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Pseudoxanthomonas icgebensis sp. nov., Isolated from the Midgut of *Anopheles stephensi* Field-Collected Larvae[§]

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A Gram-negative, aerobic, golden yellow, rod-shaped bacterium, a strain designated ICGEB-L15^T, was isolated from the larval midgut of *Anopheles stephensi* captured in District Jhajjar, Haryana, India. The strain ICGEB-L15^T grows at 30-50°C (optimum 30-37°C), pH 6.5-8.5 (optimum 7.0-8.0) and in the presence of 2% NaCl. The major fatty acids were iso-$C_{15:0}$ (22.5% of total fatty acid), anteiso-$C_{15:0}$ (16.5%), iso-$C_{17:1}\omega 9c$ (10.3%), iso-$C_{16:0}$ (7.3%), $C_{16:0}$ (6.1%), and iso-$C_{11:0}$ (5.3%). The strain showed the highest 16S rRNA gene sequence similarities with the type strains *Pseudoxanthomonas daejeonensis* KCTC 12207^T (97.4%), *Pseudoxanthomonas kaohsiungensis* J36^T (97.17%), and *Pseudoxanthomonas mexicana* AMX 26B^T (97.11%). The DNA relatedness between ICGEB-L15^T and *Pseudoxanthomonas daejeonensis* KCTC 12207^T, *Pseudoxanthomonas kaohsiungensis* J36^T and *Pseudoxanthomonas mexicana* AMX 26B^T was 24.5%, 28.2%, and 33.6%, respectively. The G+C content of genomic DNA was 69.9 mol%. The major isoprenoid quinone of strain ICGEB-L15^T was Q-8. The strain ICGEB-L15^T represents a novel species of the genus *Pseudoxanthomonas* based on physiological, biochemical and phylogenetic properties; therefore, the name *Pseudoxanthomonas icgebensis* sp. nov. is proposed. The type strain is ICGEB-L15^T (=KACC 14090^T =DSM 22536^T).

***Keywords*: *P. icgebensis*, *A. stephensi*, midgut microflora, cement tanks**

Members of the genus *Pseudoxanthomonas*, first described by Finkmann *et al.* (2000), are phylogenetically related to the genera *Xanthomonas*, *Xylella*, and *Stenotrophomonas* (Garrity and Holt, 2001; Finkmann *et al.*, 2000; Chen *et al.*, 2002; Thierry *et al*., 2004; Yang *et al*., 2005). They are Gram-negative, non-spore forming rods, consisting of straight, and branched chain fatty acids of ubiquinone with eight isoprene units (Q-8). This genus can be differentiated from related genera by the ability to reduce nitrite, but not nitrate, and the absence of $C_{13:0}$ iso 3-OH fatty acid (Yang *et al.*, 1993; Finkmann *et al.*, 2000; Assih *et al.*, 2002). At the time of writing, the genus *Pseudoxanthomonas* is comprised of twelve species: *Pseudoxanthomonas broegbernensis* (Finkmann *et al.*, 2000); *P. taiwanensis* (Chen *et al.*, 2002); *P. mexicana* (Thierry *et al.*, 2004); *P. japonensis* (Thierry *et al.*, 2004); *P. koreensis* (Yang *et al.*, 2005); *P. daejeonensis* (Yang *et al.*, 2005); *P. kaohsiungensis* (Chang *et al.*, 2005); *P. kalamensis* (Harada *et al*., 2006); *P. suwonensis* (Weon *et al*., 2006); *P. spadix* (Young *et al*., 2007); *P. yeongjuensis* (Yoo *et al*., 2007); and *P. sacheonesis* (Yoon *et al*., 2006; Lee *et al*., 2008).

In a series of earlier studies, we attempted to isolate micro-organisms from the midgut of *Anopheles stephensi* to investigate community structure based on culture dependent and culture independent methods (Rani *et al*., 2009). *Anopheles stephensi* is one of the major malaria vectors in India. While in the mosquito midgut, the parasite undergoes major developmental and maturation steps. Identification and characterization of the mosquito midgut flora may contribute to a better understanding of mosquito-pathogen interactions that are important for the development of vector control strategies. For this study, we isolated strain ICGEB-L15^T from the midgut microflora of *A. stephensi* larvae, field-collected from cement tanks in District Jhajjar, Haryana, India. The phenotypic and chemotaxonomic characteristics were examined and a phylogenetic analysis was carried out. The results suggest that the strain ICGEB-L15^T represents a novel species of the genus *Pseudoxanthomonas*, for which the name *Pseudoxanthomonas icgebensis* sp. nov. is proposed.

Materials and Methods

Collection of *A. stephensi* and midgut extraction

Anopheline larvae were collected from cement tanks in District Jhajjar, Haryana, India (28°37′N and 76°39′E) at three different times. From the total larvae collected, IV instars were separated and were transported to the laboratory in Delhi within two hours of collection, and those morphologically identified as *A. stephensi* were pooled (Rani *et al*., 2009). Cyclic colonies of *A. stephensi* were maintained in a mosquitarium maintained at 28±2°C and 70-80% humidity (Sharma *et al*., 2009). Larvae were collected in filter paper lined plastic bowls half filled with de-ionized water and left undisturbed for two days. Larvae were cultured in enamels trays and fed a mixture of dog biscuits and yeast extract in a 3:1 ratio. The larvae were surface sterilized with 95% ethanol, and larval guts were dissected aseptically using entomological needles and a stereomicroscope. The dissected

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midguts were stored at -70°C in 100 µl of sterile phosphate-buffered saline.

Isolation and culture of the bacterial strain

Strain ICGEB-L15^T was isolated on nutrient agar from the midgut of *A. stephensi* larvae collected from cement tanks (Rani *et al*., 2009). The midgut sample (1 ml) was added to 5 ml of Tryptic Soy Broth (TSB; Difco, USA) R2A, NB, and LB medium (HiMedia, India) and incubated at 30-37°C, 200 rpm for 24-48 h. Following serial dilution, the suspension was spread on TSA and LA plates and incubated at 30-37°C for 24-48 h. Single colonies were purified by transferring each colony to a fresh plate and subsequently incubating the plate. The ICGEB-L15^T strain was cultured on TSA and maintained as a glycerol suspension (20%, w/v) at -70°C. Strain ICGEB-L15^T was deposited into KACC (Korean Agriculture Culture Collection), Suwon, Korea, as KACC 14090^T and DSMZ (German Collection of Microorganisms and Cell Cultures) was deposited as DSM 22536^T.

Phenotypic characteristics

After strain ICGEB-L15^T was grown for 2-3 days on TSA plates at 30°C, cell size and morphology were studied by phase-contrast and electron microscopy. Growth was assessed at 4, 10, 15, 20, 30, 37, 40, 41, 42, 45, 50, and 55°C in TSB and in the presence of 0, 0.5, 1.5, 2, 4, 5, 7, and 8% NaCl. The optimal range of pH was investigated in liquid medium (LB with no NaCl added). The pH of the LB broth was adjusted by adding phosphate buffer and then 1 N HCl or 1 N NaOH to achieve the desired pH. Gram staining was performed using the Gram Stain kit (Difco) according to manufacturer instructions. Motility was tested on a motility medium of 0.1% yeast extract, 0.01% K_2HPO_4, and 0.2% agar. A L-alanine aminopeptidase assay was performed using Bactident aminopeptidase (Merck, USA). Oxidase activity was tested by oxidation of 1% (w/v) tetramethyl-p-phenylenediamine, and catalase activity was evaluated by production of oxygen bubbles in a 3% (v/v) aqueous hydrogen peroxide solution. Nitrate reduction as well as the hydrolysis of casein, gelatin, Tween 20 and 80, asculin, and starch was investigated using previously described methods (Lańyí, 1987; Smibert and Krieg, 1994). Urease activity was determined as described by Macfaddin (2000). Substrate utilization and enzyme activity tests of the isolate and closely related strains were examined using API 32 GN and API 20 NE kits (bioMérieux, France) according to manufacturer instructions (bioMérieux). The API 50CH kit and 50CHB medium (bioMérieux) was used to assess carbon source oxidation over a period of 3 days at 30°C. Susceptibility to antibiotics was tested using the Kirby-Bauer disc diffusion method (Bauer *et al*., 1966). Inhibition diameters were recorded after 24-48 h incubation at 37°C.

Chemotaxonomy

Cellular fatty acids were analyzed in organisms grown on TSA at 30°C for 24-48 h. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI, USA). The cellular fatty acids were analyzed by gas chromatography (Hewlett-Packard 6890) with the Microbial Identification software package (MIDI). Analyses of isoprenoid quinone and polar lipids were carried out using HPLC as described by (Groth *et al*., 1996). For measurement of the G+C content of chromosomal DNA, the genomic DNA of strain ICGEB-L15^T was extracted and purified; the DNA G+C content was determined by HPLC method described by Mesbah *et al*. (1989).

16S rRNA gene sequence and phylogenetic analysis

Genomic DNA extraction, PCR amplification using universal primers 27f and 1492r of the 16S rRNA gene and purification of the PCR product of strain ICGEB-L15^T was carried out as previously described (Marmur, 1961; Lane, 1991). Sequencing of the amplified gene was performed at Macrogen Inc. (Korea) using an ABI prism BigDye Terminator Cycle Sequencing Ready Reaction kit v.3.1 and an ABI 3730XL capillary DNA Sequencer (Applied Biosystems, USA). The sequences were assembled into a nearly complete 16S rRNA gene sequence (1492 nt) with Vector NTI software (Invitrogen, USA). The 16S rRNA gene sequence of strain ICGEB-L15^T was compared with available 16S rRNA gene sequences from GenBank using the BLAST program (http://www.ncbi. nlm.nih.gov/blast; Altschul, 1990). Alignment of the 16S rRNA gene sequences of strain ICGEB-L15^T and related type strains were conducted using CLUSTAL W software (Thompson *et al*., 1994). Sequence similarity values between the isolate and related sequences were evaluated using the EzTaxon Server (http://www.eztaxon.org; Chun *et al*., 2007). Phylogenetic trees were constructed using neighbor-joining, maximum parsimony, and maximum-likelihood methods implemented by the PHYLIP software version 3.6 (Felsenstein, 2004). The evolutionary distance matrix for neighbor-joining method was calculated using the corrections of Jukes and Cantor (1969). Trees generated were analyzed with the TREEVIEW program (Page, 1996) and tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1,000 resamplings. The taxonomic relationship between strain ICGEB-L15^T and *Pseudoxanthomonas daejeonensis* KCTC 12207^T, *Pseudoxanthomonas kaohsiungensis* J36^T, and *Pseudoxanthomonas mexicana* AMX 26B^T was further examined using DNA-DNA hybridization. Genomic relatedness was determined using the membrane filter technique (Seldin and Dubnau, 1985) according to the method described by Baik *et al.* (2006) using a DIG High Prime DNA labeling and detection starter kit II (Roche). The percentages of DNA relatedness were calculated from the mean of five replications.

Results and Discussion

Morphology and physiological characteristics

The strain ICGEB-L15^T is Gram-negative, aerobic, non-motile, catalase-positive, and oxidase-negative. It can grow in TSB medium containing 0-8% NaCl (optimum at 2% NaCl). Growth of strain ICGEB-L15^T occurred between 30 and 50°C (optimal growth at 30-37°C), but not above 50°C. The initial medium pH range that allowed growth of strain ICGEB-L15^T was 6.5-8.5; however the optimal pH was 7.0-8.0. The cells were straight rods and occurred singly, in pairs or in rosettes. Colony morphology is circular, smooth in texture, semi-translucent, non-shiny with entire edges and golden yellow on LA, R2A, and TSA plates; these colonies have diameters of approximately 1.4-3.1 mm. The physiological and biochemical properties differentiating ICGEB-L15^T from other related *Pseudoxanthomonas* strains are shown in Table 1. Other phenotypic characteristics of strain ICGEB-L15^T are presented in the species description.

Antibiotic susceptibility assay

The isolates tested had similar responses and were found to be highly resistant (high MIC values) to most of the antibiotics tested. In particular, the isolates were resistant to the amino glycosides. Strain ICGEB-L15^T had a higher MIC for penicillin G compared to other strains. The antibiotic susceptibility

profile is presented in Supplementary data Table 1.

Chemotaxonomy

Strain ICGEB-L15^T possessed iso-$C_{15:0}$ (22.5% of total fatty acid), anteiso-$C_{15:0}$ (16.5 %), iso-$C_{17:1}\omega 9c$ (10.3%), and iso-$C_{16:0}$ (7.3%), as the major cellular fatty acids (Table 2). The predominant isoprenoid quinone of strain ICGEB-L15^T was Q-8. The fatty acid iso-$C_{13:0}$ 3-OH (2.6%), reported from the strain *Pseudoxanthomonas spadix*, was also detected in the strain ICGEB-L15^T (Young *et al*., 2007). In previous studies, it has been reported that iso-$C_{15:0}$, anteiso-$C_{15:0}$, and Q-8 were the characteristic major fatty acid and ubiquinone in the genus *Pseudoxanthomonas* (Finkmann *et al*., 2000).

16S rRNA gene sequence and phylogenetic analysis

The 1,492 bp 16S rRNA gene sequence of strain ICGEB-L15^T is clearly differentiated from those of the *Pseudoxanthomonas* type species. The 16S rRNA gene sequence similarity values between strain ICGEB-L15^T and other *Pseudoxanthomonas* species ranged from 97.4 to 94.7%. The highest sequence similarity was with the type strains of *Pseudoxanthomonas daejeonensis* KCTC 12207^T, *Pseudoxanthomonas kaohsiungensis* J36^T, and *Pseudoxanthomonas mexicana* AMX 26B^T (97.4%, 97.17%, and 97.11%, respectively). A phylogenetic tree (Fig. 1), constructed using the neighbor-joining method, shows that strain ICGEB-L15^T forms a compact cluster with *Pseudoxanthomonas daejeonensis* KCTC 12207^T, *Pseudoxanthomonas mexicana* AMX 26B^T, *Pseudoxanthomonas japonensis* 12-3^T,

Table 1. Phenotypic characteristics of strain ICGEB-L15^T and closely related *Pseudoxanthomonas* species. Strains: 1, *P. icgenensis* ICGEB-L15^T; 2, *P. daejeonensis* TR6-08^T (Yang *et al*., 2005); 3, *P. mexicana* AMX 26B^T (Thierry *et al.*, 2004); 4, *P. japonensis* 12-3^T (Thierry *et al*., 2004); 5, *P. spadix* IMMIB AFH-5^T (Young *et al*., 2007); 6, *P. sacheonensis* BD-C54^T (Lee *et al*., 2008); 7, *P. yeongjuensis* GR12-1^T (Yoo *et al*., 2007); 8, [*S.*] *dokdonensis* DS-16^T (Yoon *et al*., 2006); 9, *P. kalamensis* JA40^T (Harada *et al*., 2006); 10, *P. broegbernensis* B1616/1^T (Finkmann *et al*., 2000); 11, *P. taiwanensis* ATCC BAA-404^T (Chen *et al*., 2002); 12, *P. koreensis* T7-09^T (Yang *et al*., 2005); 13, *P. kaohsiungensis* J36^T (Chang *et al*., 2005); 14, *P. suwonensis* 4M1^T (Yoo *et al*., 2007). +, positive; –, negative; w, weak reaction; nd, not determined.

Characteristic	1[a]	2[a]	3[a]	4	5	6	7	8	9	10	11	12	13	14
Habitat	Midgut	Soil	Sludge	Soil	Soil	Soil	Soil	Soil	Soil	Bio-filters	Hot spring	Soil	Soil	Compost
Motility	-	+	+	+	-	-	+	-	+	+	-	-	+	+
Catalase	+	+	+	-	+	-	+	-	+	+	+	+	-	+
Optimum growth temperature (°C)	30-37	30	30-37	30-37	30-37	25-30	28	30	37	30	50	30	35	30
Growth at 10°C	-	w	+	+	-	-	+	+	-	+	-	w	nd	+
Growth at 42°C	+	-	-	-	-	-	-	-	-	-	+	-	nd	+
Growth in 7% / 8% NaCl	+	-	-	-	-	-	-	-	-	-	-	-	nd	-
Nitrate Reduction	-	-	-	-	-	-	-	+	-	-	-	-	nd	-
Hydrolysis of Aesculin	-	+	+	nd	-	-	+	-	-	+	+	-	-	+
Casein	-	+	+	+	-	+	+	+	-	-	-	+	-	-
Gelatin	+	-	+	+	-	+	+	+	+	-	-	-	-	+
Tween 80	-	nd	+/–	+	-	-	-	+	-	+	+	nd	-	-
Urea	-	-	-	nd	-	+	-	-	-	-	nd	+	-	-
Assimilation of Glucose	+	+	+	nd	+	+	+	+	-	+	+	-	+	+
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-Arabinose	-	+	-	-	+	-	-	-	-	+	-	-	-	+
D-Mannose	-	-	+	-	-	+	-	+	-	+	-	-	-	-
D-Mannitol	-	-	nd	nd	-	-	-	-	-	-	nd	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mio-Inositol	-	-	-	-	-	-	-	-	-	-	-		-	-
N-Acetyl-glucosamine	-	+	nd	nd	+	+	+	+	-	+	nd	-	+	+
D-Maltose	-	+	+	+	+	+	+	+	-	+	-	-	+	+
Citrate	-	-	-	-	-	-		-	-	+	nd	-	-	-
DNA G+C Content (mol%)	69.9	68.7	67.8	65.2	64	67.5	63.4	65.1	68.5	66.5	70.0	69.5	60.1	66.6-68.4

[a] Data from this study utilize the same conditions as described in this paper.

Table 2. Fatty acid compositions of strain ICGEB-L15^T and type strains of related *Pseudoxanthomonas* sp. Strains: 1, *P. icgenensis* ICGEB-L15^T 2, *P. daejeonensis* TR6-08^T (Yang *et al*., 2005); 3, *P. mexicana* AMX 26B^T (Thierry *et al*., 2004); 4, *P. japonensis* 12-3^T (Thierry *et al*., 2004); 5, *P. spadix* IMMIB AFH-5^T (Young *et al*., 2007); 6, *P. sacheonensis* BD-C54^T (Lee *et al*., 2008); 7, *P. yeongjuensis* GR12-1^T (Yoo *et al*., 2007); 8, *S. dokdonensis* DS-16^T (Yoon *et al*., 2006); 9, *P. kalamensis* JA40^T (Harada *et al*., 2006); 10, *P. broegbernensis* B1616/1^T (Finkmann *et al*., 2000); 11, *P. taiwanensis* ATCC BAA-404^T (Chen *et al*., 2002); 12, *P. koreensis* T7-09^T (Yang *et al*., 2005); 13, *P. kaohsiungensis* J36^T (Chang *et al*., 2005); 14, *P. suwonensis* 4M1^T (Yoo *et al*., 2007). -, not detected.

Fatty acid	1[a]	2[a]	3[a]	4	5	6	7	8	9	10	11	12	13	14
Saturated														
$C_{14:0}$	1.6	-	0.4	0.3	3.4	0.2	-	-	0.6	12.0	6.4	-	-	-
$C_{16:0}$	6.1	1.2	0.5	0.5	16.5	1.5	2.3	1.8	2.9	6.9	4.0	-	1.5	2.2
Branched														
iso-$C_{11:0}$	5.3	-	5.4	4.8	1.3	9.4	5.5	5.6	6.9	1.4	-	-	5.3	4.5
iso-$C_{14:0}$	2.6	2.2	2.1	2.8	1.0	1.6	1.4	2.1	0.6	-	-	-	-	-
iso-$C_{15:0}$	22.5	40.5	39.7	36.8	16.1	30.6	32.0	19.6	40.0	32.4	27.3	46.2	26.0	29.9
anteiso-$C_{15:0}$	16.5	6.9	2.6	1.9	1.2	-	4.4	3.4	4.6	31.8	11.3	-	10.3	11.8
iso-$C_{16:0}$	7.3	8.0	9.7	11.7	6.4	9.5	13.3	27.6	6.9	-	35.9	6.8	9.1	5.5
Unsaturated														
iso-$C_{17:0}$	3.6	3.6	4.9	3.5	16.1	8.6	6.4	-	6.7	-	3.3	7.8	7.5	8.4
iso-$C_{17:1}\omega 9c$	10.3	20.3	18.5	19.9	2.4	12.3	14.6	11.9	-	-	-	24.4	25.2	15.9
$C_{16:1}\omega 9c$ alcohol	3.1	-	-	-	-	-	-	-	1.9	-	-	-	-	-
$C_{13:0}$ 2-OH	1.7	-	-	-	-	-	-	-	0.1	-	-	-	-	-
iso-$C_{12:0}$ 3-OH	1.4	-	-	-	-	0.3	-	2.8	0.4	-	-	-	-	-
iso-$C_{13:0}$ 3-OH	2.6	-	-	-	0.6	-	-	-	-	-	-	-	-	-
Summed feature[b] 3	6.8	-	3.2	4.5	4.0	1.4	4.0	7.8	-	-	-	-	-	-

[a] Data from this study utilize the same conditions as described in this paper. Data are expressed as percentage of total fatty acids
[b] Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system.
Summed feature 3 contains $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH.

and *Pseudoxanthomonas taiwanensis* ATCC BAA-404^T. The overall topology of the NJ tree is supported by ML and MP algorithms (Fig. 1). The DNA relatedness of strain ICGEB-L15^T with *Pseudoxanthomonas daejeonensis* KCTC 12207^T, *Pseudoxanthomonas kaohsiungensis* J36^T, and *Pseudoxanthomonas mexicana* AMX 26B^T were 24.5%, 28.2%, and 33.6%, respectively, all of which are below the 70% threshold generally accepted for species delineation (Wayne *et al*., 1987; Stackebrandt and Goebel, 1994; Rosselló-Mora and Amann, 2001). The G+C content of strain ICGEB-L15^T was 69.9 mol%. From these data, it is evident that strain ICCEB-L15^T represents a novel species within the genus *Pseudoxanthomonas*; thus, the name *Pseudoxanthomonas icgebensis* sp. nov. is proposed.

Description of *Pseudoxanthomonas icgebensis* sp. nov.

Pseudoxanthomonas icgebensis (ic.ge.ben'sis. N.L. fem. adj. icgebensis pertaining to ICGEB, the acronym of the International Center for Genetic Engineering and Biotechnology, where the type strain was isolated and characterized).

Colonies are circular, smooth, semi-translucent and golden yellow with a diameter of 1.4-3.1 mm on NA, R2A, and TSA plates. Cells are aerobic and gram-negative as well as, non-motile and non-spore forming rods that are approximately 0.5-0.8×1.0-1.4 μm long. This strain grows at temperatures from 30-50°C (optimum: 30-37°C), in pH from 6.5 to 8.5 (optimum: pH 7.0-8.0) and in salinities from 0-8% (w/v) NaCl [optimum: 2% (w/v)]. Strain ICGEB-L15^T tests positive for catalase but negative for oxidase, arginine decarboxylase, H_2S production, indole, lysine decarboxylase, methyl red, nitrate reduction, and ornithine decarboxylase. Gelatin is hydrolyzed, but aesculin, Tween 20, Tween 80, CM-cellulose, pectine, starch, and xylan are not. The strain is positive for lipase activity. In addition, D-glucose can be assimilated as a sole carbon source however, it does not assimilate, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, capric acid, adipic acid, citrate, D-maltose, D-sucrose, ascorbic acid, D-fructose, D-galactose, D-raffinose, D-xylose, glycerol, lactose, L-arginine, L-cystine, L-arabinose, and oxalic acid. Acids are produced from D-glucose but not from L-arabinose, glycogen, inositol, inulin, D-lactose, maltose, D-mannitol, D-raffinose, D-ribose, D-sorbitol, or sucrose. The major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:1}\omega 9c$, iso-$C_{16:0}$, iso-$C_{11:0}$, and iso-$C_{17:0}$. The isoprenoid quinone of strain ICGEB-L15^T is Q-8. The G+C content of the genomic DNA is 69.9 mol%. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ICGEB-L15^T is FJ838784.

The type strain, ICGEB-L15^T (=KACC 14090^T =DSM 22536^T), was isolated from the midgut of field collected larvae of *Anopheles stephensi* - an Asian malarial vector, from cement tanks in District Jhajjar, Haryana, India.

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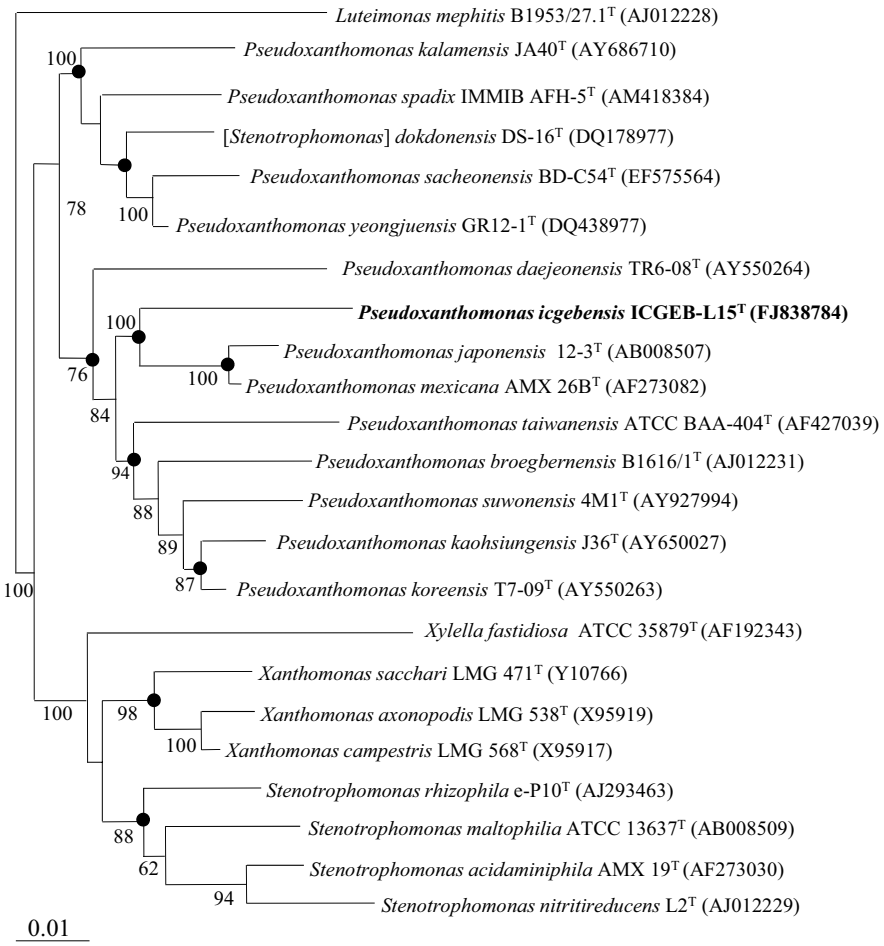

Fig. 1. Neighbor-joining tree showing the phylogenetic position of strain ICGEB-L15^T within related *Pseudoxanthomonas* strains. GenBank accession nos. of the 16S rRNA gene sequences are given in parentheses. Numbers at nodes indicate bootstrap values (based on 1,000 resamplings) if they are greater than 50%. Closed circles represent the corresponding nodes that are also recovered in both maximum-likelihood and maximum-parsimony methods. Bar, 0.01 substitutions per nucleotide position. *Luteimonas mephitis* B1953/27.1^T was used as an out-group.

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