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# *Lysobacter sedimenti* sp. nov., Isolated from the Sediment, and Reclassification of *Luteimonas lumbrici* as *Lysobacter lumbrici* comb. nov

**Xuan Zhang[1] · Ningning Wu[1] · Keke Geng[1] · Pan Yang[2] · Cuiwei Chu[2] · Jian He[1]**

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## Abstract

A bacterium, designated 50$^T$ was isolated from the sediment of a pesticide plant in Shandong Province, PR China. The strain was non-motile, Gram stain-negative, rod shaped and grew optimally on NA medium at 30 °C, pH 7.5 and with 0% (w/v) NaCl. Strain 50$^T$ showed the highest 16S rRNA gene sequence similarity with *Lysobacter pocheonensis* Gsoil 193$^T$ (96.7%), followed by *Luteimonas lumbrici* 1.1416$^T$ (96.5%). Phylogenetic analyses based on 16S rRNA indicated that strain 50$^T$ and *Luteimonas lumbrici* 1.1416$^T$ were clustered with the genus of *Lysobacter* and formed a subclade with *Lysobacter pocheonensis* Gsoil 193$^T$. In the phylogenetic analysis based on the genome sequences, strain 50$^T$ and *Luteimonas lumbrici* 1.1416$^T$ were also clustered with the type strains of the genus *Lysobacter*. The obtained ANI and the dDDH value between 50$^T$ and *Luteimonas lumbrici* 1.1416$^T$ were 80.6% and 24.0%, respectively. The respiratory quinone was ubiquinone-8 (Q-8), and the major cellular fatty acids were iso-$C_{15:0}$ (31.7%), summed feature 9 (iso-$C_{17:1}$ $\omega$9*c* or $C_{16:0}$ 10-methyl) (23.7%), iso-$C_{17:0}$ (14.3%) and iso-$C_{16:0}$ (12.6%). The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and unidentified aminophospholipid, unidentified phospholipid and unidentified lipid. The genomic DNA G + C content was 69.5 mol%. According to the phenotypic, chemotaxonomic and phylogenetic analyses, strain 50$^T$ represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter sedimenti* sp. nov. is proposed, with strain 50$^T$ (= KCTC 92088$^T$ = CCTCC AB 2022035$^T$) as the type strain. In this study, it is also proposed that *Luteimonas lumbrici* should be transferred to the genus *Lysobacter* as *Lysobacter lumbrici* comb. nov. The type strain of *Lysobacter lumbrici* is 1.1416$^T$ (= KCTC 62979$^T$ = CCTCC AB 2018348$^T$).

## Introduction

The genus *Lysobacter* (family *Xanthomonadaceae*, order *Xanthomonadales*, class *Gammaproteobacteria*, phylum Proteobacteria) was first proposed by Christensen and Cook [1]. Cells of the members of the genus *Lysobacter* are Gram stain-negative, aerobic and rod shaped, contain ubiquinone-8 as the major respiratory quinone and have high DNA G + C contents. At the time of writing, the genus comprises 66 species with validly published names, and the type species is *Lysobacter enzymogenes*. The type strains of these species were isolated from various environments, including soil, sediments and aqueous habitats. Here, we present a polyphase taxonomic study to describe a novel bacterium, named strain 50$^T$, which was isolated from the sediment of a pesticide plant. The strain is related with the genus *Lysobacter* but clearly differs from them in phenotype and genotype. We propose to establish a new species *Lysobacter sedimenti* sp. nov.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences and the whole genome of strain 50$^T$ are ON037515 and JALGCL000000000, respectively.

✉ Cuiwei Chu
20171071@zknu.edu.cn

✉ Jian He
hejian@njau.edu.cn

[1] Key Laboratory of Agricultural Environmental Microbiology, Ministry of Agriculture, College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, Jiangsu, People's Republic of China

[2] College of Life Sciences and Agronomy, Zhoukou Normal University, Zhoukou 466000, Henan, People's Republic of China

## Materials and Methods

### Strain Isolation

Strain $50^T$ was isolated from sediment in a pesticide plant (117° 97′ E, 37° 38′ N) in Bingzhou city, Shandong province, PR China, in June, 2021. To isolate the bacterial strain, 10 g of sample was suspended in 90-mL sterile water and then diluted and spread onto nutrient agar medium (NA) (beef extract 3 g, peptone 10 g, NaCl 5 g, agar 17 g, water 1000 mL, pH 7.2). After incubation at 30 °C for 4 days, single colonies were purified by repeated streaking on NA plates. A strain, designated as $50^T$, that formed yellow, sticky and circular colonies was isolated and purified. The strain was preserved at − 80 °C in NA broth supplemented with 20% (v/v) glycerol. On the basis of the analysis of 16S rRNA gene sequences and genome sequences, strain $50^T$ was screened out as a candidate novel taxon for this study.

### Phylogenetic and Genome Sequence Analyses

The 16S rRNA sequence of strain $50^T$ was amplified by PCR using the primers 27F and 1492R [2]. The 16S rRNA gene sequence of $50^T$ obtained in this study was compared with sequences from EzBioCloud using BLAST (https://www.ezbiocloud.net/) [3]. Phylogenetic analysis was performed using three tree-making algorithms: the neighbour-joining (NJ) [4], maximum-likelihood [5] and minimum-evolution (ME) [6] algorithms using the software MEGA7 [7]. Evolutionary distances were calculated using Kimura's two-parameter method [8]. The topologies of the resultant trees were evaluated using the bootstrap resampling method of Felsenstein [9] with 1000 replicates. The phylogenomic tree was also reconstructed based on amino acid sequences of core genes identified using the BPGA 1.3 pipeline [10]. Missing data and poorly aligned regions from concatenated protein sequence alignment of the core genome were removed using Gblock [11]. A ML tree was reconstructed from the resulting alignment of 16,439 amino acid sequences using IQ-Tree [12] with the best-fit substitution model (LG + F + R4) and 1000 SH-aLRT and 1000 ultrafast bootstrap replicates.

The total DNA of strain $50^T$ was extracted according to the method described by Sambrook et al. [13]. The draft genome of strain $50^T$ was sequenced by Illumina Hiseq 4000 platform at Shanghai Biozeron Biotechnology Co., Ltd (Shanghai, PR China). Paired end libraries with average insert length 350 bp were constructed and then, 100 × libraries were obtained from clean paired end read data. Raw sequencing data assembly was performed using SOAPdenovo version 2.04 [14]. The genome of *Luteimonas lumbrici* $1.1416^T$ (SRZZ00000000) was retrieved from the NCBI database, whilst the genome of *Lysobacter pocheonensis* Gsoil $193^T$ could not be retrieved. The assembled genome was annotated with the Rapid Annotation with Subsystem Technology (RAST) server [15]. Comparative genomic analysis was carried out by the compare-function-based tool of the SEED Viewer [16]. The genomic DNA G + C content of $50^T$ was determined from the genome sequence. To further clarify the taxonomic relationship between strain $50^T$ and *Luteimonas lumbrici* $1.1416^T$, average nucleotide identity (ANI) values and digital DNA–DNA hybridization (dDDH) between them were calculated using the OrthoANIu algorithm (https://www.ezbiocloud.net/tools/ani) and genome-to-genome distance calculator (http://ggdc.dsmz.de/ggdc.php/) [17], respectively.

### Chemotaxonomic Characteristics

For analysis of fatty acids, cells of strain $50^T$ were freeze-dried and then cellular fatty acid were saponified, methylated and extracted as described by the Sherlock MIS (MIDI) system [18]. The fatty acid methyl esters were separated using a gas chromatograph (6890 N; Agilent) and identified by the MIDI Sherlock MIS (Library: TSBA6; version, 6.0B). Polar lipids were examined using freeze-dried cells and appraised using two-dimensional TLC as described previously [19, 20]. Respiratory quinones were extracted and detected by HPLC as described by Komagata and Suzuki [21].

### Morphological, Physiological and Biochemical Characteristics

To analyse the biochemical and physiological characteristics, strain $50^T$ was cultivated in nutrient broth (NB) at 30 °C. The Gram stain was performed using the method described by Beveridge et al. [22]. Cell size and morphology were observed by transmission electron microscopy (H-7650, Hitachi). Growth at various temperatures was determined at 10–52 °C (10, 16, 20, 25, 30, 37, 42, 45 and 52 °C) in NA medium. The pH range for growth was assessed in NB with the pH being adjusted to 4.0–10.0 (at intervals of 0.5 pH unit) using citrate/$Na_2HPO_4$ buffer (pH 4.0–5.0), $Na_2HPO_4$/$NaH_2PO_4$ buffer (pH 6.0–8.0) and glycine/NaOH buffer (pH 9.0–10.0). Tolerance of NaCl was tested using modified NB as the basal medium supplemented with 0–10.0% (w/v) NaCl at intervals of 0.5%. Growth was monitored by measuring $OD_{600nm}$. Enzymatic activities and utilization of various carbon sources were investigated using commercial kits (API 50CH, API ZYM and API 20E) according to the manufacturer's protocol.

## Results and Discussion

### Phylogenetic and Genomic Analyses

An almost complete 16S rRNA gene sequence of $50^T$ (1449 bp) was determined. Blast in EzBioCloud indicated that strain $50^T$ showed the highest 16S rRNA gene sequence similarity with *Lysobacter pocheonensis* Gsoil193$^T$ (96.7%), followed by *Luteimonas lumbrici* 1.1416$^T$ (96.5%). Phylogenetic analyses based on the NJ (Fig. S1), ML (Fig. S2) and ME (Fig. S3) algorithms revealed that strain $50^T$ grouped with the members of the genus *Lysobacter* and formed a subclade with *Luteimonas lumbrici* 1.1416$^T$ and *Lysobacter pocheonensis* Gsoil193$^T$. Phylogenetic analyses based on genome sequences (Fig. 1) also revealed that $50^T$ and *Luteimonas lumbrici* 1.1416$^T$ clustered with the type stains of the genus *Lysobacter*.

The genome size of strain $50^T$ was approximately 2.8 Mb, which was a little larger than that of *Luteimonas lumbrici* 1.1416$^T$ (2.4 Mb); the number of contigs was 9, and the contig 50 was 1421.5 kb. The number of protein-coding genes was 2495, the number of genes assigned to clusters of orthologous groups (COGs) was 2157 and the number of genes assigned to the Kyoto Encyclopedia of Genes and Genomes (KEGG) was 1512, all of which were lager than those of *Luteimonas lumbrici* 1.1416$^T$ (2256, 1597 and 1309, respectively). The genomic DNA G + C content of $50^T$ was 69.5 mol%, whilst was a little higher than of *Lysobacter pocheonensis* Gsoil193$^T$ (64.8%) and a little lower than that of *Luteimonas lumbrici* 1.1416$^T$ (70.5%).

In addition, the obtained ANI between $50^T$ and *Luteimonas lumbrici* 1.1416$^T$ was 80.6%, which was below the 95% standard threshold recommended for novel species [23]. The dDDH value between $50^T$ and *Luteimonas lumbrici* 1.1416$^T$ was 24.0%, which was below the 70% standard threshold recommended for novel species [24]. Thus, strain $50^T$ should represent a novel species according to the results of ANI and dDDH.

### Phenotypic and Physiological Characteristics

Colonies of strain $50^T$ grown on NA plate were yellow, sticky, convex and smooth. Cells of strain $50^T$ was Gram stain-negative, non-motile, non-spore forming and rod shaped (1.8–2.9 μm in length and 0.2–0.5 μm in width) (Fig. S4). Growth was observed at 20–40 °C (optimum 30 °C), pH 6.0–9.0 (optimum 7.5) and salinity of 0%–1.0% (w/v, optimum 0%) (Table 1). Distinctive phenotypic and genotypic characteristics of $50^T$, *Luteimonas lumbrici* 1.1416$^T$ [25] and *Lysobacter pocheonensis* Gsoil 193$^T$ [26] are shown in Tables 1 and S1.

### Chemotaxonomic Analysis

The cellular fatty acid profiles of strain $50^T$ are presented in Table S2. Strain $50^T$ contained iso-$C_{15:0}$ (31.7%) and summed feature 9 (iso-$C_{17:1}$ $\omega 9c$ or $C_{16:0}$ 10-methyl) (23.7%), iso-$C_{17:0}$ (14.3%), and iso-$C_{16:0}$ (12.6%) as major fatty acids which were consistent with those of *Luteimonas lumbrici* 1.1416$^T$ [25] and *Lysobacter pocheonensis* Gsoil 193$^T$ [26]. However, some qualitative and quantitative differences in fatty acid content could be observed between $50^T$ and the two reference strains. The major polar lipids of $50^T$ were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, a unidentified aminophospholipid, two unidentified phospholipids and a unidentified lipid, which were basically consistent with those of *Luteimonas lumbrici* 1.1416$^T$, except that strain 1.1416$^T$ did not

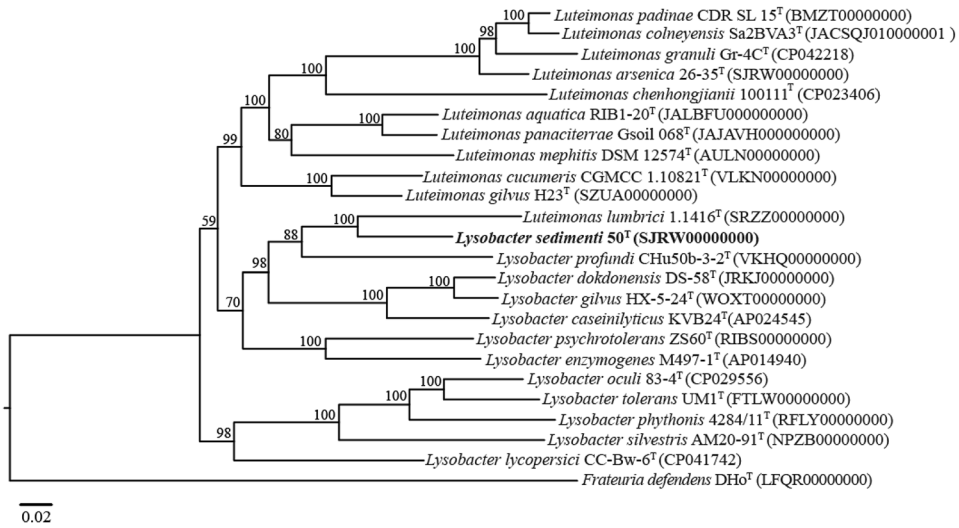


**Fig. 1** Phylogenetic tree based on the genome sequences showing the phylogenetic position between strain $50^T$ and type strains of the known species of the genera *Lysobacter* and *Luteimonas*. The tree based on concatenated amino acid sequences of the core genes are reconstructed by the maximum-likelihood method. Genome accession numbers are indicated in parentheses. Bootstrap values based on 1000 replications are indicated at branch nodes. Bar, 0.02 substitutions per nucleotide position

**Table 1** The different physiological and biochemical characteristics between $50^T$ and related strains

| Characteristics | 1 | 2 | 3 |
|---|---|---|---|
| Colony colour | Yellow | Yellow–green | Yellowish |
| Motility | Non-motile | Non-motile | Gliding |
| Temperature range for growth (°C) | 20–40 | 10–45 | 20–30 |
| pH range | 6.0–9.0 | 6.0–8.0 | 5.0–9.0 |
| Optimal pH | 7.5 | 7.0 | 6–7.0 |
| NaCl range (w/v %) | 0–1 | 0–1 | 0–0.5 |
| *Hydrolysis of* | | | |
| Casein, Gelatin | − | − | + |
| *Enzyme activities* | | | |
| Esterase lipase | + | − | + |
| Lipase, $\alpha$-Chymotrypsin, Trypsin, Arginine dihydrolase | − | + | + |
| Naphthol-AS-BI-phosphohydrolase | w | + | + |
| *Acid production from* | | | |
| Aesculin | + | − | − |
| *Assimilation of* | | | |
| D-Xylose | − | − | − |
| D-glucose | w | + | − |
| L-Arabinose | + | − | − |
| DNA G + C content mol% | 69.5 | 71.0 | 64.8 |

Strains: 1, $50^T$ (data from this study); 2, *Luteimonas lumbrici* $1.1416^T$ [25]; 3, *Lysobacter pocheonensis* Gsoil $193^T$ [26]. +, Positive; −, negative; w, weakly positive; NA, data not available. The data of strain 2 and 3 were cited by their corresponding references. Strains 1–3 are positive for activities of catalase, oxidase and hydrolysis of Tween 20, 40, 60 and 80. All strains are positive for acid phosphatase, alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase and cysteine arylamidase, but negative for $\beta$-galactosidase, $\beta$-glucuronidase, $\alpha$-glucosidase, *N*-acetyl-$\beta$-glucosaminidase and $\alpha$-mannosidase according to API ZYM test kits results. All strains are negative for indole production, inositol, D-mannitol, D-sorbitol and D-melibiose according to API 20E test kits results. In the API 50CH test strips, strains 1–2 are not able to produce acids by assimilating the following substrates: glycerol, erythritol, D-adonitol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, inositol, D-mannitol, D-sorbitol, methyl $\beta$-D-xylopyranoside, methyl $\alpha$-D-glucopyranoside, methyl $\alpha$-D-mannopyranoside, amygdalin, D-maltose, D-trehalose, inulin, D-melezitose, D-raffinose, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium 2-ketogluconate and potassium 5-ketogluconate. Strains 1–2 are able to assimilate acetic acid, acetoacetic acid, glycerol, propionic, but not bromosuccinic acid, $\alpha$-hydroxybutyric acid, $\alpha$-ketobutyric acid, γ-aminobutyric acid, dextrin, formic acid, *N*-acetyl-D-galactosamine, gentiobiose, D-gluconic acid, *N*-acetyl-D-glucosamine, methyl-$\beta$-D-glucoside, $\alpha$-ketoglutaric acid, L-pyroglutamic acid, quinic acid, D-malic acid, L-malic acid, D-maltose, D-mannose, D-melibiose, mucic acid, *N*-acetyl neuraminic acid, p-hydroxyphenylacetic acid, draffinose, D-saccharic acid, D-salicin, D-serine, L-serine, D-sorbitol, sucrose or D-trehalose according to the results from the Gen III Microplate test kit

contain unidentified aminophospholipid and unidentified lipid [25]. The respiratory quinone of $50^T$, *Luteimonas lumbrici* $1.1416^T$ [25] and *Lysobacter pocheonensis* Gsoil $193^T$ [26] were all Q-8.

## Taxonomic Conclusion

According to the phylogenic, phenotypic, and chemotaxonomic data, strain $50^T$ was considered to be a member of the genus *Lysobacter* with the family *Xanthomonadaceae*. The phylogenetic distinctiveness, together with DNA–DNA hybridization data, confirmed that strain $50^T$ represented a novel species of the genus *Lysobacter*, for which the name *Lysobacter sedimenti* sp. nov. is proposed. *Luteimonas lumbrici* $1.1416^T$ is reclassified as a member of the genus *Lysobacter* as *Lysobacter lumbrici* comb. nov. (type strain $1.1416^T$ = KCTC $62979^T$ = CCTCC AB $2018348^T$).

## Description of Lysobacter sedimenti sp. nov.

*Lysobacter sedimenti* (se. di.men′ti. L. gen.neut. n. *sedimenti* of sediment, from where the type strain was isolated).

Cells are aerobic, Gram stain-negative, non-motile and rod shaped (1.8–2.9 µm in length and 0.2–0.5 µm in width). Colonies on NA are circular with regular border, yellow, sticky, and usually 0.8–2.1 mm in diameter after incubation for 4 d at 30℃, growth at 20–40℃ (optimum, 30℃), pH 6.5–8.0 (optimum pH 7.5) and with 0–1.0% NaCl (optimum, 0%). The ubiquinone is Q-8. The major cellular fatty acids are iso-$C_{15:0}$, summed feature 9 (iso-$C_{17:1}$ $\omega 9c$ or $C_{16:0}$ 10-methyl), iso-$C_{17:0}$ and iso-$C_{16:0}$. Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and unidentified aminophospholipid, phospholipid and lipid. In API ZYM strips, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and $\alpha$-fucosidase are positive. In API 20E strips, Voges–Proskauer test, D-glucose and L-arabinose are positive. In the Biolog Gen III microPlate system, cells are positive for acetic acid, acetoacetic acid, propionic acid, sodium butyrate, glycerol, rifamycin SV, minocycline, 3-methyl glucose, tetrazolium violet, tetrazolium blue, D-cellobiose and gelatin. The genomic DNA G + C content is 69.5 mol%.

The type strain $50^T$ (= KCTC $92088^T$ = CCTCC AB $2022035^T$) was isolated from a pesticide plant in Bingzhou city, Shandong province, PR China.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain $50^T$ and the whole-genome shotgun project of strain $50^T$ has been deposited at DDBJ/ENA/GenBank under the accession number ON037515, JALGCL000000000, respectively.

## Description of Lysobacter lumbrici comb. nov.

*Lysobacter lumbrici* (lum'bri.ci. L. gen. n. *lumbrici* of an intestinal worm).

Basonym: *Luteimonas lumbrici* Cha et al. 2020.

The description is as given for *Luteimonas lumbrici* by Cha et al. 2020.

The type strain is $1.1416^T$ (= KCTC $62979^T$ = CCTCC AB $2018348^T$).

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00284-022-03084-0.

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**Data Availability** All authors have declared that all data are available.

## Declarations

**Conflict of interest** The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All the authors declare that they have no conflict of interest.

**Ethical Approval** The authors have declared that no ethical issues exist.

**Research Involving Human and/or Animal Participants** This article does not contain any studies with human participants or animals performed by any of the author.

**Consent to Participate and Consent for Publication** All authors agree to have participated in the research proposed to be published and agree to be published in the journal.

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