitrate is reduced to nitrite. Gelatin is not liquefied. Starch hydrolysis was negative, and esculin hydrolysis was positive. No crystalline dextrans are formed in rolled oat medium. Casein is weakly decomposed. Indole is not produced. Acid is produced from L-arabinose, D-xylose, β methyl-xyloside, glucose, D-fructose, D-mannose, mannitol, N acetyl glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, threolose, inulin, D-raffinose, starch, glycogen, gentibiose and turanose. Strain P121^T does not produce acid from glycerol, erythritol, D-arabinose, ribose, L-xylose, adonitol, galactose, L-sorbose, rhamnase, dulcitol, inositol, sorbitol, melezitose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2 keto-gluconate or 5 keto-gluconate. A weak reaction was observed with xylitol, α -methyl-D-glucoside, and α -methyl-D-mannoside. Assimilation of maltose and

mannitol was positive but negative for mannose and N-acetyl-glucosamine. Utilization of gluconate, caprate, adipate, malate, citrate and phenyl-acetate was not observed. Nitrogen fixation (acetylene reduction) was detected. The G-C content of the type strain is 53.9 mol%. The major cellular fatty acids are anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{15:0} and C_{14:0}. Isolated from the gut of the armored catfish *Parotocinclus maculicauda*. The type strain is LFB-Fiocruz 1636, DSM 25072 (= P121^T).

Declarations

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Author's contributions

LS and ASR conceived the study. REV performed deposition, and LS and REV performed the polyphasic taxonomy. MBFS and EAL performed genome analysis. FA provided transmission electron microscope data. LS drafted the manuscript. All authors revised the manuscript, provided comments and approved the final version of the manuscript.

Declarations Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

This article does not contain any studies with human participants. The isolation of the *Paenibacillus* strain from the gut of the armored catfish described here followed the recommendations of the Guide for the Care and Use of Laboratory Animals of the Laboratory of Biology and Fisheries Technology, Institute of Biology, UFRJ.

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Figures

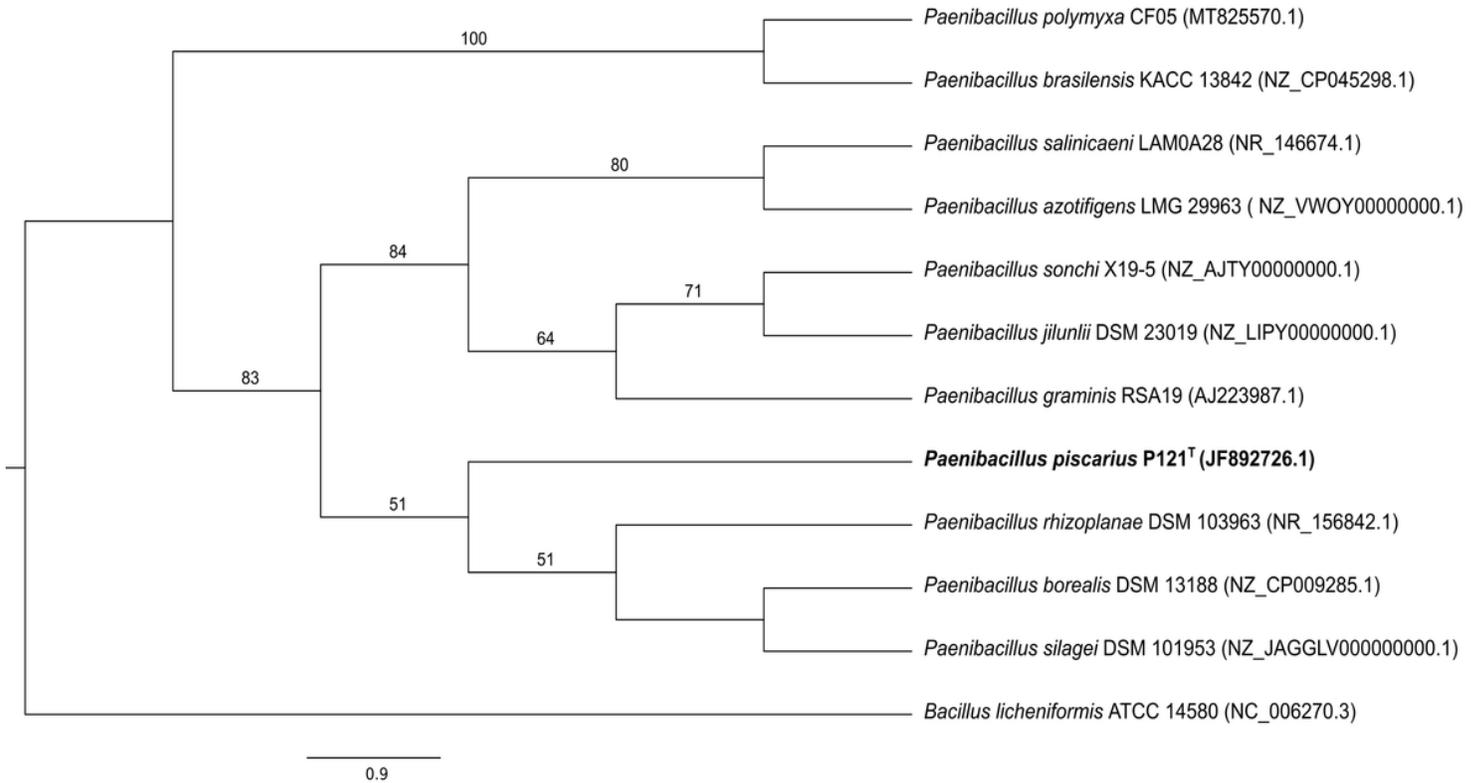


Figure 1

Maximum likelihood tree with GTRGAMMA distribution of the multiple alignment of the 16S rRNA encoding gene of *Paenibacillus piscarius* P121T and related species. The GenBank accession number of each sequence is shown in parentheses. Bootstrap values are expressed as percentages of 1000 replications and are shown at branch points. *Bacillus licheniformis* ATCC 14580 was used as outgroup. Bar = substitutions per nucleotide position.

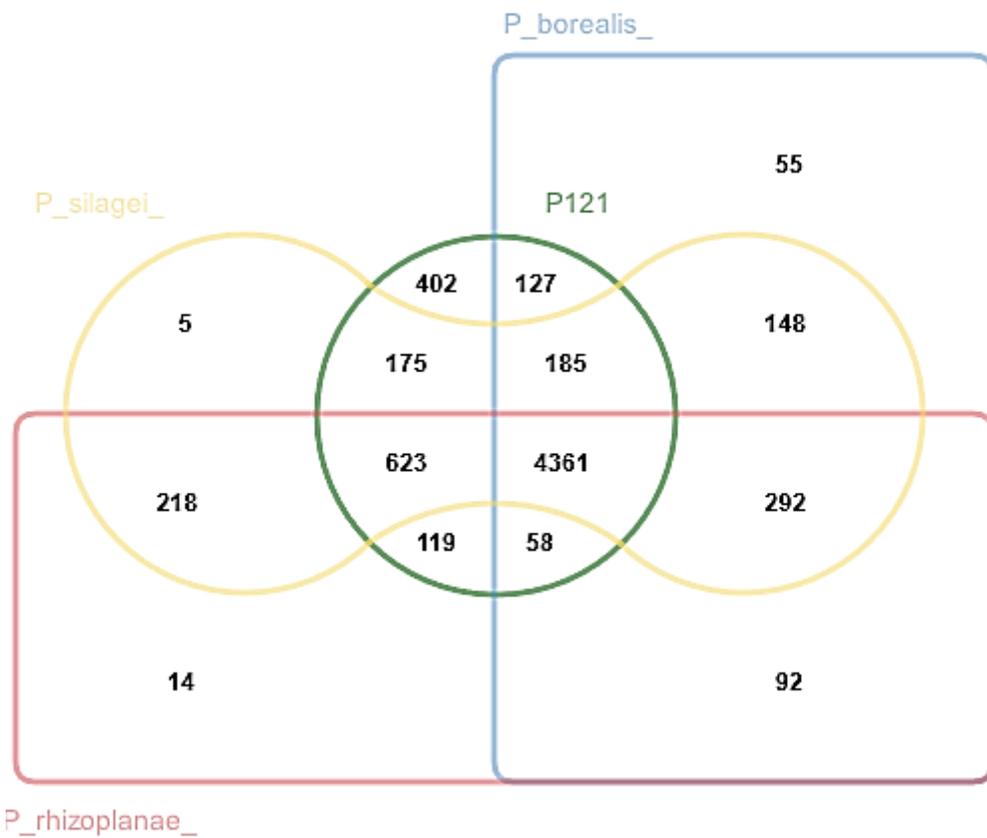


Figure 2

Comparative genome analysis for *Paenibacillus piscarius* P121T, *Paenibacillus borealis* DSM 13188T, *Paenibacillus silagei* DSM 101953T and *Paenibacillus rhizoplae* DSM 103963T performed by the OrthoVenn2 webserver. The numbers in the Venn diagram represent the number of clusters shared between strains.

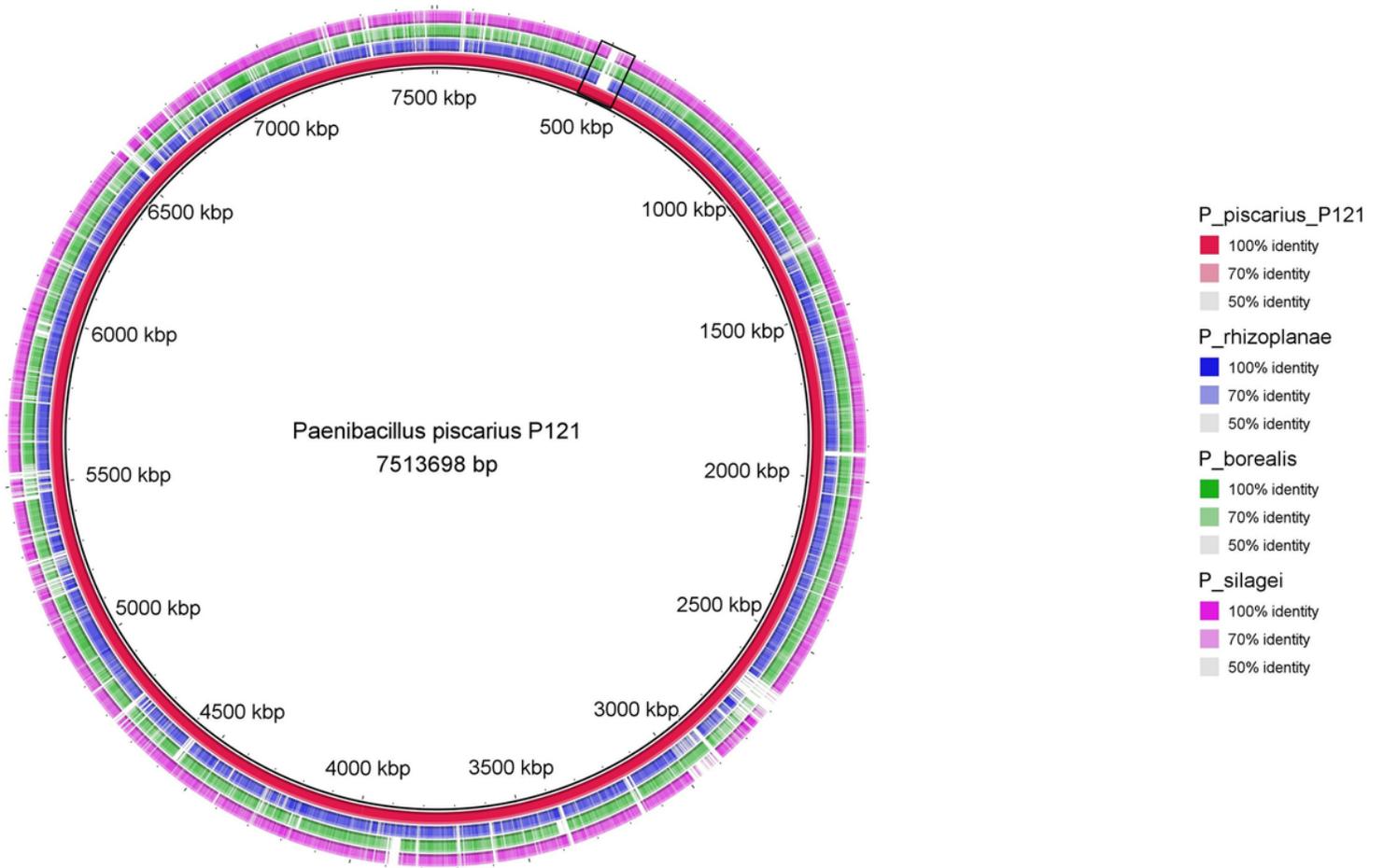


Figure 3

Circular diagram illustrating the nucleotide similarity between *P. piscarius* P121T (in red) and other *Paenibacillus* genomes represented by concentric rings. The *nif* genes region is delimited in black.

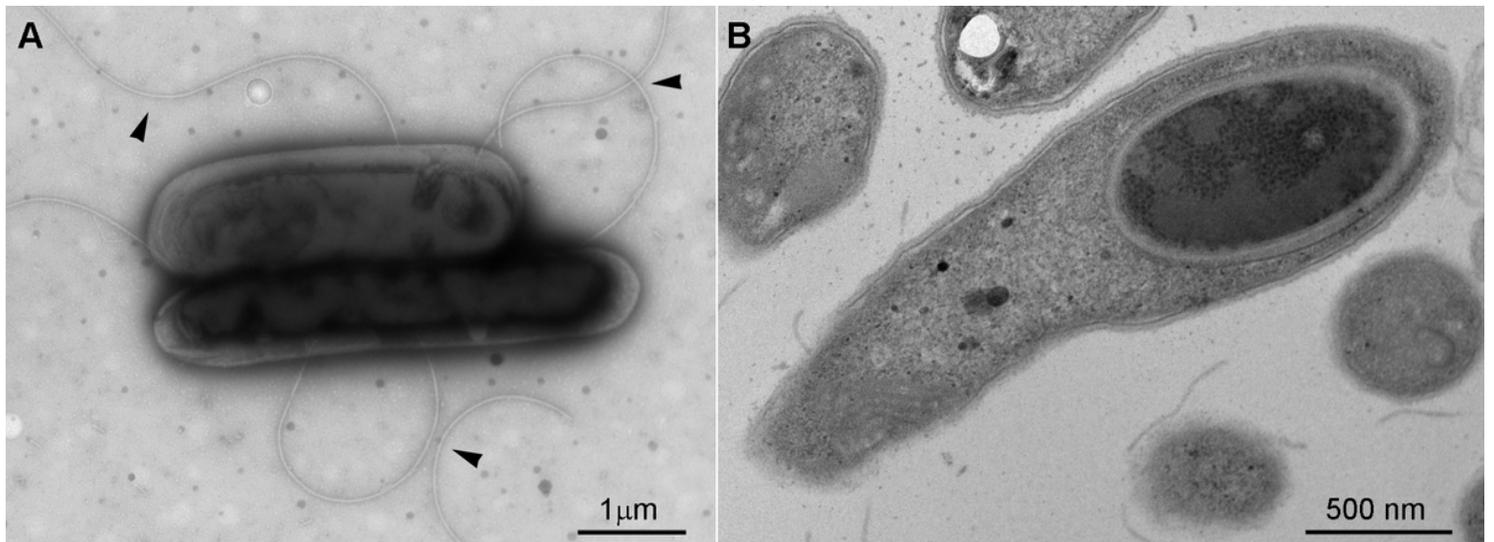


Figure 4

Transmission electron micrographs of isolate P121T. (A) Vegetative cells with flagella; (B) ellipsoidal spore, swelling the sporangia.

Supplementary Files

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