INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

RESEARCH ARTICLE

Qiu *et al.*, *Int. J. Syst. Evol. Microbiol.* 2024;74:006550

DOI 10.1099/ijsem.0.006550

MICROBIOLOGY SOCIETY

Pseudomonas machongensis sp. nov. and *Stenotrophomonas capsici* sp. nov., isolated from wilted pepper plants

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Abstract

Two Gram-stain-negative, aerobic, rod-shaped, non-endospore-forming bacteria, designated as strain MH1^T and MH2^T, were isolated from branches of wilted pepper plants (*Capsicum annuum*) collected from a farmland in Machong town, Guangdong, China, and investigated using a polyphasic approach. MH1^T grew at temperatures of 4–42 °C (optimum 28 °C), with 0–6.0% (w/v) NaCl and at pH 4.0–10.0 (optimum pH 4.0). MH2^T grew at temperatures of 4–42 °C (optimum 28 °C), with 0–6.0% (w/v) NaCl and at pH 4.0–10.0 (optimum pH 5.0). Analysis of the 16S rRNA gene sequence indicated that MH1^T belongs to *Stenotrophomonas* and MH2^T belongs to *Pseudomonas*. Genome-based phylogenetic analysis further established that MH1^T shares the closest evolutionary relationships with *Stenotrophomonas humi* DSM 18929 and *Stenotrophomonas terrae* DSM 18941, and MH2^T is sister to *Pseudomonas wayambapalatensis* RW3S1. Whole-genome comparisons between MH1^T and known *Stenotrophomonas* species revealed average nucleotide identity (ANI) values up to 84.5%, as well as digital DNA–DNA hybridization (dDDH) values up to 28.3%, both substantially lower than the accepted thresholds for species delineation (ANI: 95%; dDDH: 70%). The ANI and dDDH values between MH2^T and known *Pseudomonas* species were at most 94.6 and 59.2%, respectively. Additional biochemical and physiological analyses further support that MH1^T and MH2^T represent a novel species in *Stenotrophomonas* and *Pseudomonas*, respectively. Notably, the differences in carbon source utilization could differentiate MH1^T and its close relatives in *Stenotrophomonas*. The major fatty acids were iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{14:0}$ for MH1^T and were $C_{16:0}$, $C_{18:1}\,\omega7c/C_{18:1}\,\omega6c$ (summed feature 8), $C_{16:1}\,\omega7c/C_{16:1}\,\omega6c$ (summed feature 3) and $C_{17:0}$ cyclo for MH2^T. Therefore, we propose a new species *Stenotrophomonas capsici* sp. nov., with MH1^T (=GDMCC 1.3749^T=JCM 36317^T) as the type strain, and a new species *Pseudomonas machongensis* sp. nov., with MH2^T (=GDMCC 1.3750^T=JCM 36318^T) as the type strain. The MH1^T genome has a size of 4.18 Mb and a GC-content of 67.19 mol%, while the MH2^T genome has a size of 5.71 Mb and a GC-content of 63.12 mol%.

INTRODUCTION

The genus *Pseudomonas*, a member of the family *Pseudomonadaceae*, contains bacteria of significant importance due to their wide distribution in various environments and their diverse metabolic capabilities [1–3]. Established by Migula in 1894, the genus *Pseudomonas* has been extensively studied, with *Pseudomonas aeruginosa* being the most well-known species due to its

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Keywords: novel species; pepper; *Pseudomonas machongensis*; *Stenotrophomonas capsici*.
Abbreviations: ANI, average nucleotide identity; BLASTN, nucleotide basic local alignment search tool; BUSCO, benchmarking universal single-copy orthologs; CTG, casein-hydrolysates tryptone glucose; dDDH, digital DNA-DNA hybridization; DDH, DNA-DNA hybridization; DPG, diphosphatidylglycerol; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; GGDC, genome-to-genome distance calculator; GTDB-tk, genome database taxonomy toolkit; HPLC, high-performance liquid chromatography; LB, lysogeny broth; NCBI, National Center for Biotechnology Information; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGAP, prokaryotic genome annotation pipeline; TYGS, type strain genome server.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MH1^T is PP060478 (https://www.ncbi.nlm.nih.gov/nuccore/PP060478); for the draft genome sequence of MH1^T is JAYFUH000000000 (https://www.ncbi.nlm.nih.gov/nuccore/JAYFUH000000000); for the 16S rRNA gene sequence of strain MH2^T is PP061858 (https://www.ncbi.nlm.nih.gov/nuccore/PP060478); for the draft genome sequence of MH2^T is JAYFUI000000000 (https://www.ncbi.nlm.nih.gov/nuccore/ JAYFUI000000000https://www.ncbi.nlm.nih.gov/nuccore/ JAYFUI000000000). Strain MH1^T have been deposited at GDMCC (accession number: 1.3749) and JCM (accession number: 36317). Strain MH2^T have been deposited at GDMCC (accession number: 1.3750) and JCM (accession number: 36318).
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Three supplementary figures and four supplementary tables are available in the online version of this article.

role as a major opportunistic human pathogen and its ability to exhibit resistance to multiple antibiotics [4]. Currently, the genus comprises over 300 species with validly published names, reflecting its vast diversity and ecological significance [List of Prokaryotic Names with Standing in Nomenclature (LPSN) [5]; https://lpsn.dsmz.de/genus/pseudomonas, accessed on 19 June 2024]. *Pseudomonas* species are known for their metabolic versatility, which allows them to thrive in diverse environments, including soil, water and plant surfaces and even clinical settings [6]. Many species within this genus play beneficial roles in agriculture by promoting plant growth, suppressing plant diseases and decomposing organic materials [7]. However, some species are also known to be pathogenic to plants and animals, further highlighting their dual roles in nature [8]. The continuous exploration and identification of novel *Pseudomonas* species are essential for understanding their ecological roles and potential applications in biotechnology and agriculture.

The genus *Stenotrophomonas*, belonging to the family *Lysobacteraceae*, represents a group of bacteria that have garnered attention for their diverse roles in both environmental ecosystems and clinical settings [9]. Established by Palleroni and Bradbury in 1993, the genus has undergone significant taxonomic refinement, with the type species *Stenotrophomonas maltophilia* being identified as a notable pathogen [10]. This species remains the most well-characterized member of the genus due to its clinical relevance as an opportunistic pathogen with multidrug resistance capabilities [11]. As of the most recent updates, the genus comprises 20 validly described species [12] (LPSN; accessed on 19 June 2024). *Stenotrophomonas* species have been isolated from a variety of environments, including the rhizosphere, where they interact closely with plant roots [13–15]. While some species contribute positively to plant health by facilitating nutrient uptake and producing plant-growth-promoting factors, others may act as phytopathogens causing disease. This dual nature makes the genus particularly interesting from an ecological and agricultural standpoint. Given the implications for both animal and plant health, the ongoing discovery and characterization of novel *Stenotrophomonas* species are vital.

In this study, we isolated two bacterial strains from pepper plants (*Capsicum annuum*) showing wilt disease symptoms collected from a farmland in Machong town, Guangdong, China. Subsequent phenotypic, biochemical, physiological and genomic characterizations demonstrated that they both represent novel bacterial species. Here, we describe two novel bacteria, *Pseudomonas machongensis* MH2^T and *S. capsici* MH1^T.

Isolation and ecology

Samples of wilted *C. annuum* along with surrounding soil were collected from Gumei Ecological Agricultural Park in Dongguan City, Guangdong, China (23.047751° N 113.568133° E). The samples were carefully transported to the laboratory under ambient conditions. To prepare the samples for isolation, branches of *C. annuum* were segmented and immersed in 75% alcohol for 30 s for initial sterilization. Subsequently, the branch segments were thoroughly rinsed with sterile water, blotted dry with filter paper and further sectioned into segments of ~0.5 cm in length. The samples were then subjected to agitation in 20 ml sterile water at 28 °C for 1 h to facilitate the release of bacterial cells from the plant tissue. The resulting suspension was then serially diluted to gradients of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}; the dilutions were cultured separately on triphenyl tetrazolium chloride plates for 1 day to generate single colonies. The strain formed red-purple colonies, which were isolated for further investigation.

Table 1. Genome characteristics of MH2^T and MH1^T

Strain	MH2^T	MH1^T
Assembly size (bp)	5710696	4175974
G+C content (%)	63.12	67.19
Number of scaffolds	198	263
Scaffold N50 (bp)	81419	125146
Number of protein-coding genes	5237	3822
Completeness (%)	98.1	97.8
Number of pseudogenes	62	40
Number of rRNAs	16	9
Number of tRNAs	78	64
Number of ncRNAs	4	4

16S rRNA gene analysis

To investigate the taxonomic identities of MH2^T and MH1^T, we amplified the 16S rRNA gene fragment from a pure culture of each strain using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1504R (5′-AAGGAGGTGATC-CAGCCGCA-3′) and conducted Sanger sequencing to obtain the nearly complete 16S rRNA gene sequences. Nucleotide basic local alignment search tool (BLASTN) [16] searches against the NCBI 16S ribosomal RNA sequence database revealed high levels of sequence identity between 16S rRNA gene sequences of MH2^T and *Pseudomonas* spp., as well as between those of MH1^T and *Stenotrophomonas* spp. Specifically, MH2^T shared greater than 98.7% 16S rRNA sequence identities with types strains of 27 *Pseudomonas* species, among which a maximum identity (100%) was observed for *P. wayambapalatensis* RW3S1 (Table S1, available in the online version of this article). MH1^T showed 16S rRNA sequence identities above the proposed bacterial species threshold of 98.7% with three *Stenotrophomonas* type strains, namely *S. nitritireducens* L2 (99.52%), *S. terrae* R-32768 (99.31%) and *S. humi* R-32729 (99.17%) (Table S2). The relationships between MH1^T (or MH2^T) and closely related species were not well resolved in 16S rRNA-based phylogenetic analysis (Fig. S1). The two strains were also analysed by ProkAtlas [17], which associates metagenome-derived 16S rRNA sequences with prokaryotic habitats, thus enabling the inference of habitat environments of bacteria based on their 16S rRNA gene sequences. The results suggest that closely related strains of both MH2^T and MH1^T most likely prefer 'rhizosphere' (preference scores of 26.96 and 24.22, respectively) and 'soil' (preference scores of 9.65 and 15.66, respectively) as their habitats, while the relatives of MH2^T might adapt to a broader range of environments than those of MH1^T (48 vs 26 habitats) (Table S3).

Genomic characterization

For whole-genome sequencing, MH2^T and MH1^T were cultured in Luria–Bertani medium at 28 °C for 24 h, and DNA was isolated using the EasyPure Bacteria Genomic DNA Kit according to the manufacturer's instructions for Gram-negative bacteria. To assess the quality of isolated genomic DNA, DNA degradation and contamination were monitored on 1% agarose gels, and DNA concentration was measured by Qubit DNA Assay Kit in Qubit 3.0 Fluorometer (Invitrogen, USA). The sequencing libraries were generated from a total amount of 0.2 µg genomic DNA using NEB Next Ultra DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations. High-throughput sequencing experiments were conducted by Novogene (Tianjin, China) using the Illumina NovaSeq 6000 platform, generating 1.69 Gb (5.65 million read pairs) and 1.51 Gb (5.04 million read pairs) of 150 bp paired-end short-read sequencing data, respectively. *De novo* genome assembly of each strain was conducted using MaSuRCA v4.1.0 [18] on raw sequencing data as suggested by the assembler. For each strain, the completeness of genome assembly was assessed using both Benchmarking Universal Single-Copy Orthologs (BUSCO) v5.5.0 [19] (with the Pseudomonadales_odb10 gene set for MH2^T, and the Xanthomonadales_odb10 gene set for MH1^T) and CheckM v1.2 [20], and the genome annotation was generated using the NCBI Prokaryotic Genome Annotation Pipeline [21] v2023-05-17.build6771.

The genome characteristics of MH2^T and MH1^T are shown in Table 1. Specifically, the MH2^T genome was assembled into 198 scaffolds, with a total length of 5710696 bp, a scaffold N50 of 81419 bp and a GC-content of 63.12 mol%. BUSCO and CheckM assessments showed that 98.1 and 99.3% of marker genes are complete, respectively, and the CheckM contamination rate was only 1.60%, indicating that the MH2^T genome is of high quality. The MH2^T genome was predicted to have 5175 protein-coding genes, 16 rRNAs, 78 tRNAs and 4 ncRNAs. The MH1^T genome was assembled into 263 scaffolds, with a total length of 4,175974 bp, a scaffold N50 of 125146 bp, and a GC-content of 67.19 mol%. The completeness of the MH1^T genome was estimated to be 97.8% by BUSCO and 91.17% by CheckM, with a contamination rate of 6.15%, comparable to other sequenced genomes in the *Stenotrophomonas* genus. A total of 3782 protein-coding genes, 9 rRNAs, 62 tRNAs and 4 ncRNAs were annotated in the MH1^T genome. Analysis using the PGPg-finder pipeline and the PLaBAse (plant-associated bacteria) database identified 1219 and 797 plant growth-promoting genes in MH1^T and MH2^T, respectively (Table 2). These genes were involved in diverse functional categories such as 'Osmotic Stress Neutralization' (261 genes in MH1^T and 145 genes in MH2^T), 'Motility Chemotaxis' (166 genes in MH1^T and 162 genes in MH2^T), 'Heavy Metal Detoxification' (156 genes in MH1^T and 74 genes in MH2^T), 'Surface Attachment' (119 genes in MH1^T and 128 genes in MH2^T) and 'Vitamin Production' (118 genes in MH1^T and 71 genes in MH2^T).

The genome sequences of the two strains were analysed by GTDB-Tk v2.4.0 [22] (with GTDB Release R220); the results confirmed that MH2^T and MH1^T belong to *Pseudomonas* and *Stenotrophomonas*, respectively, but no matches against any known species were found for either strain. Additionally, the two genomes were examined on the Type Strain Genome Server [23], and the results also showed that MH2^T and MH1^T likely each represent a novel species in *Pseudomonas* and *Stenotrophomonas*, respectively. A systematic genome comparison was then carried out between MH2^T (or MH1^T) and all representative strains of *Pseudomonas* (or *Stenotrophomonas*) in the NCBI Genome database, including all the above-mentioned species sharing high 16S rRNA sequence identity with MH2^T (or MH1^T). For each pair of genomes, the whole-genome average nucleotide identity (ANI) value was calculated using pyani v0.2.13 (the 'ANIb' method) [24], and the digital DNA–DNA hybridization (dDDH) [25] value was calculated using the Genome-to-Genome Distance Calculator 3.0 [26] provided by DSMZ. The results showed MH2^T exhibited the highest ANI (94.6%) and dDDH (59.2%) values with *P. wayambapalatensis*

Table 2. Summary of plant growth-promoting genes identified in MH2^T and MH1^T

Functional category	Gene count	
	MH2^T	**MH1^T**
Nitrogen fixation	23	10
Denitrification	9	6
Nitrate reduction	10	2
Urea metabolism	9	0
Carbon dioxide fixation	19	9
P-solubilization gluconic acid-PQQ	15	6
Osmotic stress neutralization	261	145
P-mineralization	9	15
Fe-siderophores	67	47
Heavy metal detoxification	156	74
Phytohormone-IAA	22	19
Phytohormone-cytokinins	33	13
Phytohormone-GABA	30	4
Organic volatiles	53	31
Vitamin production	118	71
Insecticidal compounds	11	0
Nematicidal compounds	1	0
Root colonization	25	18
Motility chemotaxis	166	162
Surface attachment	119	128
Secretion systems	63	35

RW3S1, which were below the respective species boundaries (95% for ANI and 70% for DDH), and considerably lower genomic similarities were found between MH2^T and other *Pseudomonas* species (Table 3). At the same time, the ANI values between MH1^T and all representative *Stenotrophomonas* genomes were no greater than 84.5%, and the dDDH values were up to 28.3%, substantially lower than the commonly accepted thresholds for bacterial species delineation (Table 3).

Table 3. Average nucleotide identity (ANI) and digital DNA–DNA hybridization values between MH2^T and MH1^T and representative genomes of *Pseudomonas* and *Stenotrophomonas*

	Strain	**ANI (%)**	**dDDH (%)**
MH2^T	*Pseudomonas wayambapalatensis* RW3S1	94.64	59.20
	Pseudomonas peradeniyensis BW13M1	85.26	29.90
	Pseudomonas muyukensis COW39	85.03	29.50
	Pseudomonas faucium BML-PP048	84.99	29.40
MH1^T	*Stenotrophomonas humi* DSM 18929	84.49	28.30
	Stenotrophomonas nitritireducens	84.14	27.90
	Stenotrophomonas daejeonensis JCM 16244	84.04	27.80
	Stenotrophomonas acidaminiphila T0-18	84.01	27.60

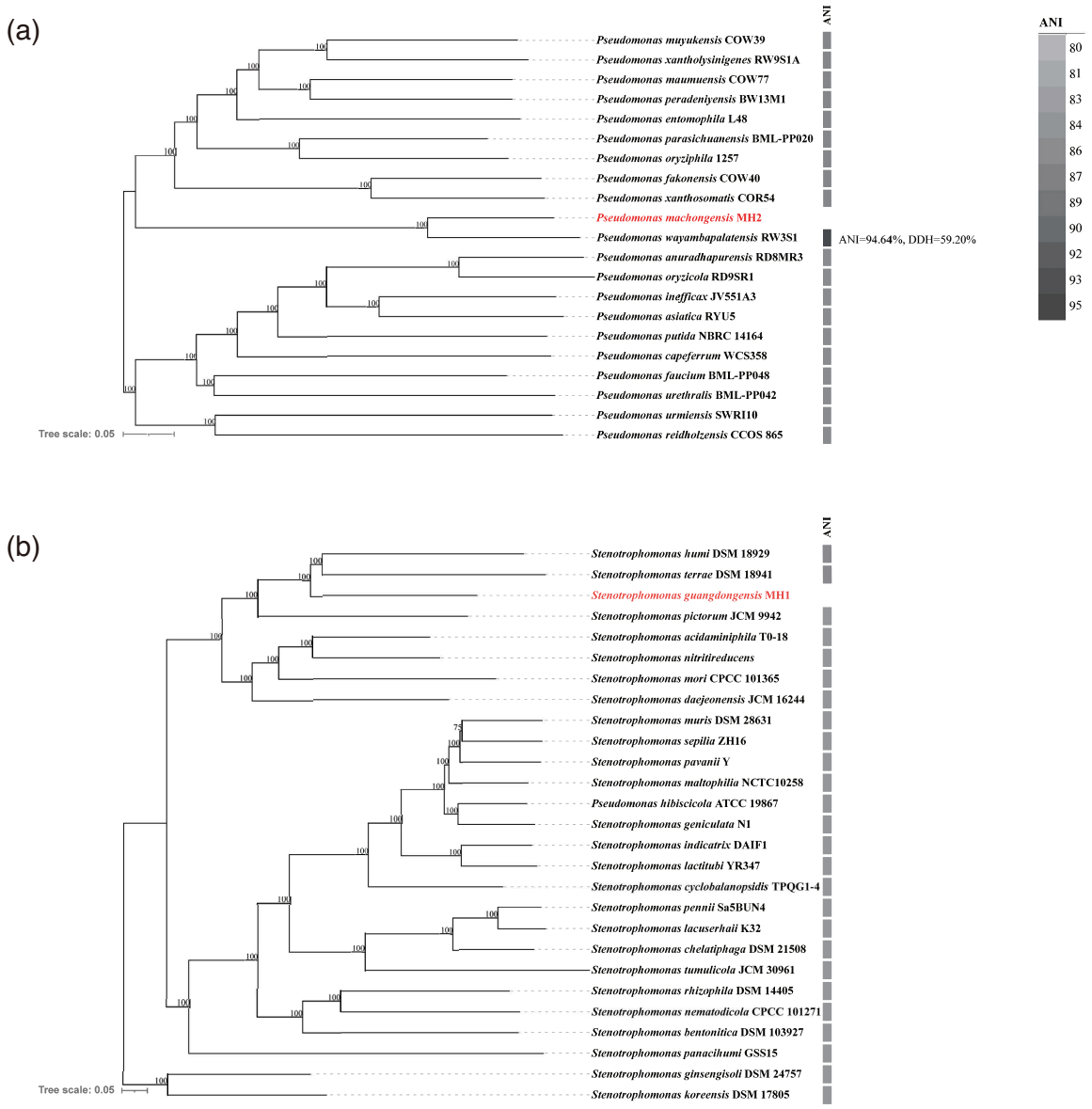

Fig. 1. Genome-based phylogenetic analyses of (a) MH2^T and its close relatives in *Pseudomonas* and (b) MH1^T and its close relatives in *Stenotrophomonas*. The trees were reconstructed based on tANI, a genome-to-genome distance, using the balanced minimum evolution algorithm implemented in the FastME function of APE. The branch support values are based on 100 bootstrap replicates. ANI values between each genome and MH2^T or MH1^T are shown next to the tree in colour scale.

To further investigate the evolutionary relationships between MH2^T and MH1^T with their close relatives in the respective genus (as indicated by the ANI and dDDH analyses), we performed separate whole-genome-based phylogenetic analyses for MH2^T and MH1^T using tANI_tool v1.3.0 [27, 28]. The software first calculated ANI values between examined genomes using BLASTN, which were then weighted by alignment fraction and corrected for saturation, arriving at an overall distance measurement called total ANI (tANI). A distance-based phylogenetic tree was inferred from all pairwise tANI values using the balanced minimum evolution algorithm, and the reliability of the tree topology was estimated by bootstrap replicate analysis. Well-resolved and highly supported phylogenies were obtained in both cases. The results showed that MH2^T is most closely related to *P. wayambapalatensis* RW3S1, which was isolated from exorhizosphere of rice grown in Sri Lanka (Fig. 1a). MH1^T is clustered with *S. humi* DSM 18929 and *S. terrae* DSM 18941, both of which were isolated from soil in Belgium (Fig. 1b) [29].

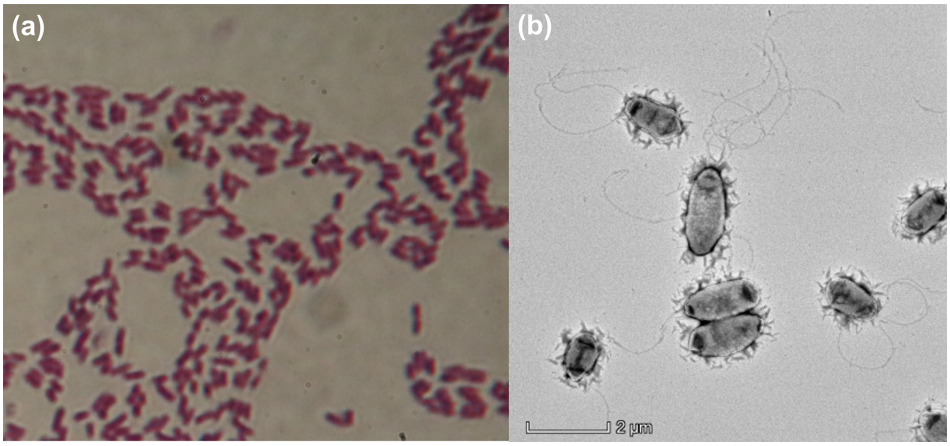

Fig. 2. Cell morphology (a) and transmissive electron microscopy image (b) of strain MH2^T.

The results of our genome sequencing and analyses indicate that MH2^T represents a novel species in the genus *Pseudomonas*, for which the name *P. machongensis* sp. nov. is proposed, and MH1^T represents a novel species in the genus *Stenotrophomonas*, for which the name *S. capsici* sp. nov. is proposed. We then carried out further phenotypic and chemotaxonomic characterizations on the two novel strains.

Physiology and chemotaxonomy

For each of MH2^T and MH1^T, the strain was cultured on LB plates to observe its colony morphology. Cell morphology was examined via transmission electron microscopy using Talos L120C (FEI, Czechia) at the Instrumental Analytical and Research Center of South China Agricultural University. Gram staining was conducted using the Gram staining solution (BKMAM, China). To investigate the optimal growth conditions of MH2^T and MH1^T, each strain was grown on LB at temperatures of 4, 15, 20, 28, 37 and 42 °C, with NaCl concentrations of 0–6.0% (w/v) with an interval of 1.0% (supplemented to the medium) and pH values of 4.0–10.0 at intervals of 1.0. Anaerobic growth capability was assessed using the anaerobic culture bag. Catalase activity was determined using a 30% (v/v) hydrogen peroxide solution. Oxidase activity was tested with the Oxidase Test Strip (HuanKai, China). MH2^T and MH1^T were also examined for their utilization of various carbon sources and sensitivity to inhibitory chemicals using the Biolog GEN III Microplate test panel.

MH2^T formed white round and flat colonies with a diameter of 1.1–2.4 mm after growth on LB at 28 °C for 1–2 days (Fig. S2a). Cells were Gram-negative (Fig. 2a), strictly aerobic, non-spore-forming, rod-shaped (1.2–2.2 μm long, 0.8–0.9 μm wide) and motile with a small number of polar flagella (Fig. 2b). Cell growth occurred at 4–42 °C (optimum 28 °C), 0–6.0 % NaCl (optimum 0%) and at pH 4.0–10.0 (optimum pH 5.0). The strain was positive for both catalase and oxidase activities. According to the results of the Biolog assay (Table 4), MH2^T can assimilate 34 of the 71 tested carbon sources. Comparison with the Biolog database showed that the phenotypic fingerprints of MH2^T are most similar to that of *P. syringae* pv. *pisi* with moderate confidence (SIM [similarity index value]=0.516, DIST [distance]=7.275).

MH1^T formed yellow, round and flat colonies with a diameter of 1.2–2.5 mm after growth on LB at 28 °C for 1–2 days (Fig. S2b). Cells were Gram-negative (Fig. 3a), strictly aerobic, non-spore-forming, rod-shaped (1.3–3.2 μm long and 0.5–0.6 μm wide) and motile with a small number of polar flagella (Fig. 3b). Cell growth occurred at 4–42 °C (optimum 28 °C), 0–6.0% NaCl (optimum 0%) and at pH 4.0–10.0 (optimum pH 4.0). The strain was positive for catalase and negative for oxidase activities. According to the results of the Biolog assay (Table 4), MH1^T can assimilate 24 of the 71 tested carbon sources. Comparison with the Biolog database showed that the phenotypic fingerprints of MH1^T are most similar to that of *S. maltophilia* with moderate confidence (SIM=0.617, DIST=5.602). Notably, MH1^T could utilize D-cellobiose, D-turanose and gentiobiose, which can be used to differentiate MH1^T from its closest relatives, *S. humi* and *S. terrae*.

To characterize their chemotaxonomic properties, MH2^T and MH1^T were cultured on LB plates at 28 °C for 48 h. For each strain, cellular fatty acid methyl esters were extracted from 100 mg of fresh colonies and analysed using the Sherlock Microbial Identification System (MIDI, version 6.0) as per the system's standard protocol. Polar lipids were extracted using a chloroform–methanol mixture and identified through two-dimensional TLC. Respiratory quinones were extracted following the method previously described and analysed using high-performance liquid chromatography. Analyses of whole-cell fatty acids, polar lipids and respiratory quinones were all conducted at the Guangdong Institute of Microbiology (Guangdong, China).

Table 4. Physiological characteristics of strain MH1^T and MH2^T

Carbon source reactions	MH2^T	MH1^T	Carbon source reactions	MH2^T	MH1^T
Polymer			Amides		
Dextrin	–	w	Glucuronamide	+	w
Gelatin	–	w	Amino acids, peptides and related chemicals		
Sugars and sugar derivatives			D-Aspartic acid	+	–
D-Cellobiose	–	w	Glycyl-L-proline	–	w
D-Fructose	w	+	L-Alanine	w	–
D-Fucose	+	w	L-Arginine	w	–
D-Galactose	w	–	L-Aspartic acid	+	–
D-Maltose	–	+	L-Glutamic acid	+	–
D-Mannitol	w	–	L-Histidine	w	–
D-Mannose	w	+	L-Pyroglutamic acid	w	–
D-Turanose	–	w	L-Serine	+	–
Gentiobiose	–	+	γ-Amino-butryric acid	w	–
L-Fucose	+	w	Alcohols		
N-Acetyl-D-galactosamine	–	w	D-Fructose-6-PO_4	+	w
N-Acetyl-D-glucosamine	–	w	Else		
α-D-Glucose	+	+	1 % Sodium lactate	w	+
Methyl esters			Fusidic acid	w	–
Methyl pyruvate	w	–	Troleandomycin	+	w
Carboxylic acids			Rifamycin SV	+	w
Acetic acid	w	w	Minocycline	w	–
Acetoacetic acid	–	w	Lincomycin	+	+
Bromo-succinic acid	w	–	Guanidine HCl	+	+
Citric acid	+	w	Niaproof 4	+	w
D-Galacturonic acid	+	w	Vancomycin	+	+
D-Gluconic acid	w	–	Tetrazolium violet	+	+
D-Glucuronic acid	w	w	Tetrazolium blue	+	+
D-Malic acid	w	–	Nalidixic acid	+	–
D-Saccharic acid	w	–	Lithium chloride	–	w
L-Galactonic acid lactone	+	w	Potassium tellurite	+	–
L-Lactic acid	+	w	Aztreonam	w	+
L-Malic acid	+	w			
Mucic acid	w	–			
Propionic acid	w	–			
Quinic acid	+	–			
α-Keto-glutaric acid	w	–			

No growth was observed on these carbon/sugar sources or carboxylic acids/alcohols: Pectin, Tween 40, 3-methyl glucose, D-arabitol, melibiose, raffinose, D-salicin, D-sorbitol, trehalose, L-rhamnose, myo-inositol, *N*-acetyl neuraminic acid, *N*-acetyl-*β*-D-Mannosamine, stachyose, sucrose, lactose, *β*-methyl-D-glucoside, D-lactic acid methyl ester, formic acid, *p*-hydroxy-phenylacetic acid, α-hydroxy-butyric acid, α-keto-butyric acid, D-serine, inosine, glycerol, D-glucose-6-PO_4, sodium butyrate and sodium bromate.

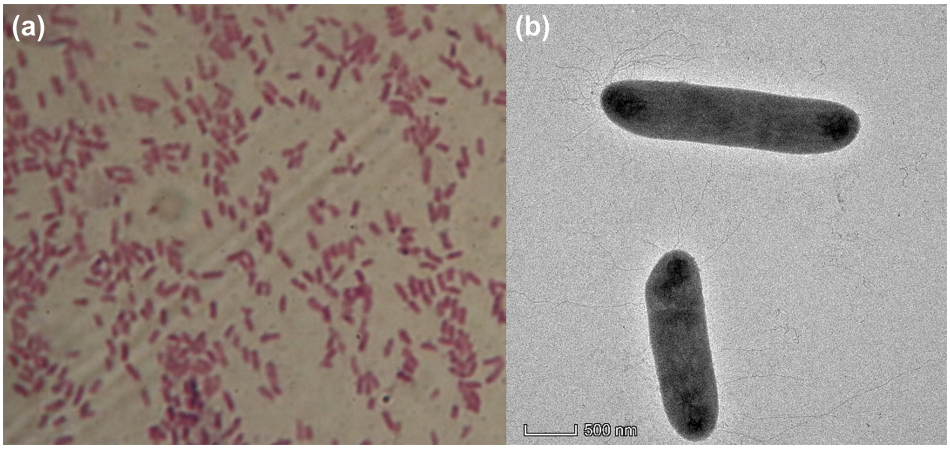

Fig. 3. Cell morphology (a) and transmissive electron microscopy image (b) of strain MH1^T.

The major cellular fatty acids (≥10%, same below) of MH2^T were $C_{16:0}$ (30.7%), $C_{18:1}$ ω7c/$C_{18:1}$ ω6c (summed feature 8, 20.5%), $C_{16:1}$ ω7c/$C_{16:1}$ ω6c (summed feature 3, 14.6%) and $C_{17:0}$ cyclo (13.6%) (Table S4) similar to the compositions previously reported for *Pseudomonas* strains [30]. The respiratory quinone of MH2^T was determined to be Q-9, the same as its close relatives in the phylogeny (Fig. 1a) [30]. The dominant polar lipids of MH2^T included diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and an unidentified aminolipid (Fig. S3). For MH1^T, the major cellular fatty acids were iso-$C_{15:0}$ (28.8%), iso-$C_{16:0}$ (14.8%) and iso-$C_{14:0}$ (12.1%) (Table S4), largely consistent with the fatty acid profiles of *S. humi* DSM 18929 and *S. terrae* DSM 18941 [28]. The respiratory quinone of MH1^T was found to be Q-8, which is common in *Stenotrophomonas*. The main polar lipid components of MH1^T included DPG, PE and PG, as well as unidentified aminolipid, aminophospholipids, lipids and phospholipids (Fig. S3). The polar lipid profiles of MH2^T and MH1^T are also typical to their respective genus.

DESCRIPTION OF *PSEUDOMONAS MACHONGENSIS* SP. NOV.

Pseudomonas machongensis (ma.chong.en'sis. N.L. fem. adj. *machongensis*, pertaining to Machong Town, Guangdong, China, the geographical origin of the type strain).

Cells are Gram-stain-negative, strictly aerobic, non-spore-forming, motile and rod-shaped (1.2–2.2 µm long, 0.8–0.9 µm wide). The colonies are white, circular, smooth and the edges are regular after incubation on LB broth at 28°C for 2 days. Growth occurs at 4–42 °C (optimum, 28°C), pH 4.0–10.0 (optimum, pH 5.0) and with 0–6% (w/v) NaCl (optimum, 0%). Catalase and oxidase activities are both positive. Biolog GEN III Microplate assay indicates that the cells can utilize acetic acid, bromo-succinic acid, citric acid, D-aspartic acid, D-fructose, D-fructose-6-phosphate, D-fucose, D-galactose, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, D-malic acid, D-mannitol, D-mannose, D-saccharic acid, D-serine, glucuronamide, L-alanine, L-arginine, L-aspartic acid, L-fucose, L-galactonic acid lactone, L-glutamic acid, L-histidine, L-lactic acid, L-malic acid, L-pyroglutamic acid, L-serine, methyl pyruvate, mucic acid, propionic acid, quinic acid, α-D-glucose, α-keto-glutaric acid and γ-amino-butyric acid. Cells are insensitive to aztreonam, fusidic acid, guanidine HCl, lincomycin, minocycline, nalidixic acid, niaproof 4, potassium tellurite, rifamycin sv, tetrazolium blue, tetrazolium violet, troleandomycin and vancomycin. The major cellular fatty acids are $C_{16:0}$, $C_{18:1}$ ω7c/$C_{18:1}$ ω6c (summed feature 8), $C_{16:1}$ ω7c/$C_{16:1}$ ω6c (summed feature 3) and $C_{17:0}$ cyclo, the respiratory quinone is Q-9, and the major polar lipids include DPG, PE, PG and an unidentified aminolipid.

The type strain, MH2^T (=GDMCC 1.3750^T=JCM 36318^T), was isolated from *C. annuum* showing wilt disease symptoms collected from a farmland in Machong town, Guangdong, China. The genome has a size of 5.71 Mb and a GC-content of 63.12 mol%. The GenBank accession number for the 16S rRNA gene sequence of strain MH2^T is PP061858; for the draft genome sequence of MH2^T is JAYFUI000000000.

DESCRIPTION OF *STENOTROPHOMONAS CAPSICI* SP. NOV.

Stenotrophomonas capsici (cap'si.ci. N.L. neut. gen n. *capsici*, referring to *Capsicum*, the genus name of pepper).

Cells are Gram-negative, strictly aerobic, non-spore-forming, motile and rod-shaped (1.3–3.2 µm long, 0.5–0.6 µm wide). Colonies are yellow, circular, smooth and edges are regular after incubation on LB at 28°C for 2 days. Growth occurs at 4–42 °C (optimum, 28°C), pH 4.0–10.0 (optimum, pH 4.0) and with 0–6% (w/v) NaCl (optimum, 0%). Catalase and oxidase

activities are negative and positive, respectively. Biolog GEN III Microplate assay indicates that the cells can utilize acetic acid, acetoacetic acid, citric acid, D-cellobiose, dextrin, D-fructose, D-fructose-6-phosphate, D-fucose, D-galacturonic acid, D-glucuronic acid, D-maltose, D-mannose, D-turanose, gelatin, gentiobiose, glucuronamide, glycyl-L-proline, L-fucose, L-galactonic acid lactone, L-lactic acid, L-malic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine and *α*-D-glucose. Cells are insensitive to aztreonam, guanidine HCl, lincomycin, lithium chloride, niaproof 4, rifamycin sv, tetrazolium blue, tetrazolium violet, troleandomycin and vancomycin. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{14:0}$, the respiratory quinone is Q-8, and the major polar lipids include DPG, PE, PG, as well as unidentified aminolipid, aminophospholipids, lipids and phospholipid.

The type strain, MH1^T (=GDMCC 1.3749^T=JCM 36317^T), was isolated from *C. annuum* showing wilt disease symptoms collected from a farmland in Machong town, Guangdong, China. The genome has a size of 4.18 Mb and a GC-content of 67.19 mol%. The GenBank accession number for the 16S rRNA gene sequence of strain MH1^T is PP060478; for the draft genome sequence of MH1^T is JAYFUH000000000.

Funding information
This study was supported by the National Key Area Research and Development Program of China (2022YFA1304401) and the Basic and Applied Basic Research Foundation of Guangdong Province (2022A1515010223).

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

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