Isolation and characterization of a novel Gram-negative bacterium *Chromobacterium alkanivorans* sp. nov., strain IITR-71T degrading halogenated alkanes

Abhay Bajaj,† Anand Kumar,† Shivani Yadav, Gurwinder Kaur, Monu Bala, Nitin Kumar Singh, Rajendran Mathan Kumar, Natesan Manickam and Shanmugam Mayilraj

1Environmental Biotechnology Division, CSIR-Indian Institute of Toxicological Research (IITR), Lucknow, 226 001, India
2Microbial Type Culture Collection & Gene Bank (MTCC), CSIR-Institute of Microbial Technology (IMTECH), Chandigarh, 160 036, India

The taxonomic position of a Gram-stain negative, non-violaceinpigmented bacterium isolated from an insecticide-contaminated site was characterized by a polyphasic approach. The bacterium was able to grow on three different halogenated compounds namely 1-chlorobutane, 1-chloropropane and 1,2-dichloroethane. As a critical step in the degradation of these haloalkanes, stoichiometric amounts of dechlorination were estimated. Based on selective enrichment method for three months, using a highly contaminated mixed chemical soil, a bacterium was obtained and designated as IITR-71T. Its versatility and novelty led us to further characterize it by polyphasic taxonomy. The 16S rRNA gene sequence (1446 bases) comparison showed highest similarity with those of members of the genus *Chromobacterium* with the most closely related species to strain IITR-71T being *Chromobacterium aquaticum* (99.3%) followed by *Chromobacterium haemolyticum* (98.6%) and *Chromobacterium piscinae* (97.1%). The major ubiquinone was Q-8. Predominant polar lipids are phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG). The DNA G+C content of IITR-71T was estimated to be 61.2 mol %. The genotypic and phenotypic distinctiveness of IITR-71T and its phylogenetic relationships indicate that IITR-71T represents a novel species, for which the name *Chromobacterium alkanivorans* sp. nov. is proposed. The type strain is IITR-71T (=MTCC 11059T=JCM 30068T=KCTC 52433T).

The genus *Chromobacterium* is part of the family *Chromobacteriaceae* of the class *Betaproteobacteria* of the phylum *Proteobacteria*. The genus includes generally Gram-stain-negative, soil- and water-associated organisms (Hungria et al., 2005), one, the type species of the genus, *Chromobacterium violaceum* (Boisbaudran, 1882) is well known for the production of a violet pigment known as violacein, while other non-pigmented species *Chromobacterium haemolyticum* (Han et al., 2008) and *Chromobacterium aquaticum* (Young et al., 2008) also represent the genus, along with several other members. In recent decades, industrial chemicals have flooded the environment and have seeped into various compartments of the air, water and soil. Among them, halogenated aliphatic hydrocarbons are considered serious environmental pollutants, however, owing to their continued use in India and other developing countries, additional sites are being contaminated. These compounds include 1-chlorobutane, 1-chloropropane and 1,2-dichloroethane. These are often encountered as groundwater pollutants which need to be eliminated from the environment. Many organisms have evolved to degrade these aliphatic compounds and they have been reported from soil and activated sludge, etc., Some of the efficient bacteria isolated for degradation were, *Xanthobacter autotrophicus* GJ10 (Janssen et al., 1985), *Ancylobacter aquaticus* and methylotrophs (Van den Wijngaard et al., 1992) for 1,2-dichloroethane.
Mycobacterium aurum L1 for vinyl chloride (Hartmans & De Bont, 1992), Pseudomonas cichorii 170 for 1,3-dichloropropane (Poelarends et al., 1998) and Mycobacterium strain GP1 for the degradation of 1,2-dibromoethane (Poelarends et al., 1999). It has also been reported that different types of haloalcohols and haloalkanoates have been degraded by species of the genus Agrobacterium and other Rhizobia having dehalogenation activities (Effendi et al., 2000; Hartmans & De Bont, 1992). A member of the genus Thauera belonging to the Betaproteobacteria was found to mineralize 1,2-dichloroethane to CO₂ under nitrate-reducing conditions (Dinglasan-Panilio et al., 2006). Recently, a member of the genus Pseudomonas isolated from contaminated soil was studied in detail and found to utilize 2,3-dichloro-1-propa-nol (DCP) and several aliphatic haloacids and haloalcohols as sole carbon and energy sources for growth (Arif et al., 2012). Biochemical studies on the degradation of chlorinated compounds reveals dechlorination as a major step which is catalyzed by dehalogenases (Higgins et al., 2005). To date, to our knowledge, no study has reported using members of the genus Chromobacterium for degradation of chlorinated recalcitrant compounds. Previous reports on genetic insights from the whole genome of Chromobacterium violaceum have revealed the presence of three sets of genes related to arsenic resistance, cyanate degradation and acid dehalogenation, which have been suggested to have a role in both environmental pollution control and bioremediation (Carepo et al., 2004). Also, recently genes for phenol monooxygenase have been characterized from the same species (Perpetuo et al., 2009).

In our previous studies, we have reported bacteria that could be used for the biodegradation of chlorinated pesticides (Manickam et al., 2007, 2008) and in our continued effort to isolate potential degraders for chlorinated contaminants the present study was undertaken. Here, we report isolation, identification and characterization of a novel species of the genus Chromobacterium. Strain IITR71T was found to be able to utilize three different compounds with moderate dechlorination efficiency under aerobic conditions.

Soil and sediment samples used in this study were collected from Hindustan Insecticides Limited (HIL), Cochin, India. A creek carries the chemical wastes to Periyar River, which ultimately enters the Arabian Sea at the Cochin Coast. The industry (HIL) is manufacturing multiple chlorinated pesticides and has used many solvents in the process since 1954. The collected samples were placed in Zip-Loc bags, placed in a cooler and transported to the laboratory. Using 10 g mixed soil and sediment samples, an enrichment culture was set up in 50 ml minimal medium (Ahuja et al., 2001) containing chlorinated alkanes and pesticides in separate Erlenmeyer flasks. Every 10 days, 5 ml culture was transferred to a fresh flask containing the same substrates and minimal medium as above and this was repeated five times. After the last enrichment, a 1 ml portion was serially diluted and plated on LB-agar medium (peptone 10 g; yeast extract 5.0 g; NaCl 5.0 g; and agar 15.00 g in 1000 ml distilled water, pH 7.5) and incubated at 30 °C for two days. Six distinguishable colonies were obtained on plates and strain IITR-71T was taken for further studies to verify its ability to grow and dechlorinate alkanes and other pesticides.

The isolated colonies obtained as described above were grown using 3 mM substrates in a chloride-free mineral salt medium (Manickam et al., 2006) (KH₂PO₄ 34 g; Na₂HPO₄ 19.6 g; (NH₄)₂SO₄ 2.0 g; MgSO₄ 97.4 mg; FeSO₄ 0.10 mg; CaCO₃ 0.40 mg; ZnSO₄ 0.16 mg; CuSO₄·5H₂O 0.032 mg; H₃BO₃ 0.012 mg; dissolved in 1000 ml of distilled water, pH 7.6) supplemented with 0.01 % yeast extract and incubated at 30 °C on a rotary shaker.

The isolated bacterium was tested on nine chemicals, namely 1-chlorobutane, 1-chloropropane, 2-chlorobenzoic acid, 3-chlorobenzoic acid, 4-chlorobenzoic acid, benzoic acid, 1,2-dichloroethane, 2,5-dichloro-1,4-benzquinone and hexachlorocyclohexane (HCH). From the reaction mixture, a 2 ml portion was withdrawn every two days for growth and chloride measurements. Growth was maintained at 30 °C and spectrophotometrically monitored at 600 nm. The free chlorine ions were measured at 460 nm using a colorimetric method as described previously (Manickam et al., 2006).

To study its phenotypic characters, the isolate was routinely cultivated on TSA medium at 30 °C and maintained as glycerol stocks at −70 °C. The reference type strains of C. aquaticum (MTCC 11259T), C. haemolyticum (MTCC 11261T) and Chromobacterium piscinae (MTCC 11258T) were obtained from Microbial Type Culture Collection & Gene Bank (MTCC), Chandigarh, India. Colony morphology, cell morphology, motility (hanging drop method) and Gram reaction of the strain were determined by using standard methods (Barrow & Feltham, 1993; Murray et al., 1994). Phenotypic characterization was performed using TSA as a basal medium and strains were incubated at their optimum growth temperatures. Physiological tests such as growth at different temperatures (12, 15, 20, 25, 30, 37 and 42 °C), pH values (5.0, 6.5, 7.0, 8.0, 9.0 and 10.0) using different biological buffers and NaCl concentrations (0, 2, 5, 7, 9 and 10 % w/v) were determined using TSA as a basal medium and acid production from various carbohydrates and other biochemical tests were performed as described (Barrow & Feltham, 1993; Powers, 1995; Smibert & Krieg, 1994; Stanier et al., 1966; Xu et al. 2005). For anaerobiosis, TSA plates were streaked and placed in an anaerobic jar (MART), which was evacuated and flushed using an Anoxomat unit (MART) with an anaerobic gas mixture consisting of nitrogen (85 %), carbon dioxide (10 %) and hydrogen (5 %). Plates were incubated at 30 °C for 5–6 days. Oxidation of various substrates was determined by using Biolog GN2 MicroPlates by methods described by the manufacturer. For different enzymatic reactions VITEK 2-GN cards were used in triplicates using 36 h old culture, incubated at 37 °C as per the manufacturer’s instructions (bioMérieux). Sensitivity of
the strain to antibiotics was tested by using antibiotic susceptibility discs (HiMedia) after incubation for 48 h.

Freeze-dried cells for chemotaxonomic analysis (except for fatty acid study) were prepared by harvesting the bacterial cells in the late exponential phase following their growth in tryptic soy broth (TSB; HiMedia) at 30 °C for 48 h. Isoprenoid quinones were extracted and purified as described by Saha et al. (2005). Fatty acid methyl ester analysis was performed by using gas chromatography according to the instructions of the Sherlock Microbial Identification System (MIDI) as described previously (Pandey et al., 2002). Extraction of polar lipids was done based on the modified protocol of Bligh & Dyer (1959). Two-dimensional TLC was run for identification of polar lipids according to the procedure described by Komagata & Suzuki (1987). Lipid spots were detected using the following spray reagents: molybdophosphoric acid (5 % w/v) in absolute ethanol, molybdenum blue spray reagent (1.3 %, Sigma), ninhydrin (0.2 % w/v) in acetone and anisaldehyde reagent (Sigma) for detection of total lipids, phospholipids, aminolipids and glycolipids respectively.

For the MALDI-TOF analysis, protein extraction, sample preparation MALDI-TOF mass spectra analysis was performed using Microflex III instrument (Bruker Daltonik) and methods described by Kumar et al. (2015). For 16S rRNA gene sequencing, the genomic DNA extraction and amplification was performed as described previously (Mayilraj et al., 2006). Identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequencing similarities were achieved using the EzTaxon server (Kim et al., 2012). The 16S rRNA gene sequence of IITR-71 \(^T\) and the members of the closely related genera were retrieved from EzTaxon server (Kim et al., 2012) and aligned using CLUSTAL W in MEGA version 6.0 (Tamura et al., 2013). Phylogenetic trees were reconstructed using the neighbour-joining as well as maximum-parsimony and maximum-likelihood algorithms. Bootstrap analysis was performed to assess the confidence limits of the branching. Evolutionary analyses were carried out in MEGA 6.0. The neighbour-joining tree was reconstructed using a standard parameter of CLUSTAL W alignment with a gap opening penalty 15 and gap extension penalty of 6.66 for both pairwise and multiple alignments. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood model (effective nucleotide substitution model with rate uniformity, pattern homogeneity, rate variation among sites, pattern heterogeneity between lineages, rate variation and pattern heterogeneity) and are in the units of base substitutions per site. The optimal tree with the sum of branch lengths of 0.09323015 was retrieved. The analysis involved eight nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1396 positions in the final dataset.

The 16S rRNA gene sequence (1446 bp) of IITR-71 \(^T\) was determined for phylogenetic analysis. IITR-71 \(^T\) showed the highest degree of sequence similarity with C. aquaticum (99.3 %) followed by C. haemolyticum (98.6 %) and C. piscinae (97.1 %). The sequence similarities with the rest of the species of the genus Chromobacterium with validly published names were less than 97.1 %. A neighbour-joining tree (Fig. 1) based on 16S rRNA gene sequences showed that IITR-71 \(^T\) formed a distinct branch with the most closely related species, C. aquaticum and C. haemolyticum, separated from the other members of the genus Chromobacterium. The G+C content of genomic DNA was determined spectrophotometrically (Lambda 35, Perkin-Elmer) using the thermal denaturation method (Mandel & Marmur, 1968). DNA–DNA hybridization was performed each time with freshly isolated genomic DNA and was repeated three times by the membrane filter method (Tourouva & Antonov, 1987).

In the initial experiments, IITR-71 \(^T\) was tested for its substrate utilization with a wide range of compounds as the soil samples used for isolation of the bacteria contained mixed chemical residues. It was found that IITR-71 \(^T\) was able to

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**Fig. 1.** Phylogenetic position of IITR-71 \(^T\) along with the other closely related species of the genus Chromobacterium. The tree was reconstructed using neighbor-joining analysis based on 1446 bases of aligned 16S rRNA gene sequences. *Aquitalea magnusoni* TRO-001DR8 (DQ018117) was used as an outgroup. Bootstrap values (expressed as percentage of 100 replications) greater than 70 % are given at the nodes. Filled circles indicate that corresponding nodes were also recovered in the tree generated with maximum-parsimony and maximum-likelihood algorithms. Bar 5 % sequence variation.
grow and dechlorinate 1-chlorobutane, 1-chloropropane and 1,2-dichlorehane in mineral salt medium supplemented with 0.01 % yeast extract. Among the three compounds the growth on 1,2-dichlorehane and 1-chloropropane was similar but on 1-chlorobutane less growth was attained (Fig. S1, available in the online Supplementary Material). *C. aquaticum* MTCC 19852 was used as a reference strain as the 16S rRNA gene analysis revealed it to be closely related to IITR71T. However, *C. aquaticum* failed to show growth on any of the compounds studied. Growth of the isolated bacteria on all the three compounds indicated the utilization of these compounds as sources of carbon and energy. The chloride released into the medium was also estimated and it was found that all the three compounds undergo dechlorination. A maximum of 83.19 % from 1-chloropropane, 79.64 % from 1-chlorobutane and 37.09 % from 1,2-dichlorehane chloride release was obtained under the experimental conditions by isolated bacteria. These results indicated that the strain has biotransformation capability for mono- and di-chlorinated compounds. In order to verify the ability of the bacterium to degrade other halogenated alkanes and aromatic compounds, the strain was grown on different compounds. After 8 days of incubation under similar conditions to those described above, the bacteria could not grow and dechlorinate a large number of compounds (Table 1). Because IITR-71T grew on three different halogenated alkanes, it was selected for detailed taxonomic characterization.

The detailed differential phenotypic properties are shown in Table 2 and also listed in the species description. Phenotypic data presented in the table indicate that IITR-71T differs from the closely related species with respect to at least by 40 characters, which include acid production from carbohydrates, sensitivity to antibiotics, casein hydrolysis, nitrate reduction, hydrogen sulphide production, assimilation/oxidation of different carbon sources and other mentioned tests. Most of the chemotaxonomic properties of IITR-71T (presented in the species description) were typical of members of the genus *Chromobacterium*. The major fatty acids C16:1ω7c/C16:1ω6c, C16:0 and C18:1ω7c detected in the novel strain (presented in the species description) were consistently found in members of the genus *Chromobacterium* (Table 3). The major polar lipids present in IITR-71T and the other three closely related reference strains were phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE) and six unknown phospholipids (PL) whereas *C. piscinae* do not possess unknown phospholipid PL 7. (Fig. S2). The major ubiquinones detected in IITR-71T were Q-8 and minor amounts of Q-7. The DNA G+C content of IITR-71T was estimated to be 61.2 mol%.

MALDI-TOF measurements were made for IITR-71T followed by *C. aquaticum*, *C. haemolyticum* and *C. piscinae* using a Microflex III instrument (Bruker Daltonik) for the automatic acquisition of mass spectra in the linear positive mode within a range of 2–20 kDa, according to the manufacturer’s instructions. The peak from the generated mass spectra and their matching against the reference spectra of the integrated database was performed using MALDI Biotyper Software (Bruker Daltonics). MALDI profile of IITR-71T showed unique peaks represented in red at position 6420.919 m/z which were very much evident from the gel view (Fig. S3). MALDI-TOF mass spectrum profile (MSP) dendrogram analyses indicated the closeness of IITR-71T and other related strains *C. aquaticum*, *C. haemolyticum* and *C. piscinae* (Fig. S4). 16S rRNA-gene-based phylogenetic analysis and MALDI-TOF-based MSP dendrogram analysis between the strain IITR-71T and other related species *C. aquaticum*, *C. haemolyticum* and *C. piscinae* show the same grouping pattern.

The 16S rRNA gene sequence (1446 bp) of IITR-71T was determined (GenBank accession number JN210566) and compared with those of other closely related taxa retrieved from the Ez-taxon server. Sequence analysis revealed that IITR-71T shared 99.3 % 16S rRNA gene sequence identity with *C. aquaticum*. On the basis of the 16S rRNA gene sequence identity, the strain could be assigned to the genus *Chromobacterium*. The unified phylogenetic tree of neighbour-joining, maximum-likelihood and maximum parsimony-algorithms indicated that IITR-71T formed a separate lineage along with the closely related species (Fig. 1). However, genomic relatedness, as shown by DNA–DNA hybridization, of IITR-71T with *C. aquaticum* (60.2±0.3 %), *C. haemolyticum* (43.5±0.5 %) and *C. piscinae* (30.5±0.2 %), was below the 70 % threshold value recommended for the delineation of bacterial species (Wayne et al., 1987). DNA–DNA relatedness values between IITR-71T and the remaining type strains of the genus *Chromobacterium* were not determined, since organisms with more than 1.4–3 % 16S rRNA gene sequence dissimilarity are classified as

### Table 1. Qualitative assessment of growth and chloride release by *Chromobacterium alkanivorans* IITR-71T on various test substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Growth</th>
<th>Chloride release</th>
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<tbody>
<tr>
<td>1-Chlorobutane</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1-Chloropropane</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2-Chlorobenzoic acid</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3-Chlorobenzoic acid</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4-Chlorobenzoic acid</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1,2-Dichloroethane</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,5-Dichloro-1,4-benzoquinone</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4,4-Dichlorobenzenophenone</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hexachlorocyclohexane</td>
<td>–</td>
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</table>
All the strains were positive for growth at pH 5.0–10.0, 25–37°C and tolerated up to 2.0% NaCl. Positive for hydrolysis of casein, catalase and citrate utilization and negative for hydrolysis of starch, methyl red, Voges–Proskauer and indole production. Acid is produced from trehalose, fructose and glucose; negative for maltose, raffinose, cellobiose, sucrose, salicin, rhamnose, galactose, xylose, arabinose, lactose, melibiose, inulin, adonitol and dulcitol. In VITEK GN2 cards all the four strains were positive for adonitol and dulcitol. In VITEK GN2 cards all the four strains were positive for turanic acid, -melibiose, -trehalose, acetic acid, -cellobiose, -glutamyl transferase, tyrosine arylamidase, urease, -galactosidase, glycine arylamidase, ornithine decarboxylase, l-histidine assimilation, β-glucoronidase, glu-gly-arg-arylamidase, l-malate assimilation and Ellman. In utilization of carbon sources using Biolog GN2 microplates all the strains are positive for Tween 40, Tween 80, N-acetyl-d-glucosamine, d-fructose, α-d-Glucose, l-proline, d-serine, D-alanine, L-alanine, l-glutamic acid, l-histidine, d,l-lactic acid, succinic acid, l-asparagine, l-aspartic acid, l-serine, d-trehalose, acetic acid, d-gluconic acid and propionic acid. Negative for α-cyclodextrin, l-arabinose, l-galactose, i-erythritol, α-D-lactose, maltose, d-melibiose, β-methyl-D-glucoside, d-psicose, D-raffinose, L-rhamnose, xylitol, D-glucosaminic acid, malonic acid, quinic acid, d-saccharic acid, succinamic acid, glycyld-l-aspartic acid, l-pyrogulamic acid, d,l-carnitine, inosine, uridine, thymidine, 2-aminoethanol, N-acetyl-D-galactosamine, adonitol, D-cellobiose, l-fucose, gentibiose, lactulose, sucrose, turanose, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, sebacic acid, glucuronamide, l-ornithine, urocanic acid, phenylacetilamine, glycerol, glucose-1-phosphate and 2,3-butanediol. +, Positive; −, negative.
A novel bacterium, *Chromobacterium alkanivorans* with the potential to biodegrade persistent organochlorine pollutants was isolated and characterized in detail. The potential biodegradation was investigated for different chloroalkanes and it was found that the bacterium possesses the ability to grow and dechlorinate three haloalkanes. The results indicate that the strain showed growth adaptation towards the di-chlorinated compound 1,2-dichlorethane, as more growth and concomitant chloride release was observed. Furthermore, more detailed studies and genetic characterization would reveal the degradation capability of the bacteria as species of the genus *Chromobacterium* have not been reported to biodegrade chloroalkanes. Also, enzyme-based studies using this bacterium are envisaged for further understanding the degradation pathway of chloro-aliphatic pollutants and its applicability for bioremediation purposes.

Subsequent taxonomic characterization of the bacterium based on a polyphasic approach indicated that it may be a novel species of the genus *Chromobacterium*. Based on the analysis of phenotypic and genotypic results confirmed that IITR-71 sp. nov. should be considered to represent a novel species of the genus *Chromobacterium*. Table 2 lists the main features that distinguish IITR-71 sp. nov. from its close relatives. Therefore, the polyphasic evidence obtained in this study indicates that IITR-71 sp. nov. has the potential for biodegradation of environmental pollutants like persistent chloro-aliphatic compounds, represents a novel species of the genus *Chromobacterium*, for which the name *Chromobacterium alkanivorans* sp. nov. is proposed.

### Description of *Chromobacterium alkanivorans* sp. nov.

*Chromobacterium alkanivorans* (al.ka.ni.vo’rans. N.L. neut. n. alkanum alkane; L. part. adj. vorans devouring; N.L. part. adj. alkanivorans alkane-degrading).

Cells are facultatively anaerobic, Gram-stain-negative and motile rods (0.3–0.6 × 0.8–2.4 μm). Colonies are circular, convex, smooth and creamish-yellow pigmented. Grows at temperatures between 12 and 37 °C (optimum, 30 °C), at pH 5.0–10.0 (optimum, pH 8.0) and tolerates up to 2% NaCl. Casein and gelatin are hydrolysed, but not starch. Positive for catalase, urease and citrate. Negative for H₂S, indole production, methyl red, Voges–Proskauer and oxidation–fermentation tests. Nitrate is not reduced to nitrite. Positive for acid production from trehalose, fructose, mannose, *myo*-inositol, sorbitol and glucose; negative for mannitol and maltose, raffinose, cellobiose, sucrose, salicin, rhamnose, galactose, xylose, arabinose, lactose, melibiose, inulin, adonitol and dulcitol. Capable of degrading 1-chlorobutane, 1-chloropropane and 1,2-dichlorethane. In VITEK GN2 cards positive for L-pyrroldonyl-arylamidase, glutamyl arylamidase pNA, γ-glutamyl-transferase, D-glucose, fermentation/glucose, D-mannose, tyrosine arylamidase, L-lactate alkalisation, succinate alkalisation, β-N-acetyl-glucosaminidase, phosphatase, comaurase, 0/129 resistance, urease and citrate (sodium) and negative for ala–phe–pro-arylamidase, L-arabitol, β-galactosidase, β-glucosidase, adonitol, D-cellobiose, β-xylosidase, β–alanine arylamidase pNA, L-proline arylamidase, lipase, palatinose, D-tagatose, saccharose/sucrose, D-maltose, malonate, 5-keto-D-gluconate, α-glucosidase, β-N-acetyl-
galactosaminidase, α-galactosidase, glycine arylamidase, ornithine decarboxylase, lysine decarboxylase, L-histidine assimilation, β-glucoronidase, glu–gly–arg–arylaminidase, L-malate assimilation, L-lactate assimilation and Ellman. In utilization of carbon sources using Biolog GN2 microplates positive for dextrin, glycen, D-arabitol, putrescine, L-lysine, myo-inositol, β-hydroxybutyric acid, α-ketoglutaric acid, hydroxy-y-l-proline, nonomethyl succinate, bromosuccinic acid, L-alanamidne, D-glucose-6-phosphate, γ-aminobutyric acid, Tween 40, Tween 80, N-acetyl-D-glucosamine, D-fructose, α-D-galactose, L-proline, D-serine, D-alanine, L-alanine, L-glutamic acid, L-histidine, D,L-lactic acid, succinic acid, L-asparagine, L-aspartic acid, L-serine, D-trehalose, acetic acid, D-glucolic acid and propionic acid. Negative for α-cyclodextrin, L-arabinose, D-galactose, α-D-lactose, maltose, D-melibiose, D-mannitol, glycyln-L-glutamic acid, methyl pyruvate, β-methyl-D-glucoside, D-psicose, D-raffinoside, L-rhamnose, L-phenylalanine, L-alanyl-glycine, xylitol, D-glucosaminic acid, malonic acid, quinic acid, D-saccaric acid, succinic acid, glycyln-L-aspartic acid, L-propylgulutamic acid, D,L-carantine, inosine, thymidine, 2-aminoethanol, N-acetyl-D-galactosamine, adonitol, D-cellobiose, L-fucose, gentiobiose, lactulose, D-mannose, sucrose, turanose, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucuronic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylactic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, sebacic acid, glucononamide, L-ornithine, urocanic acid, L-threonine, phenethylamine, glycerol, glucose-1-phosphate, D,L-α-glycol-olerase and 2,3-butanediol. The major fatty acids are C₁₆:ω7c/C₁₆:ω6c, C₁₆:0 and C₁₈:1ω7c. The major ubiquinone is Q-8. Predominant polar lipids are phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and diphostatidylglycerol (DPG).

The type strain is IITR-71ᵀ (= MTCC 11059ᵀ = JCM 30068ᵀ = KCCT 52433ᵀ) isolated from soil collected from an industrial site in Eloor in the Cochin region of Southern India. The DNA G+C content of the type strain was estimated to be 61.2 mol%.

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