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Lysobacter arvi sp. nov. Isolated from Farmland Soil

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Abstract
A bacterial strain designated as UC was isolated from farmland soil. Strain UC^T formed a pale yellow colony on nutrient agar. Cell morphology revealed it as the rod-shaped bacterium that stained Gram-negative. The 16S rRNA gene sequence analysis identified strain UC^T as a member of the genus *Lysobacter* that showed high identity with *L. soli* DCY21^T (99.5%), *L. panacisoli* CJ29^T (98.7%), and *L. tabacisoli* C8-1^T (97.9%). It formed a distinct cluster with these strains in the neighbor-joining phylogenetic tree. A similar tree topology was observed in TYGS-based phylogenomic analysis. However, genome sequence analyses of strain UC^T showed 87.7% average nucleotide identity and 34.7% digital DNA–DNA hybridization similarity with the phylogenetically closest species, *L. soli* DCY21^T. The similarity was much less with other closely related strains of the genus *Lysobacter*. The G + C content of strain UC^T was 68.1%. Major cellular fatty acids observed were $C_{14:0}$ iso (13.4%), $C_{15:0}$ iso (13.6%), and $C_{15:0}$ anteiso (14.8%). Quinone Q-8 was the major respiratory ubiquinone. Predominant polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylglycerol. Production of xanthomonadin pigment was observed. Based on phenotypic differences and phylogenomic analysis, strain UC^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter arvi* is proposed. The type strain of the novel species is UC^T (= KCTC 92613^T = JCM 23757^T = MTCC 12824^T).

Introduction

Members of the genus *Lysobacter* are ubiquitous as they were isolated from water and soil, including the rhizosphere [1–5]. It belongs to the class *Gammaproteobacteria* of the phylum *Pseudomonadota*. The *Lysobacter* genus belongs to the family *Lysobacteraceae* under the taxonomically complex order *Lysobacterales* [6]. Christensen and Cook [1] first proposed the genus *Lysobacter* for non-fruiting bacteria that resemble cells of myxobacteria, but do not display the morphology of multi-cellular fruiting bodies and contained high guanine-plus-cytosine content in the deoxyribonucleic acid (DNA). *Lysobacter* is distinguished from myxobacteria due to its gliding feature and high G + C mol% content. The genus *Lysobacter* is profoundly related to species of *Xanthomonas* and *Stenotrophomonas*, as some of the strains were reclassified from these genera [7, 8]. They are known to produce antimicrobial metabolic products that were found to inhibit the growth of pathogenic bacteria, fungi, and nematodes [2, 3]. The ability of *Lysobacter* species to control crop pathogens emphasizes their relevance in the field of agriculture. Extracellular lytic enzymes are secreted by members of the genus *Lysobacter* that display broad-spectrum inhibition and degraded the cell walls of various pathogenic strains of plants, including nematodes [9]. Species of the genus *Lysobacter* are found to play an important role in biocontrol and therefore, new species of this genus have been increasingly reported in the recent past. As of the time of writing, there are 71 species with validly published and correct names according to the LPSN-List of Prokaryotic names with Standing in Nomenclature [10] (website accessed on 2 August 2023). The present work includes the polyphasic characterization of the strain designated as UC to propose it as a novel species within the family *Lysobacteraceae* and to determine its taxonomic status in the genus *Lysobacter*.

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Material and Methods

Phenotypic Characterization

Cell morphology was visualized under a phase contrast microscope (Axiophot, ZEISS, USA) using an oil immersion objective (100X). Further, the cell morphology was confirmed using transmission electron microscopy at an in-house facility [11]. Logarithmic phase-grown culture in nutrient broth (NB) was assigned for Gram staining using a kit (HiMedia, India). Motility was checked using the hanging drop method. Physiological properties, including growth at different temperatures and pH values, anaerobic growth, and NaCl tolerance (0–10%), were determined using either nutrient agar (NA) or nutrient broth (NB) medium. For pH growth experiments, medium pH was adjusted using biological buffers, including citrate buffer (pH 4–6), phosphate buffer (pH 6–8), $NaHCO_3$/Na_2CO_3 buffer (pH 8–10), and KCl/NaOH buffer system (pH 11–12). Strain UC^T grown in NB medium under optimal conditions was used for biochemical tests. Oxidase and catalase were performed as described by Cowan and Steel [12]. Sugar utilization and acid production from different sugars by the strain UC^T were carried out using different sugar disks in 24 well plates, as described earlier [13]. Citrate utilization was tested using Simmons citrate agar (HiMedia, India). Starch, esculin, gelatine, casein hydrolysis, and other biochemical tests were determined following standard methods [14, 15]. Additionally, various enzyme reactions of strain UC^T and acid production from sugars were also tested using VITEK2 (Version 05.02, bioMerieux, USA). The analysis of fatty acid methyl esters (FAMEs) was performed on GC following instructions as per the Sherlock Microbial Identification System (MIDI) version 6.1 [16]. A loopful of freshly grown culture on TSA medium was employed for the extraction of FAMEs. For other chemotaxonomy analyses, strain UC^T was grown to the logarithmic phase, cells were harvested, and the cell pellet obtained was lyophilized to obtain the mass. Ubiquinone and different polar lipid extraction were performed following the method given by Minnikin et al. [17] and polar lipids profile was analyzed using a two-dimensional TLC. Respiratory ubiquinone extraction and analysis were performed using the method described by Collins et al. [18].

Phylogenetic Analysis

Genomic DNA extraction from the strain UC^T was performed using a DNA isolation kit (Zymo, USA). The 16S rRNA gene was amplified using universal primers, including 27 forward and 1492 reverse [12]. The amplified product was further extracted using the HiYield™ Gel/PCR DNA mini kit (Real Biotech Corporation, Taiwan) and used for sequencing PCR. The sequencing was carried out using an in-house 3130xL genetic analyzer (Applied Biosystems, USA). The sequence obtained was visualized using FinchTV software version 1.4.0 (Geospiza Inc.) and analyzed manually. The final corrected sequence was BLAST analyzed using the EZTaxon database [19]. The 16S rRNA gene sequence of strain UC^T and its phylogenetically closest strains were subjected to alignment by both pairwise and multiple methods using ClustalW [20]. The evolutionary distance was calculated using the Kimura two-parameter method for strain UC^T with phylogenetically related strains and a neighbor joining-based phylogenetic tree [21] was constructed using the MEGA-7 program [22] with bootstrap values based on 1000 replications. Similarly, phylogenetic trees were constructed using maximum likelihood [23] and minimum evolution [24] methods. All the gaps and missing data were deleted by considering the complete deletion parameter.

Genomic Analysis

Whole-genome sequence data were generated using Illumina Novaseq as the platform facility (MedGenome, Hyderabad, India). The raw reads generated from Illumina were further assessed for good quality using FastQC version 0.11.9 (https://qubeshub.org/resources/fastqc). Reads in the form of paired end were de novo assembled using SPAdes version 3.13.0 [25]. The genome assembly was evaluated using QUAST version 5.2.0 [26]. The completeness and contamination of the genome were checked using Checkm version 1.2.2 [27]. The genome annotation was performed using the Prokaryotic Genome Annotation Pipeline (PGAP) (www.ncbi.nlm.nih.gov/genome/annotation_prok/) and also using RAST prokaryotic genome annotation services [28]. Average nucleotide identities of strain UC^T to other species of *Lysobacter* were calculated using Eztaxon ANI [19]. The genomic relatedness of UC^T with other species was also determined using digital DNA–DNA hybridization (dDDH) values computed from the DSMZ server (https://ggdc.dsmz.de/). Phylogenomic analysis was carried out using a type (strain) genome server (TYGS) [29]. The genomic phylogeny of the strain UC^T along with the genomes of its closest strains was analyzed to determine its taxonomic position. Genome blast distance phylogeny (GBDP) was used for comparison of the UC^T genome with the type strains and intergenomic distances were accurately estimated using the trimming algorithm and the d5 distance formula. These intergenomic distances are used to construct a balanced minimum evolution tree using FASTME 2.1.4 with branch

support determined through 100 pseudo-bootstrap replicates [30].

Results and Discussion

Phenotypic Description

Strain UC^T was isolated using the spread plate method from a farmland sample collected in Chandigarh, India. The pure isolate of the strain was preserved as 20% (w/v) glycerol stocks in the − 80 °C deep freezer and also as lyophilized ampules for further characterization. Cells were stained Gram-negative, grew aerobically, and displayed a rod shape in TEM analysis (Fig. 1A). Growth was observed under anaerobic conditions only after 72 h of incubation. Sporulation was not observed but displayed gliding motility. Strain UC^T was able to grow in the range of 15–40 °C temperature and 5.0 to 12.0 pH. The salt tolerance of strain UC^T showed growth up to 2% NaCl. Freshly grown colonies on NA showed the presence of catalase and oxidase enzyme activity. Indole, MR, VP, and urease tests showed negative reactions. Hydrolysis of starch was not observed, but gelatine, casein, and esculin were hydrolyzed. It showed a positive reaction for the reduction of nitrate to nitrite. Tween 40 and 60 hydrolysis were positive while it was negative for tween 20 and tween 80. The fatty acid composition of the strain UC^T revealed saturated fatty acids as the predominant fatty acids, including $C_{11:0}$ iso 3OH (4.8%), $C_{14:0}$ iso (13.4%), $C_{15:0}$ iso (13.6%), $C_{15:0}$ anteiso (14.8%), and $C_{16:0}$ iso (4.2%). Most of the phenotypic characteristics of the strain UC^T were similar to the described features of other members of the genus *Lysobacter*. However, there are some differences observed in phenotypic properties with its phylogenetically closely related strains *L. soli* DCY21^T and *L. panacisoli* CJ29^T (Table 1). The predominant polar lipids in the cell wall of the strain UC^T were phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphoaminolipid (PAL1) (Supplementary Fig. S1), and Q-8 was the major ubiquinone. Results of these chemotaxonomy studies indicated that the strain UC^T displayed similar chemical markers to those of closely related strains.

Phylogenetic Analysis

The processed 16S rRNA gene sequence of the strain UC^T was identical to the 16S rRNA gene sequence extracted from the genome sequence, which showed a higher degree of similarity in the sequence with species from the genus *Lysobacter,* including *L. soli* DCY21^T (99.5%), *L. panacisoli*

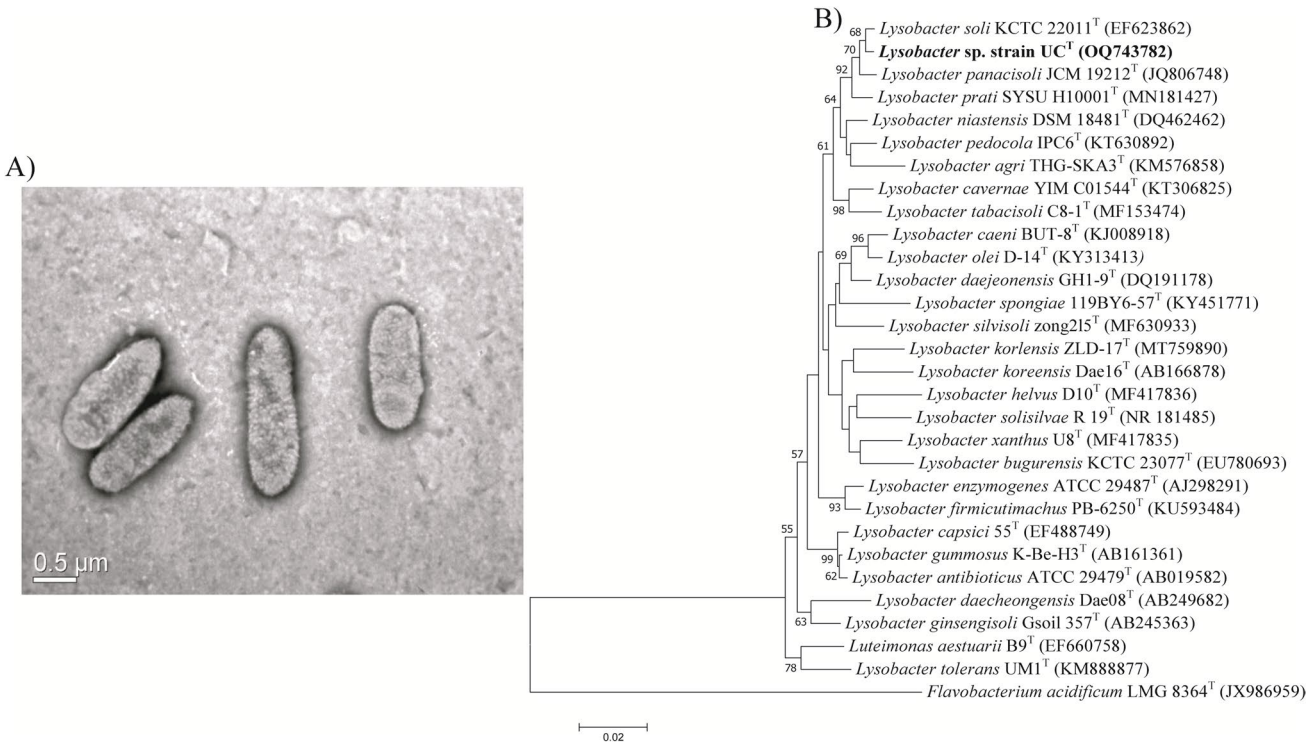

Fig. 1 Characterization of strain UC^T. **a** Transmission electron micrograph of *Lysobacter* sp. strain UC^T showing rod shape. **b** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequence showing the relationship between *Lysobacter* sp. strain UC^T and its closely related species. *Flavobacterium acidificum* LMG8364^T was used as an out-group. Bootstrap values greater than 50% are provided at the branch nodes

Table 1 Differential phenotypic characteristics of strain UC^T and its phylogenetically closely related species *Lysobacter soli* DCY21^T and *L. panacisoli* CJ29^T

Characteristic features	*Lysobacter* sp. UC^T	*L. soli* DCY21^{T†}	*L. panacisoli* CJ29^{T‡}
Length (μm)	0.5–0.7	0.6–0.9	0.4–0.45
Colony color	Pale yellow	Yellowish	Bright yellow
pH	6.8	7.0–7.5	7.0
NaCl (%)	2	NA	0–1
Nitrate reduction	+	+	−
Gelatin	+	NA	+
Starch	−	+	−
Casein	+	NA	+
Citrate	−	NA	−
TSI (H_2S production)	−	+	NA
Fructose	+	−	+
Arabinose	+	+	−
Cellobiose	−	+	−
Mannose	+	W	+
Trehalose	+	+	−
Sucrose	−	−	+
Mannitol	+	−	−
Dextrose	+	+	−
Galactose	+	+	−
DNA G + C content (%)	68.1	65.4	65.6
Major Polar Lipids (total > 50%)	DPG, PE, PG	DPG, PE, PG, PME	PE, PG, DPG
Major Fatty acid (each with > 10%)	$C_{15:0}$ iso, $C_{15:0}$ anteiso, and $C_{14:0}$ iso	$C_{15:0}$ iso, $C_{17:1}\omega9c$ iso, and $C_{17:0}$ iso	$C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:1}\omega9c$

All strains grew at 30 °C, positive for aesculin hydrolysis and acid production from xylose, and negative reaction for indole test

†Data from Srinivasan et al. [4]; ‡Data from Choi et al.[5]

CJ29^T (98.7%), *L. tabacisoli* C8-1 ^{T} (97.9%), *L. prati* SYSU H10001^T (97.7%), *L. niastensis* DSM18481^T (97.6%), and other species showed lesser than 97% identity. The phylogenetic tree constructed based on the neighbor-joining phylogenetic method for the strain UC^T with its closely related type strains of the genus *Lysobacter* formed a monophyletic cluster with *L. soli* DCY21^T, *L. panacisoli* CJ29^T, and *L. prati* SYSU H10001^T but formed a clade with *L. soli* DCY21^T (Fig. 1B). Similar tree topology was observed with maximum likelihood and minimum evolution phylogenetic analyses with minor differences in bootstrap values (Supplementary Fig. S2).

Genomic Analyses

The genome sequence de novo assemblies of *Lysobacter* sp. UC^T resulted in a draft genome of 4.2 Mb. Contigs smaller than 500 bp in length were removed. The draft genome of strain UC^T was assembled in 52 contigs with a genome size of 4,181,175 bp, representing average genome coverage of 822X. The N50 value was 556,993 bp, and the total number of RNAs was 59, including 55 tRNA and 4 rRNA genes. The draft form of the strain UC^T genome was submitted to the NCBI GenBank database under the accession number JARUHH000000000. The 16S rRNA gene sequence of the strain UC^T was extracted from the assembled genome using RNAmmer [31], and it was found to be 100% identical to that obtained from the Sanger sequencing of the amplified gene. The total number of coding sequences included 3845 genes within the genome of strain UC^T. The genomic GC content was 68.1%, which is within the range of G + C content observed in different species from the genus *Lysobacter*. Genomic analysis of the strain UC^T revealed distinguishing features from its closest genomes. The ANI values of UC^T with its closest neighbors *L. soli* DCY21^T and *L. panacisoli* CJ29^T were found to be 87.7 and 83.6%, respectively, which is well below the threshold cut-off (95–96%) for description of a novel species [32]. Further for in silico dDDH, the identity between strain UC^T and its closest strains *L. soli* DCY21^T and *L. panacisoli* CJ29^T was found to be 34.7 and 25.9%, respectively. Again, the dDDH identity value is far below the 70% threshold recommended for species delineation [33]. Type strains of *L. prati* SYSU H10001^T and *L.*

niastensis DSM 18481 ^{T} showed 82% ANI and 78.9% ANI was observed for *L. tabacisoli* C8-1 ^{T}. However, *L. prati* SYSU H10001^{T}, *L. niastensis* DSM 18481 ^{T}, and *L. tabacisoli* C8-1 ^{T} displayed dDDH values of 23.4%, 23.2%, and 22.2%, respectively. The genome-based phylogenomic tree formed a similar pattern as observed with the16S rRNA gene, where strain UC^{T} formed a monophyletic cluster with its closest neighbors. Further, it formed a distinct clade with *L. soli* DCY21^{T} that is out-grouped by *L. panacisoli* CJ29^{T} (Fig. 2). The cluster also contained the strains *L. prati* SYSU H10001^{T} and *L. niastensis* DSM 18481 ^{T}. The coding sequence observed was 3844 from the genome of UC^{T} using the RAST annotation tool and showed the distribution of different functional categories, with the majority of genes encoding primary metabolism. Biosynthetic gene cluster (BGC) analysis of secondary metabolites showed genes encoding different enzymes involved in auxin biosynthesis. Further, the antiSMASH [34]-based secondary metabolite prediction analysis revealed that strain UC^{T} contained BGC associated with xanthomonadin pigment production. Circular genomic representation of the strain UC^{T} analysis by Proksee [35] using *L. soli* DCY21^{T} as a reference showed a colored depiction of genomic features in the form of different rings arranged in inward directions (Fig. 3A). The outermost two rings are the genomes of strain UC^{T} and its phylogenetic closest strain *L. soli* DCY21^{T}, followed by CDS (including sense and antisense). The xanthomonadin BGC (contig shown in yellow in Fig. 3A) sequence showed 57% similarity with the BGC of *Xanthomonas oryzae*; however, the gene arrangement found in strain UC^{T} was in alignment with xanthomonadin BGC of *Xanthomonas oryzae* [36] except gene encoding putative acetyltransferase (Fig. 3B).

Pan-genome analysis of strain UC^{T} and closely related strains *L. soli* DCY21^{T}, *L. panacisoli* CJ29^{T}, and *L. prati* SYSU H10001^{T} were performed using the ultra-fast computational pipeline BPGA [37]. The pan-genome analysis of strain UC^{T} and its closely related type strains displayed a core genome comprising 2490 genes. The accessory genes between the two species are mentioned in Supplementary Fig. S3A. The number of unique genes observed between type strains was 612, 374, 802, and 575 for strains UC^{T}, *L. soli* DCY21^{T}, *L. prati* SYSU H10001^{T}, and *L. panacisoli* CJ29^{T}, respectively. In the pan-core plot diagram, a stepwise increase in the pan-genome (new gene families) was observed with every new genome addition, while the core genome decreased with the addition of a second genome and so on (Supplementary Fig. S2B). The expansion of the pan-genome with the addition of new genome sequences infers the open pan-genome of *Lysobacter* sp. strain UC^{T}. With the addition of its closest strain genomes, the number of new gene counts was also found to keep on increasing. The new gene addition between *L. panacisoli* CJ29^{T} and *L. prati* SYSU H10001^{T} is 1010, between *L. prati* SYSUH10001^{T} and *L. soli* DCY21^{T} is 642, and between *L. soli* DCY21^{T} and UC^{T} is 612, but the core genes among the genomes decreased from 3554 to 2491 (Supplementary Fig. S3B).

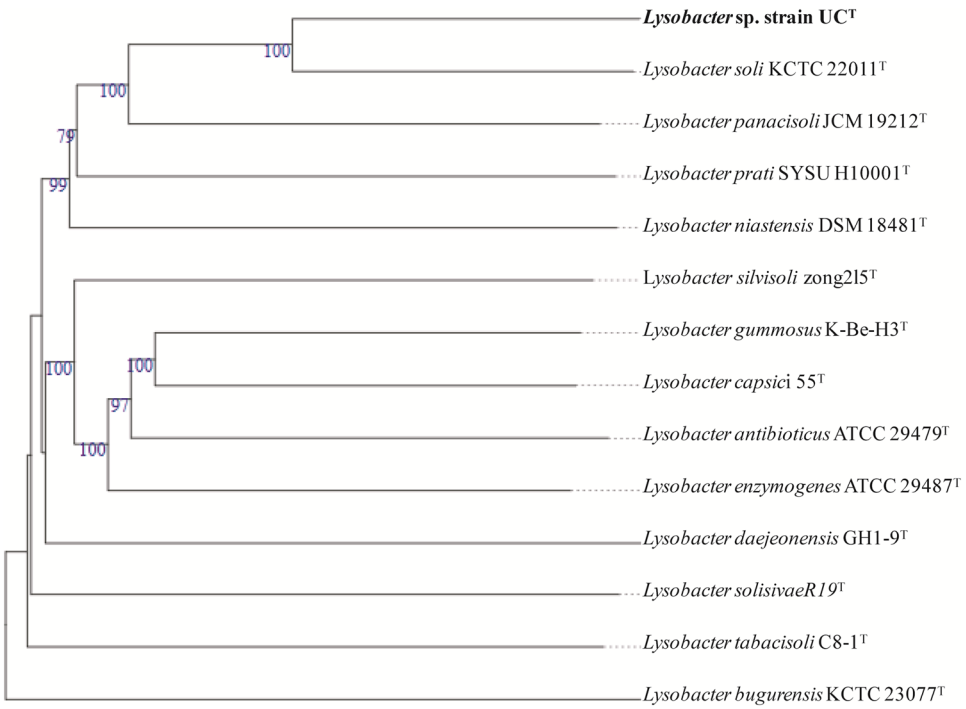

Fig. 2 Phylogenomic tree constructed using TYGS server showing the relationship between strain UC^{T} and its closest type strain. The tree was deduced from GBDP distances calculated for genome sequences. The bootstrap percentage values greater than 50% are provided at the branches

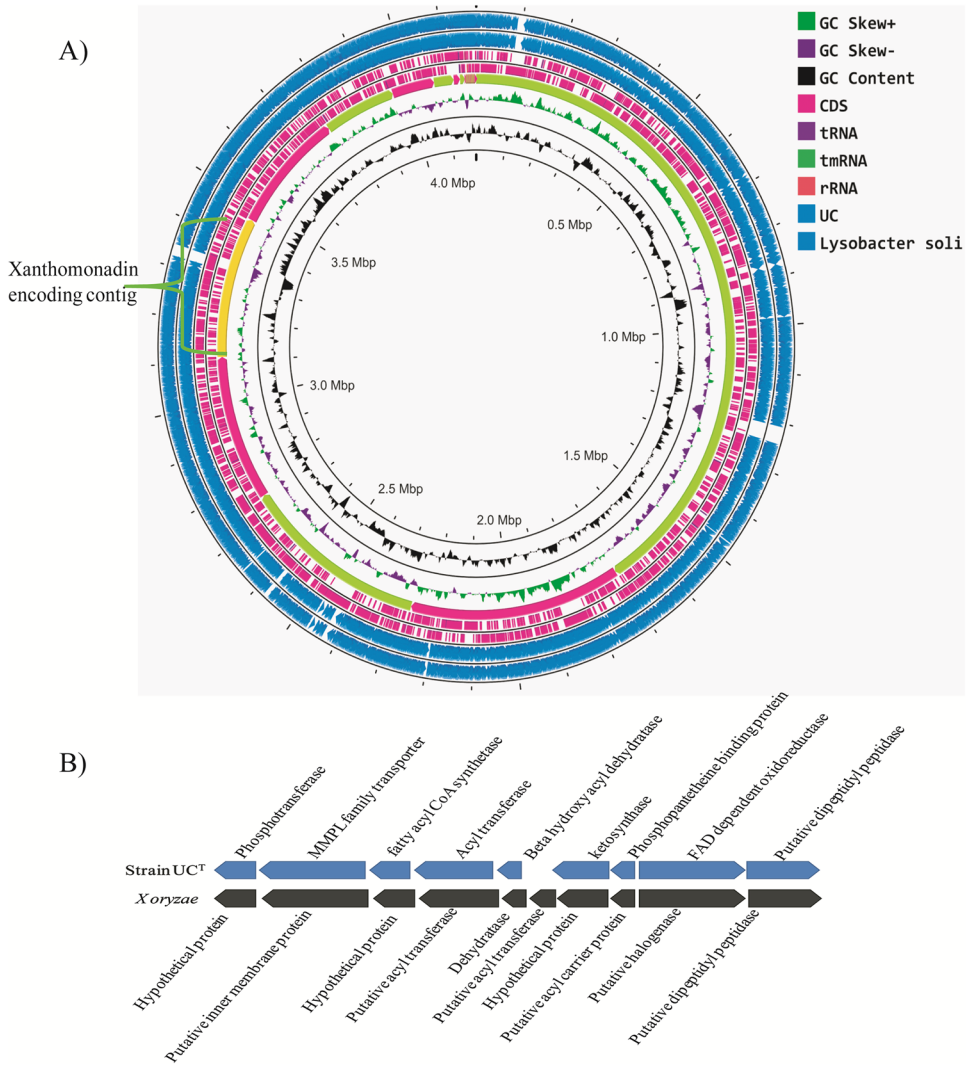

Fig. 3 Genomic analysis of *Lysobacter* sp. strain UC^T. **a** Circular map representation of the strain UC^T genome circles from outside to inward directions. Outermost circles represent the genome of strain UC^T and *L. soli* DCY21^T (blue color), coding sequences (both sense and antisense pink color), different contigs are shown in green and pink color, circle 6 represents GC skew positive (green color) and GC skew negative (purple color), and circle 7 (black) represents GC content in the genome of strain UC^T *Lysobacter* sp. The xanthomonadin encoding cluster is shown in the contigs section (yellow color). **b** Gene arrangement of xanthomonadin biosynthetic gene cluster in the strain UC^T genome and comparison with *X. oryzae* using antiSMASH database (Color figure online)

Taxonomic Conclusion

Apart from low ANI and dDDH values observed for strain UC^T with any phylogenetic relative of the genus *Lysobacter*, strain UC^T also showed differences in morphological features, nitrate reduction, starch hydrolysis ability, H_2S production, and acid production from arabinose, cellobiose, dextrose, fructose, galactose, mannose, mannitol, sucrose, and trehalose with the closest relatives. The major whole-cell fatty acids $C_{14:0}$ iso (13.4%) and $C_{15:0}$ anteiso (14.8%) observed in strain UC^T were differed with closest phylogenetic relative, *L. soli* DCY21^T despite cultivated under similar conditions. Thus, we conclude that strain UC^T is a new species of the genus *Lysobacter* and propose with name *Lysobacter arvi* sp. nov.

Description of *Lysobacter arvi* sp. nov.

Lysobacter arvi **(ar´vi. L. gen. n.** ***arvi*** **of soil field, farmland)**

Cells are rod-shaped and Gram-stained negative. Colony morphology is concave with an entire margin and yellow pigmented. Growth occurs aerobically, but slow growth is observed under anaerobic conditions. The optimal growth conditions are pH 6.8 and 30 °C temperature. It shows positive reactions for both catalase and oxidase enzyme tests. The hydrolysis of esculin, casein, and gelatine is positive, while the hydrolysis of starch is negative. Nitrate reduction to nitrite is observed. Sugars including maltose, lactose, fructose, arabinose, mannose, trehalose, sorbitol, melibiose, mannitol, dextrose, xylose, and galactose were utilized, but

not inulin, dulcitol, cellobiose, sucrose, raffinose, adonitol, rhamnose, and inositol. The whole-cell fatty acids are $C_{11:0}$ iso 3OH, $C_{14:0}$ iso, $C_{15:0}$ iso, $C_{15:0}$ anteiso, and $C_{16:0}$ iso. Polar lipids present are diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphoaminolipid (PAL1), and phospholipid (PL1). Q-8 is the major ubiquinone. The type strain is UC^T (= KCTC 92613^T = JCM 23757^T = MTCC 12824^T), isolated from farmland soil collected in Chandigarh.

The Genbank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and whole-genome sequence for the strain UC^T are OQ743782 and JARUH000000000, respectively. The whole-genome sequence bioproject number is PRJNA951362.

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Author Contributions SC and SK contributed to conceptualization, designing experiments, and analysis. SC performed chemotaxonomy. SC and HV performed biochemical experiments. SC, HV, and SK performed phenotypic analysis. SC, RR, and PBP performed genome sequencing. SC, HV, RR, PBP, and SK performed genomic analysis. SC and SK wrote the manuscript.

Data Availability Strain and sequence deposition: The 16S rRNA GenBank accession number for strain UC^T (= MTCC 12824^T = KCTC 92613^T = JCM 23757^T) submitted at NCBI with OQ743782. The whole-genome sequence of strain UC^T deposited NCBI with Accession Number JARUH000000000 and bioproject no. PRJNA951362.

Declarations

Conflict of interest The authors declare no conflict of interest.

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