Mesorhizobium thiogangeticum sp. nov., a novel sulfur-oxidizing chemolithoautotroph from rhizosphere soil of an Indian tropical leguminous plant

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The bacterial strain SJT^T, along with 15 other mesophilic, neutrophilic and facultatively sulfur-oxidizing chemolithotrophic isolates, was isolated by enrichment on reduced sulfur compounds as the sole energy and electron source from soils immediately adjacent to the roots of *Clitoria ternatea*, a slender leguminous herb of the Lower Gangetic plains of India. Strain SJT^T was able to oxidize thiosulfate and elemental sulfur for chemolithoautotrophic growth. 16S rRNA and *recA* gene sequence-based phylogenetic analyses showed that the Gram-negative rod-shaped bacterium belonged to the genus *Mesorhizobium* and was most closely related to *Mesorhizobium loti*, *Mesorhizobium plurifarum*, *Mesorhizobium amorphae* and *Mesorhizobium chacoense*. Unequivocally low 16S rRNA (<97%) and *recA* (≤88%) gene sequence similarities to all existing species of the most closely related genera, a unique fatty acid profile, a distinct G+C content (59.6 mol%) and phenotypic characteristics all suggested that strain SJT^T represents a novel species. DNA–DNA hybridization and SDS-PAGE analysis of whole-cell proteins also confirmed the taxonomic uniqueness of SJT^T. It is therefore proposed that isolate SJT^T (=LMG 22697^T=MTCC 7001^T) be classified as the type strain of a novel species, *Mesorhizobium thiogangeticum* sp. nov.

Taxonomy of the family *Rhizobiaceae* has undergone extensive revision in the last couple of decades and the description of a bacterium as a member of the family usually includes its ability to nodulate a leguminous host. However, it has also been found that symbiotic properties in some rhizobia are genetically unstable and the possibility of non-symbiotic rhizobia (Segovia *et al.*, 1991; Rogel *et al.*, 2001) existing as significant components of rhizobial populations in the soil has been indicated (Sullivan *et al.*, 1996).

From soil adjacent to the root of *Clitoria ternatea*, a slender leguminous herb (family *Papilionaceae*) that occurs on almost every piece of waste ground and in village forests of the Lower Gangetic plains of India, the mesophilic, neutrophilic, facultatively sulfur-oxidizing chemolithotrophic

bacterial strain SJT^T was isolated by enrichment, along with 15 other sulfur-oxidizing strains, on reduced sulfur compounds as the sole energy and electron source. Numerous plants were uprooted from a single plot of land and soil that adhered loosely to the roots was dislodged by gently striking the roots with sterile forceps. The collected pool of loose soil that was devoid of any root nodules was supplemented with Na₂S₂O₃·5H₂O (5%), Na₂S (1%) and elemental sulfur powder (5%) and incubated at 30 °C for 2 weeks with intermittent sprinkling of sterile water. Enriched soil samples were added (1%, w/v) to MS-thiosulfate-yeast extract (MSTY; 20 mM Na₂S₂O₃·5H₂O supplemented with 5.0 g yeast extract l⁻¹) liquid medium (pH 7.0–7.5) based on a modified basal and mineral salts (MS) solution that contained the following (l⁻¹ distilled water): 1 g NH₄Cl, 4 g K₂HPO₄, 1.5 g KH₂PO₄, 0.5 g MgSO₄·7H₂O and 5.0 ml trace metals solution (Vishniac & Santer, 1957). Mixtures were incubated at 30 °C on a rotary shaker until the colour of the phenol red indicator added to the medium changed to yellow. Serial dilutions from the soil-MSTY broth mixture were plated on MSTY agar and incubated at 30 °C. Strain SJT^T, which could be distinguished in terms of colony morphology and the rate and extent of acid production in chemolithoautotrophic MS-thiosulfate (MST; 20 mM Na₂S₂O₃·5H₂O plus 50 mg yeast extract l⁻¹ as growth factor

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *recA* gene sequences of the strain SJT^T are AJ864462 and AM040610, respectively.

Fatty acid profiles, a phenogram derived from UPGMA/S_{SM} analysis and SDS-PAGE analysis of whole-cell proteins of strain SJT^T and related species are available as supplementary material in IJSEM Online.

supplement) and mixotrophic MSTY media, was isolated as a pure culture.

All growth experiments were performed at 30 °C and yeast extract (50 mg l⁻¹) or a vitamin mixture (10 mg each of nicotinic acid, pantothenic acid, pyridoxine, thiamin, *p*-aminobenzoic acid, riboflavin and biotin l⁻¹) was always added to the medium. Cells were tested for their ability to use thiosulfate (12–20 mM), sulfide (2 mM), sulfite (3 mM), thiocyanate (2 or 5 mM), elemental sulfur (0.5 and 1.0 %, w/v) or tetrathionate (10 mM) as a substrate for chemolithotrophic growth. For testing mixotrophic utilization of sulfur compounds, yeast extract (2.5 or 5.0 g l⁻¹) was added to the above formulations. The level of thiosulfate or tetrathionate in the medium was estimated by the cyanolytic method described by Kelly & Wood (1994). Strain SJT^T was facultatively chemolithotrophic and could grow on 12–20 mM thiosulfate as the sole energy and electron source, but not on soluble sulfide, thiocyanate, tetrathionate or sulfite under the experimental conditions used. It also utilized elemental sulfur (10 g l⁻¹) for chemolithotrophic growth, with a corresponding increase in the OD₆₀₀ of the broth cultures by an order of 0.2, which is attributable to oxidation and the disappearance of colloidal sulfur, along with lowering of the pH of the medium from 7.5 to 6.2 over an incubation period of 5 days. Details of chemolithotrophic growth and thiosulfate consumption by SJT^T in MST or MSTY medium containing 20 mM sodium thiosulfate, equivalent to 40 µg sulfur atoms ml⁻¹, are shown in Table 1.

All other phenotypic tests were performed using standard techniques described elsewhere (Gerhardt *et al.*, 1994). The bacterium could grow in complex media like Luria–Bertani (LB) broth, nutrient broth (NB) and MS-peptone casein hydrolysate. Poor growth was observed on yeast

extract-mannitol agar (YMA). In synthetic heterotrophic media, i.e. MS solution plus one organic carbon source (5 g l⁻¹) supplemented with vitamin mixture, the isolate could utilize only a few carbon compounds.

The 16S rRNA gene was amplified and its sequence was determined from PCR products using universal primers (Gerhardt *et al.*, 1994). Evolutionary distances were calculated by pairwise comparison of the aligned 16S rRNA gene sequences (Jukes & Cantor, 1969) by the program DNADIST. A consensus neighbour-joining tree (Saitou & Nei, 1987) was constructed following majority rule and strict consensus out of 100 phylogenetic trees produced using the program NEIGHBOR in PHYLIP version 3.572c (Felsenstein, 1993). Bootstrap values (100 replicates) were calculated by the method of Felsenstein (1985) to validate the reproducibility of the branching pattern of the tree. 16S rRNA gene sequence-based phylogenetic analysis of SJT^T showed that the strain had maximum 16S rRNA gene sequence similarity (96.2–96.8 %) to species of *Mesorhizobium*, e.g. *Mesorhizobium loti*, *Mesorhizobium plurifarium*, *Mesorhizobium amorphae* and *Mesorhizobium chacoense*, and occupied the same phylogenetic branch as *Mesorhizobium* species (Fig. 1). Species of other genera, i.e. *Sinorhizobium fredii* (96 %), *Aminobacter aminovorans* (95.7 %), *Aminobacter aganoensis* (95.6 %) and *Aminobacter niigataensis* (95.6 %), had still lower 16S rRNA gene sequence similarity to SJT^T, whereas strains of *Pseudaminobacter salicylatoxidans* exhibited ≤94 % rRNA gene sequence similarity to SJT^T. Strain KCT001, a thiosulfate- and tetrathionate-oxidizing facultatively chemolithotrophic member of *Pseudaminobacter salicylatoxidans* (Deb *et al.*, 2004) exhibited 94 % 16S rRNA gene sequence similarity to SJT^T. A Mexican soil isolate, designated Ls29 and identified as a strain of *M. plurifarium*, showed slightly higher 16S rRNA gene sequence similarity (97.1 %) to SJT^T.

Phylogeny of the *recA* gene (encoding part of the DNA recombination and repair system) in rhizobial taxa (Gaunt *et al.*, 2001; Weir *et al.*, 2004; Vinuesa *et al.*, 2005) has been shown to be congruent with that of the small-subunit rRNA genes with well-supported clades for members of *Mesorhizobium* species having unequivocal bootstrap supports (Gaunt *et al.*, 2001). Moreover, large-scale phylogenies of *recA* in the *Bacteria* as a whole have been found to be consistent with the corresponding rRNA gene sequence-based phylogenies (Eisen, 1995; Young, 1998).

A *recA* gene fragment was amplified by PCR from isolate SJT^T using published *recA* primers (Gaunt *et al.*, 2001) [*recA* 6 (forward; CGKCTSGTAGAGGAYAAATCGGTGGA) and *recA* 555 (reverse; CGRATCTGGTTGATGAAGATCACCAT)], where mixtures of bases used at certain positions are as follows: K, T or G; S, G or C; Y, C or T; R, A or G]. The *recA* gene sequence-based phylogenetic affinities of strain SJT^T were reconstructed using the neighbour-joining method (Saitou & Nei, 1987) with distances estimated using Jukes–Cantor as well as Kimura's two-parameter models with 100 bootstrap replications.

Table 1. Utilization of thiosulfate and concomitant decrease in pH of the medium over 4 days of growth by the novel strain SJT^T in chemolithotrophic medium at 30 °C

The initial concentration of thiosulfate (Na₂S₂O₃) in both media was 20 mM (equivalent to 40 µg sulfur atoms ml⁻¹). The increase in OD₆₀₀ is attributed to thiosulfate utilization and values presented were calculated after eliminating background growth of the bacteria in basal salts medium without thiosulfate. The initial pH of thiosulfate-containing media was 7.5.

Parameter	MST	MSTY
Thiosulfate consumption after incubation for:		
1 day	8	12
2 days	12	20
3 days	24	28
4 days	30	34
Final OD ₆₀₀	0.10–0.15	—
Final pH of spent medium	5.5	5.8

Fig. 1. Neighbour-joining tree of the novel isolate *Mesorhizobium thioangeticum* SJT^T and phylogenetically related bacteria constructed on the basis of their 16S rRNA gene sequences. Bar, 1% nucleotide difference. GenBank accession numbers are given beside the strain numbers and bootstrap probability values (based on 100 replications) are indicated at the major branch-points.

Phylogenetic analysis based on the *recA* gene sequence also placed SJT^T in the same evolutionary branch as *Mesorhizobium* species (Fig. 2). The partial *recA* gene sequence of strain SJT^T had maximum similarity (88%) to strains of *M. loti*, whereas other rhizobial isolates awaiting specific designation and tentatively described as *Mesorhizobium* species (Weir *et al.*, 2004) also exhibited 88% nucleotide sequence similarity to the *recA* gene of SJT^T. The translated amino acid sequences of the *recA* genes of these organisms had 97% identity to that of SJT^T. The partial *recA* gene sequences of strains of *M. plurifarium*, *Mesorhizobium huakuii*, *Mesorhizobium tianshanense*, *Mesorhizobium ciceri*, *Mesorhizobium mediterraneum*, *M. amorphae* and *M. chacoense* had 85–87% similarity to that of strain SJT^T. The translated amino acid sequences of the *recA* genes of all these *Mesorhizobium* species had 96–97% identity to that of SJT^T. *recA* gene sequence similarities of the novel sulfur-oxidizing chemolithotroph with species of other genera like

Rhizobium, *Sinorhizobium*, *Agrobacterium*, *Azorhizobium*, *Bradyrhizobium*, *Rhodopseudomonas*, *Mycoplana* and *Brucella* were much lower, between 80 and 84% (Fig. 2), whereas the translated amino acid sequences of the *recA* genes of these organisms had obviously lower identities (<96%) to that of strain SJT^T.

High-molecular-mass DNA was prepared as described by Ezaki *et al.* (1988) with minor modifications. Dot-blot hybridization experiments were carried out with the DIG DNA Labelling and Detection system as described previously (Labrenz *et al.*, 2000) to determine DNA–DNA binding values. The taxonomic uniqueness of SJT^T was supported by its low DNA–DNA binding values with the four phylogenetically closest species: *M. plurifarium* LMG 11892^T (28%); *M. loti* LMG 17826 t2 (24%); *M. amorphae* LMG 18932 (18%); and *M. chacoense* LMG 19008^T (22%). The phylogenetically related sulfur-oxidizing chemolithotrophic

Fig. 2. Neighbour-joining tree of strain SJT^T and closely related bacteria constructed on the basis of their *recA* gene sequences. Bar, 10% nucleotide difference. GenBank accession numbers are given beside the strain numbers and bootstrap probability values (based on 100 replications) are indicated at the major branch-points.

Pseudaminobacter salicylatoxidans KCT001 (Deb *et al.*, 2004) exhibited an even lower DNA–DNA binding value (10 %) with SJT^T.

In this connection, it is noteworthy that homologues of the sulfur oxidation (Sox) genes discovered by virtue of genetic studies with alphaproteobacteria like *Paracoccus pantotrophus*, *Pseudaminobacter salicylatoxidans* strain KCT001 and *Rhodovulum sulfidophilum* (Friedrich *et al.*, 2000; Mukhopadhyaya *et al.*, 2000; Rother *et al.*, 2001; Appia-Ayme *et al.*, 2001; C. Lahiri, S. Mandal, W. Ghosh, B. Dam and P. Roy, unpublished observations) have also been revealed from whole genome sequence analyses of several species of the order *Rhizobiales* (Kaneko *et al.*, 2002; see NCBI Microbial Genomes Annotation Project, accession no. NZ_AAAF01000001 for *Rhodopseudomonas palustris*). However, despite the close phylogenetic relationship of the two organisms, no hybridization was detected, even under low stringency conditions, when the genomic DNA of SJT^T was probed by Southern hybridization with DIG-labelled *soxT*, *soxYZ* or *soxBC* gene fragments from *Pseudaminobacter salicylatoxidans* KCT001 (Mukhopadhyaya *et al.*, 2000; GenBank/EMBL accession number AJ404005) (data not shown). A distinct DNA G + C content of 59.6 mol%, determined using HPLC as described by Mesbah *et al.* (1989), was observed for SJT^T, which differs from the G + C contents of most of the closely related species of *Mesorhizobium*, thus reiterating the distinction of the isolate as a unique member of the mesorhizobia.

At the same time, the reference strains *M. plurifarium* LMG 11892^T, *M. loti* LMG 17826 t2, *M. amorphae* LMG 18932 and *M. chacoense* LMG 19008^T were tested for their ability to oxidize or chemolithotrophically utilize thiosulfate in autotrophic MST or mixotrophic MSTY media. However, these mesorhizobial strains phylogenetically closest to SJT^T could neither oxidize nor grow in MST or MSTY medium under the experimental conditions used.

Nodulating ability of SJT^T was tested on *Clitoria ternatea*, as well as two other economically important leguminous plants (*Pisum sativum* and *Cicer arietinum*). Seeds were surface-sterilized in concentrated H₂SO₄ for 20 min. After the acid was drained, seeds were washed thoroughly with 10 changes of sterile water and placed in moistened steam-sterilized sand to germinate at 28 °C for 3–5 days. Germinating seedlings, 3–4 cm long, were inoculated at the base of the stem or the radicle–plumule junction with 1 ml SJT^T inoculum (heavy suspension of LB broth-grown exponential-phase culture in 10 ml nitrogen-free Jensen solution, pH 6.8) and placed in a steam-sterilized sand–vermiculite mixture in 12 cm diameter pots and left to establish in the greenhouse at 28 °C. The surface was covered with polyurethane beads to prevent evaporation and contamination. Plants were watered with a sterile nitrogen-free nutrient solution once in 3 days. Saplings were checked for the presence of nodules after 10 weeks of growth. None of the plant species (10–15 individual saplings were taken for each plant species) inoculated with SJT^T were found to develop

nodules. Positive controls of *Cicer arietinum* seedlings inoculated with *M. ciceri* ATCC 51585^T developed nodules, whereas uninoculated plants did not. Since the negative observations found in these cases cannot be taken as attributes of the organism, it cannot be confirmed that SJT^T does not have the potential to nodulate any plant host.

The inability to utilize maltose, raffinose, L-alanine, rhamnose, fructose, L-arabinose, sucrose or lactose as single carbon sources, fast growth rates, growth in LB medium and chemolithotrophic utilization of thiosulfate and sulfur are some of the phenotypic characters that discriminate strain SJT^T from its phylogenetic relatives. Numerical analysis of all the available comparative phenotypic characteristics was performed using the simple matching coefficient (*S*_{SM}) (Sneath & Sokal, 1973) followed by generation of phenograms using the unweighted pair group with mathematical averages (UPGMA) algorithm. These analyses largely corroborated the phylogenetic and genetic distinctiveness of strain SJT^T. Table 2 shows the key phenotypic characters that distinguish SJT^T from the most closely related *Mesorhizobium* species and *Pseudaminobacter salicylatoxidans*; the phenogram derived from UPGMA/*S*_{SM} analysis of the phenetic characteristics of SJT^T with respect to the nearest mesorhizobia is available as Supplementary Fig. S1 in IJSEM Online. Polyphasic evaluation that included almost 70 % phenotypic similarity to standard strains of *M. plurifarium* (Wang *et al.*, 2003) and high 16S rRNA gene sequence similarity (98.75 %) to the type strain (LMG 11892^T) of the species identified Ls29 as a strain of *M. plurifarium*. However, SJT^T had several phenotypic dissimilarities from Ls29 (Table 2) and a lower *S*_{SM} value (60 %).

SDS-PAGE analysis of whole-cell proteins also yielded distinct profiles for SJT^T when compared with *Pseudaminobacter salicylatoxidans* KCT001 or the four nearest mesorhizobia, *M. plurifarium* LMG 11892^T, *M. loti* LMG 17826 t2, *M. amorphae* LMG 18932 and *M. chacoense* LMG 19008^T (Supplementary Fig. S2).

Cellular fatty acid profiles have also been shown to discern species of *Mesorhizobium* accurately and to distinguish between related genera (de Lajudie *et al.*, 1998; Tighe *et al.*, 2000). After an incubation period of 40 h at 30 °C on LB agar, a loopful of well-grown cells was harvested and the preparation, separation and identification of fatty acids were performed using the Sherlock Microbial Identification system (Microbial ID) at the DSMZ (Braunschweig, Germany). Strain SJT^T synthesized 16:0, 17:0, 18:0, 17:0 iso, 17:1 ω 8c, 18:1 ω 7c and 19:0 cyclo ω 8c fatty acids, thus confirming its classification as a species of *Mesorhizobium*. On the other hand, SJT^T was distinct from all related mesorhizobia by its unique possession of 14:0, 18:0 3-OH, 15:0 iso 3-OH, relatively large amounts (77.41 %) of 18:1 ω 7c, and the absence of 13:0 iso 3-OH, 12:0 3-OH and 17:0 cyclo. The fatty acid profile also set SJT^T apart from species of other phylogenetically related genera like *Aminobacter*, *Pseudaminobacter* and *Sinorhizobium* (Supplementary Table S1).

Table 2. Phenotypic characteristics that are useful for differentiation of *Mesorhizobium thiogangeticum* SJT^T from related taxa

Species: 1, *M. thiogangeticum* sp. nov. SJT^T; 2, *M. loti*; 3, *M. plurifarium* Ls29 (a Mexican strain); 4, *M. plurifarium* (characters observed for standard strains); 5, *M. amorphae*; 6, *M. chacoense*; 7, *M. mediterraneum*; 8, *M. huakuii*; 9, *Pseudaminobacter salicylatoxidans*. +, Positive; –, negative; d, strain-dependent; NA, data not available. The table was adapted from Wang *et al.* (2003). Some other information was taken from de Lajudie *et al.* (1998), Wang *et al.* (1999), Velázquez *et al.* (2001), Kämpfer *et al.* (1999) and Deb *et al.* (2004).

Character	1	2	3	4	5	6	7	8	9
Growth on LB medium	+	–	–	+	–	NA	–	–	+
Generation time (h)*	2–3	6–13	5	6–13	6–13	10–24	5–6	4–6	2–3
Range of pH tolerance	5.5–8.5	4.0 to <10.0	4.5–8.0	4.5–9.0	5.0–8.5	NA	5.0 to <10.0	5.0–9.5	5.5–8.5
Tolerance of 2% (w/v) NaCl	–	+	–	+	–	NA	+	–	–
Utilization of maltose, rhamnose, fructose, sucrose and lactose as single carbon sources	–	+	NA	+	+	+	+	+	–
Utilization of:									
Raffinose	–	d	NA	d	+	+	–	+	–
L-Alanine	–	d	NA	d	+	NA	–	–	+
DL-Malate	–	+	NA	+	+	+	+	+	+
Growth above 37 °C	–	+	–	+	–	–	+	+	+
Chemolithotrophic growth†	+	–	NA	–	–	–	NA	NA	+
DNA G+C content (mol%)	59.6	59–64	63.2	63–64	64	62	63–65	59–64	63.9

*Generation time determined on LB medium for strain SJT^T and *Pseudaminobacter salicylatoxidans* KCT001, YMA medium for *M. chacoense* and *M. loti* and PY medium for the remaining species.

†Chemolithotrophic oxidation of sulfur compounds was tested with the type strains of *M. loti*, *M. plurifarium*, *M. amorphae* and *M. chacoense* in the present study.

From the above findings, it is evident that the novel mesophilic, neutrophilic and facultatively sulfur-oxidizing chemolithoautotrophic isolate constitutes a unique taxonomic entity that belongs to the genus *Mesorhizobium*, but is distinct from all known species of the genus. Strain SJT^T is thus classified as the type strain of a novel species, *Mesorhizobium thiogangeticum* sp. nov. However, in the absence of an adequate number of strains required to describe a bacterial species in general and a rhizobial species in particular, the complete description and delimitation of the novel species can only be finalized when more isolates are discovered or become available.

Description of *Mesorhizobium thiogangeticum* sp. nov.

Mesorhizobium thiogangeticum (thi.o.gan.ge'ti.cum. Gr. neut. n. *thion* sulfur; L. neut. adj. *gangeticum* from Ganga; N.L. neut. adj. *thiogangeticum* because the bacterium is so far the only sulfur-lithotrophic member of *Mesorhizobium* and is a native of the Gangetic plains of India).

Gram-negative, aerobic, non-spore-forming, irregularly elongated or rod-shaped bacterium (cells 1.2–1.5 × 0.2–0.4 µm). Colonies on LB or MSTY media are shining white, convex, opaque and reach 1–3 mm in diameter within 4–5 days at 30 °C. Maximum temperature for growth is

37 °C and range of pH tolerance is 5.5–8.5. Catalase-positive, but oxidase-negative. Strain SJT^T cannot liquefy gelatin, produce indole or solubilize phosphate. It can grow heterotrophically in complex media like LB, NB, YMA and MS-peptone casein hydrolysate. In synthetic media supplemented with 50 mg yeast extract l^{–1} or vitamin mixture, the isolate utilizes only a few carbon compounds, D-glucose, L-arabinose, D-galactose, D-mannitol, L-histidine, L-leucine, L-isoleucine, L-glutamine, succinate and sodium benzoate, as the sole source of energy for chemo-organoheterotrophic growth. No growth is observed with citrate, oxalate, malate, acetate, maltose, sucrose, glycerol, *myo*-inositol, mandelate, DL-lactate, D-fructose, D-raffinose, D-xylose, D-lactose, L-aspartic acid, L-lysine, L-cysteine, L-cystine, L-tyrosine, L-threonine, L-alanine, L-serine, D-mannose, L-arginine or tryptophan. No growth is observed in nitrogen-free Burk medium under aerobic conditions. Chemolithoautotrophic growth is observed with thiosulfate and elemental sulfur, but not tetrathionate, thiocyanate, soluble sulfides, sulfite or arsenite. Synthesizes the following fatty acids: 14:0 (0.88%), 15:0 iso (8.23%), summed feature 3 (16:1ω7c/15:0 iso 2-OH) (1.82%), 16:0 (2.46%), 15:0 iso 3-OH (0.41%), 17:0 iso (0.85%), 17:1ω8c (0.51%), 17:0 (0.81%), 18:1ω7c (77.41%), 18:0 (1.28%), 11-methyl 18:1ω7c (4.23%), 19:0 cyclo ω8c (0.64%) and 18:0 3-OH (0.47%). The DNA G+C content of the type strain is 59.6 mol%, as determined by HPLC.

The type strain, strain SJT^T (=LMG 22697^T=MTCC 7001^T), is the only known strain of the species, isolated from soil adjacent to the roots of the leguminous plant *Clitoria ternatea*, a native of the lower Gangetic plains of India.

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