

Lysobacter capsici sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus *Lysobacter*

Joo Hwang Park, Rumi Kim, Zubair Aslam, Che Ok Jeon and Young Ryun Chung

Correspondence
Young Ryun Chung
yrchung@gnu.ac.kr

Division of Applied Life Science (BK 21), PMBBRC and EB-NCRC, Gyeongsang National University, Jinju 660-701, Republic of Korea

The taxonomic position of a novel bacterial strain, YC5194^T, with antimicrobial activity, isolated from the rhizosphere of pepper in Jinju, South Korea, was studied using a polyphasic approach. Cells of the strain were Gram-negative, rod-shaped, facultative anaerobes. It grew at a temperature of 15–37 °C (optimum 28 °C). Growth of the strain occurred between pH 5.5 and 8.5, with an optimum of pH 7.0–7.5. The strain inhibited mycelial growth of *Pythium ultimum*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Botrytis cinerea*, *Rhizoctonia solani* and *Botryosphaeria dothidea* and growth of *Bacillus subtilis*. The G+C content of the total DNA was 65.4 mol%. The 16S rRNA gene sequence of the strain was most closely related to species of the genus *Lysobacter* (<94.0 to >99.0% sequence similarity). Chemotaxonomic data (major quinone, Q-8; major polar lipids, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidyl-N-methylethanolamine; major fatty acids, iso-C_{15:0}, summed feature 3, C_{16:0}, iso-C_{17:1ω9c} and C_{18:1ω7c}) supported the affiliation of strain YC5194^T to the genus *Lysobacter*. Phylogenetic analysis based on 16S rRNA gene sequences, DNA–DNA hybridization data and biochemical and physiological characteristics strongly supported the genotypic and phenotypic differentiation of strain YC5194^T from species of *Lysobacter* with validly published names. Strain YC5194^T therefore represents a novel species, for which the name *Lysobacter capsici* sp. nov. is proposed. The type strain is YC5194^T (=KCTC 22007^T =DSM 19286^T).

The genus *Lysobacter* is grouped in the family *Xanthomonadaceae*, belonging to the *Gammaproteobacteria*. *Lysobacter* species are typically found in soil and water habitats and are defined by characteristics that include gliding motility, a high G+C content and lytic activity against other micro-organisms, including fungi and nematodes (Christensen & Cook, 1978). The initial description for the genus *Lysobacter* reported in the 1970s was based primarily on phenotypic characteristics; recently, the genus *Lysobacter* has been recognized in the phylum *Proteobacteria* with the help of polyphasic taxonomy (Lee *et al.*, 2006; Weon *et al.*, 2006; Yassin *et al.*, 2007).

Some *Lysobacter* strains have been reported to have antifungal and antimicrobial activities. *Lysobacter* sp. XL1

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YC5194^T is EF488749.

Transmission electron micrographs of cells of strain YC5194^T, TLC of polar lipids of two related type strains, fatty acid profiles and API ZYM results for strain YC5194^T and related type strains and a detailed antimicrobial spectrum for strain YC5194^T are available as supplementary material with the online version of this paper.

produces lysoamidase, from which a drug is derived that is used in the treatment of external infections caused by pathogenic Gram-positive bacteria. It is also effective against many yeast and fungi groups (Ryazanova *et al.*, 2005). *Lysobacter enzymogenes* was shown to be a potential biocontrol agent for plant fungal pathogens (Folman *et al.*, 2004; Kilic-Ekici & Yuen, 2003). *Lysobacter* sp. SB-K88, previously known as a *Stenotrophomonas* strain (Nakayama *et al.*, 1999), produces three antifungal compounds, xanthobaccins A, B and C; xanthobaccin A suppresses the sugar beet damping-off disease (Islam *et al.*, 2005). Some *Lysobacter* isolates had strong antibiotic activity against Gram-positive bacteria, especially methicillin-resistant *Staphylococcus aureus* (Ahmed *et al.*, 2003; Chohnan *et al.*, 2002; Hashizume *et al.*, 2001, 2004; Kato *et al.*, 1998).

Strain YC5194^T was identified on the basis of 16S rRNA gene sequences, DNA–DNA hybridization data and other chemotaxonomic and genotypic characteristics as belonging to a novel species in the genus *Lysobacter*.

In the process of screening biological control agents for plant-pathogenic fungi, especially those active against

Pythium ultimum and *Colletotrichum gloeosporioides*, soil samples were collected from the rhizosphere of pepper (*Capsicum annuum* L.) in Jinju, Korea, in July 2003. Ten grams of each soil sample was serially diluted in 100 ml distilled deionized water. Plates containing 2 % tryptic soy agar (2 % TSA; Difco) (pH 7.2) were inoculated with 0.1 ml aliquots and incubated at 28 °C for 3 days. Only bacterial strains with antifungal or antibacterial activity were selected (Chung *et al.*, 1999, 2000). The details of fungal, yeast and bacterial strains used to check antimicrobial activity are given in Supplementary Table S3 (available in IJSEM Online). Strain YC5194^T, with a broad antifungal spectrum, was further investigated for its taxonomic position because the 16S rRNA gene sequence of the strain was most closely related to those of species of the genus *Lysobacter* (<94.0 to >99.0 % sequence similarity).

Cell morphology was observed under a Nikon light microscope at ×1000 magnification and by using a transmission electron microscope (Hitachi H-600) with cells grown for 1 day at 28 °C on 2 % TSA. Catalase and oxidase tests were performed by the procedures outlined by Cappuccino & Sherman (2002). Carbohydrate assimilation and enzyme activities were determined by using API ID 32 E and API ID32 STREP at 30 °C and API ZYM at 37 °C according to the instructions of the manufacturer (bioMérieux). Growth at different temperatures (4, 15, 20, 28, 37 and 45 °C) was tested on 2 % TSA. Anaerobic growth was tested at 28 °C by pouring a thick layer of vaspar (50 % petrolatum, 50 % paraffin) on the surface of inoculated 2 % TSA in 35 ml screw-capped glass tubes (Costilow, 1981). Degradation of chitin, starch, gelatin and Tween 20 was investigated by using the protocols outlined by Atlas (1993). Growth at different temperatures and pH was assessed after 3 days incubation. Salt tolerance was tested in 2 % TSA broth medium supplemented with 1–10 % (w/v) NaCl after 7 days incubation. Duplicate antibiotic-sensitivity tests were done using filter-paper discs containing the following: tetracycline, kanamycin, ampicillin (Sigma) and rifampicin, each at concentrations of 10, 50 and 100 µg ml⁻¹. Discs were placed on 2 % TSA plates that had been spread with strain YC5194^T or reference strains and the plates were then incubated at 28 °C for 3 days. Almost all tests were also performed with reference strains *Lysobacter gummosus* KCTC 12132^T, *Lysobacter antibioticus* KCTC 12129^T, *Lysobacter enzymogenes* KCTC 12131^T, *Lysobacter koreensis* KCTC 12204^T, *Lysobacter daejeonensis* DSM 17634^T and *Lysobacter yangpyeongensis* DSM 17635^T.

Extraction of genomic DNA was done using a commercial genomic DNA extraction kit (Core Biosystem). The 16S rRNA gene of strain YC5194^T was PCR-amplified from a small amount of purified genomic DNA by using primers 27F and 1492R (Lane, 1991). The PCR product obtained was purified and sequenced according to Chung *et al.* (1999). 16S rRNA gene sequence was compiled using SeqMan software (DNASTAR) and the sequences of related taxa were obtained from the GenBank database. Multiple

alignments were performed by CLUSTAL_X (Thompson *et al.*, 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in the MEGA3 program (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

For the measurement of G + C content of the chromosomal DNA, genomic DNA of strain YC5194^T was extracted and purified as described by Ausubel *et al.* (1995). DNA was then enzymically degraded into nucleosides and the G + C content was determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Cellular fatty acids were analysed using *Lysobacter* strains grown on TSA for 2 days at 28 °C. Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System version 4.0 software (MIDI). Fatty acids analysed by GC (Hewlett Packard 6890) were identified using the Microbial Identification software package (Khan *et al.*, 1996). The quinone system and polar lipids were determined by TLC, as described by Lechevalier *et al.* (1977) and Tindall (1990).

DNA–DNA hybridization was carried out to evaluate the genomic DNA relatedness between strain YC5194^T and those *Lysobacter* strains that had high 16S rRNA gene sequence similarity (>97.0 %) to strain YC5194^T (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). Extracted genomic DNA was fragmented with *Hae*III for slot hybridization. Digested DNAs were diluted serially and loaded into slots with three replications for each sample and DNAs were used individually as labelled probes for cross-hybridization. Randomly primed DNA labelling with digoxigenin-dUTP and hybridization (hybridization temperature, 53 °C; washing temperature, 65–68 °C) were performed using a DIG High Prime DNA labelling kit (Roche Applied Science) according to the manufacturer's instructions and standard procedures (Lim *et al.*, 2005; Sambrook & Russell, 2001). The signals of the dilution series were quantified using Bio-Rad GelDoc scanning software. The signals produced by self-hybridization were inferred as 100 %, and hybridization values (%) were calculated from the results of three experiments.

The novel strain was Gram-negative and non-motile and had gliding activity. Cells were rod-shaped (0.3–0.5 × 2.0–20 µm) and occurred singly and in pairs and irregular filament-like forms (Supplementary Fig. S1). Colonies grown on 2 % TSA plates for 3 days were smooth, circular and creamy white to yellow, 2–4 mm in diameter, and they changed form as they grew older due to motility. Fruiting bodies were not observed. Growth was facultatively anaerobic. Only strain YC5194^T of seven tested *Lysobacter* strains had α-galactosidase activity. Physiological characteristics of strain YC5194^T are summarized in the species

Table 1. Differential phenotypic characteristics between strain YC5194^T and related *Lysobacter* type strains

Strains: 1, YC5194^T (*L. capsici* sp. nov.); 2, *L. gummosus* KCTC 12132^T; 3, *L. antibioticus* KCTC 12129^T; 4, *L. enzymogenes* KCTC 12131^T; 5, *L. koreensis* KCTC 12204^T; 6, *L. daejeonensis* DSM 17634^T; 7, *L. yangpyeongensis* DSM 17635^T. All data were obtained in this study unless indicated. +, Positive; –, negative; ±, weak; ND, not determined; EMB, eosin methylene blue. All of the strains were able to hydrolyse casein, gelatin and Tween 20 but not agar or urea and all strains showed optimal growth in the absence of NaCl.

Characteristic	1	2	3	4	5	6	7
Cell size (µm)	0.3–0.5 × 2.0–20	0.4 × 2.0	0.4 × 6.5	0.5 × 38.0	0.5–0.8 × 1.5–2.0	0.4–0.6 × 3.0–4.0	0.4–0.6 × 3.0–4.0
Colony type	Smooth	Smooth	Mucoid	Smooth	Smooth	Smooth	Smooth
Colony colour*	Y–C	PY	C	DY–C	C	DY	Y–G
NaCl concentration for growth (% w/v)							
Range	0–2	0–2	0–1	0–1	0–2	0–3	0–1
No growth	3	3 ^{a†}	3 ^a	3 ^a	3 ^b	ND	ND
Catalase	+	+	+	+	+	–	–
Oxidase	+	+	+	+	–	+	+
α-Galactosidase	+	–	–	–	–	–	–
Trypsin	+	–	–	+	–	+	+
α-Glucosidase	+	–	–	+	–	+	+
N-Acetyl-β-glucosaminidase	–	+	+	–	–	–	+
Hydrolysis of:							
Chitin	+	+	+	+	–	–	–
Aesculin	+	+	–	+	+	+	+
Starch	–	–	–	–	–	–	+
Hippurate	+	–	–	–	–	–	+
Growth on/at:							
Citrate	+	+	+	+	+	–	–
D-Glucose	–	+	–	+	–	+	–
Arabinose	–	+	–	–	+	–	–
Maltose	–	+	+	+	–	+	–
pH 4	–	+	–	±	ND	–	–
pH 10	–	–	–	+	ND	–	–
MacConkey agar	–	–	–	+	–	–	–
EMB agar‡	+	(Pi)	+	(Pi)	+	(Pi)	–
G + C content (mol%)	65.4	65.7 ^a	69.2 ^a	69.0 ^a	68.9 ^b	61.7 ^c	67.3 ^c

*C, Cream-coloured; DY, deep yellow; G, green; PY, pale yellow; Y, yellow.

†Data taken from other studies as indicated: *a*, Christensen & Cook (1978); *b*, Lee *et al.* (2006); *c*, Weon *et al.* (2006).

‡Colour of growth indicated as: Pi, pink; Po, pinkish-orange.

description and a comparison of selective characteristics with related type strains is shown in Table 1.

The cellular fatty acid profiles of strain YC5194^T and related *Lysobacter* type strains are shown in Supplementary Table S1. The major cellular fatty acids in strain YC5194^T included iso-C_{15:0} (23.3 %), summed feature 3 (20.4 %), C_{16:0} (10.8 %), iso-C_{17:1ω9c} (9.3 %), C_{18:1ω7c} (6.5 %), iso-C_{11:0} 3-OH (3.8 %), iso-C_{17:0} (3.7 %), iso-C_{11:0} (2.3 %), C_{16:1ω11c} (2.2 %), C_{14:0} (1.9 %), C_{15:0} (1.7 %) and trace amounts (<1.0 %) of some other fatty acids were detected, which are shown in Supplementary Table S1. Although the major fatty acids were similar to those of other members of the genus *Lysobacter*, significant differences were found between different species in the genus and the novel strain. C_{9:0} and C_{16:1ω11c} (2.2 %) were detected only in strain YC5194^T (Supplementary Table S1). The following polar lipids are present: diphosphatidylglycerol,

phosphatidylethanolamine, phosphatidylglycerol and phosphatidyl-N-methylethanolamine. An unknown polar lipid AL1 (amino group-containing lipid) was also detected (Fig. 1). The polar lipids of two closely related type strains, *L. gummosus* KCTC 12132^T and *L. antibioticus* KCTC 12129^T, were also determined. The major polar lipids were the same, but the unknown amino group-containing lipids differed from species to species (Fig. 1 and Supplementary Fig. S2).

Strain YC5194^T inhibited growth of the fungi *Botrytis cinerea*, *Botryosphaeria dothidea*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Aspergillus fumigatus*, the yeast *Candida albicans* and yeast-like *Cryptococcus neoformans* and the Gram-positive bacterium *Bacillus subtilis*. It has particularly strong inhibition activity against *Colletotrichum gloeosporioides*, *Colletotrichum coccodes*, *Colletotrichum orbiculare* and *Pythium ultimum*.

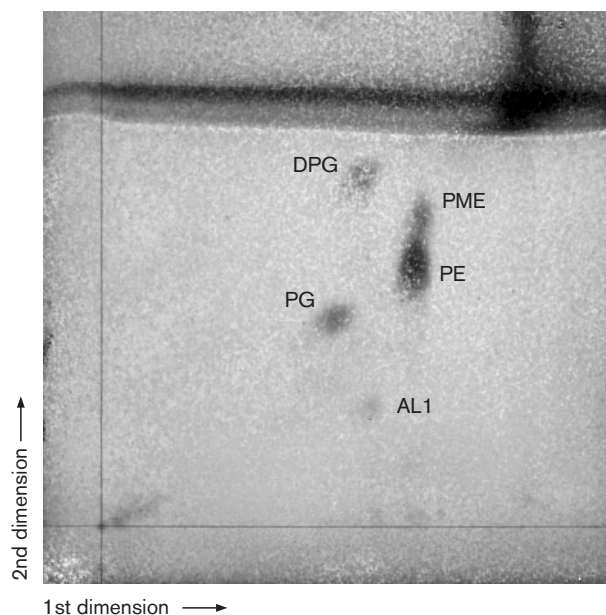


Fig. 1. Two-dimensional TLC of polar lipids from strain YC5194^T. Ascending solvent system: 1st dimension, chloroform/methanol/water (65:25:4, by vol.); 2nd dimension, chloroform/acetic acid/methanol/water (80:13:10:1.6, by vol.). For detection of polar lipids, molybdatophosphoric acid [for phosphatidylethanolamine (PE), phosphatidyl-*N*-methylethanolamine (PME), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG)] was applied. AL1, Unknown amino group-containing lipid.

(Supplementary Table S3). Other reference strains had no inhibitory activity against *Pythium ultimum* or *Colletotrichum gloeosporioides* except *L. gummosus*, which had a weak inhibitory effect on *Colletotrichum gloeosporioides* (Fig. 2).

The DNA G + C content of strain YC5194^T was 65.4 mol%. This value is within the range for the genus *Lysobacter* (65.4–70.1 mol%; Christensen & Cook, 1978). Q-8 was the major quinone in strain YC5194^T and in all type strains of recognized *Lysobacter* species.

The 16S rRNA gene sequence of strain YC5194^T was a continuous stretch of 1470 bp. Sequence similarity calculations after a neighbour-joining analysis indicated that the closest relatives of the novel strain were *L. gummosus* ATCC 29489^T (99.59 % similarity), *L. antibioticus* DSM 2044^T (99.40 %), *L. enzymogenes* DSM 2043^T (97.35 %) and *L. koreensis* KCTC 12204^T (97.0 %); low levels of similarity (<97.0 %) were found with other type strains of the genus *Lysobacter*. Phylogenetic trees based on the neighbour-joining and maximum-parsimony methods (Fig. 3) showed that strain YC5194^T formed a cluster supported with a high bootstrap value (100 %).

Strain YC5194^T exhibited 8, 10, 25 and 40 % DNA–DNA relatedness, respectively, to *L. koreensis* KCTC 12204^T, *L. enzymogenes* DSM 2043^T, *L. gummosus* ATCC 29489^T and *L. antibioticus* DSM 2044^T. The DNA–DNA relatedness values with *L. daejeonensis* DSM 17634^T and *L. yangpyeongensis* DSM 17635^T were below 5 %, which showed that strain YC5194^T belongs to a distinct genomic species (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). According to Fox *et al.* (1992) and Stackebrandt *et al.* (2002), 16S rRNA gene sequences, protein-coding gene sequences and DNA–DNA hybridization should be considered as molecular criteria for species delineation. In cases of very high similarity (16S rRNA gene sequences), DNA–DNA hybridization is a more powerful and helpful tool to distinguish strains at the species level.

Thus 16S rRNA gene sequence similarity, phylogenetic analysis, G + C content, Gram-staining behaviour (Gram-negative), gliding activity and other enzyme activities clearly indicate that strain YC5194^T belongs to the genus *Lysobacter*. To the best of our knowledge, this is the first classified member of the genus *Lysobacter* that has antifungal activity against *Pythium ultimum*. Strain YC5194^T also showed different results in antibiotic-sensitivity tests compared with reference strains (*L. gummosus* KCTC 12132^T, *L. antibioticus* KCTC 12129^T and *L. enzymogenes* KCTC 12131^T). Strain YC5194^T is positive for chitinase, gelatinase and lipase but negative for hydrolysis of starch, agar and cellulose. Thus, low DNA–DNA relatedness with other *Lysobacter* species, significant differences in fatty acid profiles (Supplementary

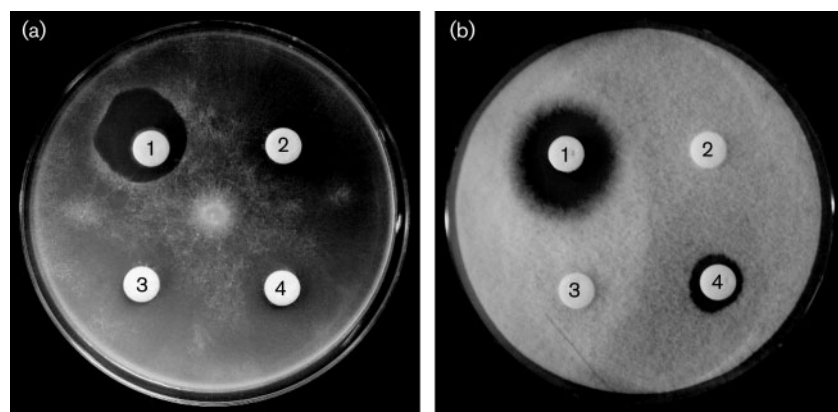


Fig. 2. Effect on mycelial growth of *Pythium ultimum* (a) and *Colletotrichum gloeosporioides* (b) of culture filtrates of strain YC5194^T (1), *L. antibioticus* KCTC 12129^T (2), *L. enzymogenes* KCTC 12131^T (3) and *L. gummosus* KCTC 12132^T (4).

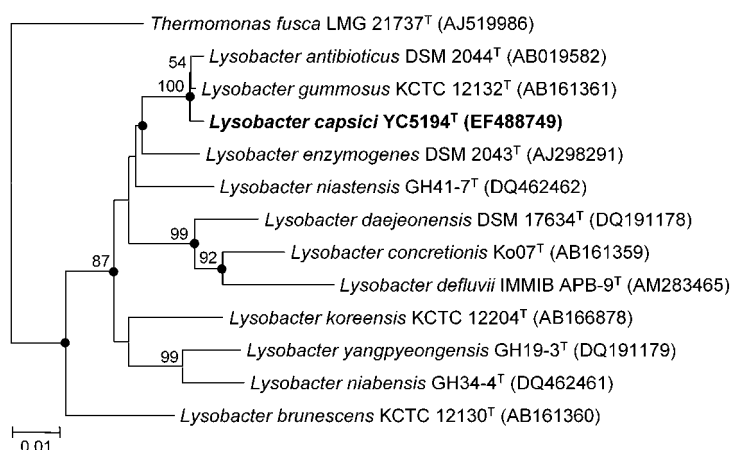


Fig. 3. Phylogenetic tree constructed from comparative analysis of 16S rRNA gene sequences showing the relationships of strain YC5194^T with other related species. The tree was constructed by using the neighbour-joining method and Jukes and Cantor evolutionary distance matrix data obtained from unambiguously aligned nucleotides. Solid circles indicate that the corresponding clades were also recovered in maximum-parsimony trees. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Bar, 1 substitution per 100 nucleotide positions.

Table S1), polar lipid differences (Fig. 1 and Supplementary Fig. S2), phylogenetic trees (Fig. 3), antibiotic sensitivities and some physiological and biochemical characteristics (Table 1, Supplementary Table S2 and Supplementary Fig. S2) clearly distinguish strain YC5194^T as a member of a novel species of the genus *Lysobacter*, for which the name *Lysobacter capsici* sp. nov. is proposed.

Emended description of the genus *Lysobacter*

Characteristics are the same as given in the original description of the genus *Lysobacter* (Christensen & Cook, 1978) with the following amendment. The genus *Lysobacter* has the following polar lipids: diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidyl-*N*-methylethanolamine. Some unknown amino group-containing lipids are also present, which may differ from species to species (Fig. 1 and Supplementary Fig. S2).

Description of *Lysobacter capsici* sp. nov.

Lysobacter capsici (cap'si.ci. N.L. gen. n. *capsici* of *Capsicum*, referring to the isolation of the type strain from the rhizosphere of *Capsicum annuum* L.).

Cells are Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming and non-motile but having gliding activity, 0.3–0.5 µm wide by 2.0–20 µm long. Cells occur singly and in pairs and can form irregular filament-like structures. Colonies grown on 2 % TSA at 30 °C for 3 days are 2.0–4.0 mm in diameter, creamy white to yellow, smooth and circular. The temperature range for growth is 15–37 °C (very weak growth at 4 °C); no growth occurs at 45 °C. The optimum temperature for growth is 28 °C. The pH range for growth is pH 5.5–8.5, with an optimum of pH 7.0–7.5. Growth occurs in the absence of NaCl and in the presence of 1.0–2.0 % (w/v) NaCl, but not in 3.0 % (w/v) NaCl. Sensitive to 10 µg rifampicin, 50 µg kanamycin and 50 µg tetracycline ml⁻¹, but resistant to 100 µg ampicillin ml⁻¹. Catalase- and oxidase-positive. Uses 5-bromo-3-indoxyl nonanoate, 4-nitrophenyl β-D-glucopyranoside and 4-nitrophenyl β-D-galactopyranoside

as sole carbon sources but not the following: L-arabitol, galacturonic acid, potassium 5-ketogluconate, sodium pyruvate, D-mannitol, maltose, adonitol, palatinose, L-tryptophan, 5-bromo-4-chloro-3-indolyl *N*-acetyl-β-D-glucosaminide, D-glucose, sucrose, L-arabinose, D-arabitol, trehalose, D-rhamnose, inositol, cellobiose and D-sorbitol. Acid is produced from ribose, mannitol, sorbitol, lactose, trehalose, raffinose, glycogen, pullulan, maltose, melibiose, melezitose, methyl β-D-glucopyranoside and tagatose. Urea is not hydrolysed. Cannot produce acid from sucrose, L-arabinose, D-arabitol or cyclodextrin. Tests for β-glucuronidase and β-mannosidase are positive. Acetoin is not produced. Enzyme activities for chitinase, alkaline phosphatase, esterase (C-4), esterase lipase (C-8), lipase (C-14), leucine arylamidase, trypsinase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase and β-glucosidase are present, but valine arylamidase, cystine arylamidase, α-chymotrypsin, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Q-8 is the major quinone. Has the major polar lipids listed in the amended genus description. The DNA G + C content of the type strain is 65.4 mol%.

The type strain, YC5194^T (=KCTC 22007^T =DSM 19286^T), was isolated from the rhizosphere of pepper in Jinju, Korea.

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