Mucilaginibacter gossypii sp. nov. and *Mucilaginibacter gossypiicola* sp. nov., plant-growth-promoting bacteria isolated from cotton rhizosphere soils

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Two isolates from rhizosphere soil of cotton, designated Gh-67^T and Gh-48^T, which produced large amounts of extracellular polysaccharide and possessed plant-growth-promoting traits, were characterized phenotypically and genotypically. The strains were Gram-negative and cells were non-motile rods that grew optimally at 28 °C and grew between pH 4 and 7. 16S rRNA gene sequence analysis of strains Gh-67^T and Gh-48^T placed them in the genus *Mucilaginibacter*, with pairwise sequence similarity between them and type strains from related genera ranging from 93.9 to 98.2%. The major fatty acids were iso-C_{15:0}, C_{16:0} and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH). The strains contained MK-7 as the major isoprenoid quinone. The DNA G+C contents of strains Gh-67^T and Gh-48^T were 46.7 and 44.2 mol%, respectively. The low DNA–DNA hybridization value (18%) and a number of phenotypic differences between strains Gh-48^T and Gh-67^T indicated that they represent two separate species. Results of phenotypic, phylogenetic and genotypic analysis revealed that the strains were separated from the species of *Mucilaginibacter* described to date. Therefore, strains Gh-67^T and Gh-48^T represent novel species of *Mucilaginibacter*, for which we propose the names *Mucilaginibacter gossypii* sp. nov. (type strain Gh-67^T = NCIMB 14470^T = KCTC 22380^T) and *Mucilaginibacter gossypiicola* sp. nov. (type strain Gh-48^T = NCIMB 14471^T = KCTC 22379^T).

Beneficial plant–microbe interactions can result in the promotion of plant health and development and play a significant role in low-input sustainable agriculture applications. Several bacterial species are known to exert beneficial effects on plant growth (Vessey, 2003; Egamberdieva *et al.*, 2008). The plant-growth-promoting effects of beneficial bacteria may be through direct or indirect mechanisms that include production of phytohormones or enzymes that

promote plant growth, increased nutrient uptake and protection from deleterious phytopathogens (Glick *et al.*, 1999; Whipps, 2001). Members of the phylum *Bacteroidetes* are widely distributed in aquatic and terrestrial habitats and have a key role in degrading various biopolymers (Floyd *et al.*, 2005). The genus *Mucilaginibacter* Pankratov *et al.* 2007, a member of the family *Sphingobacteriaceae* Steyn *et al.* 1998, was proposed to group Gram-reaction-negative, non-spore-forming, non-motile rods that produced large amounts of extracellular polymeric substances (Pankratov *et al.*, 2007). At the time of writing, the genus *Mucilaginibacter* includes seven species with validly published names, *Mucilaginibacter paludis* (the type species), *M. gracilis*, *M. kameinonensis*, *M. daejeonensis*, *M. ximonensis*, *M. oryzae* and *M. rigui*, which were isolated from an acidic sphagnum peat bog, dried rice straw, wetland fresh water and soil samples (Pankratov *et al.*, 2007; Urai *et al.*, 2008; An

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate; EPS, extracellular polysaccharide.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA sequences of strains Gh-67^T and Gh-48^T are EU672804 and EU672805.

Scanning electron micrographs of cells of strains Gh-67^T and Gh-48^T are available as supplementary material with the online version of this paper.

et al., 2009; Luo *et al.*, 2009; Jeon *et al.*, 2009; Baik *et al.*, 2010). In this paper, we describe two *Mucilaginibacter* strains, Gh-67^T and Gh-48^T, that are extracellular polysaccharide (EPS)-producing, plant-growth-promoting bacteria isolated from rhizosphere soil of cotton. Based on phenotypic, phylogenetic and genotypic characteristics, we suggest that strains Gh-67^T and Gh-48^T represent two novel species of the genus.

Samples of rhizosphere soil of cotton (*Gossypium hirsutum* L. 'MCU-12') were collected from an experimental field of the Cotton Breeding Station, Tamilnadu Agricultural University (TNAU), Coimbatore, Tamilnadu, India. Two highly distinctive types of either cream- or pink-coloured, raised, semi-transparent and slimy colonies developed on selective ammonium mineral salts (AMS) medium (Whittenbury *et al.*, 1970) supplemented with filter-sterilized cycloheximide (10 µg ml⁻¹) and 0.5 % (v/v) methanol or R2A agar (Difco) with 1 % (v/v) methanol, inoculated with the terminal dilution of a soil suspension.

Morphological properties were studied and nutritional and phenotypic characterization was carried out according to previously described protocols (Gerhardt *et al.*, 1994; Green & Bousfield, 1982). Gram staining was performed by using a Gram staining kit (Difco) and bacterial suspensions were examined by phase-contrast microscopy for cell morphology and motility. Growth was tested on nutrient (NA; Difco), tryptic soy agar (TSA) (3 g tryptic soy broth l⁻¹; Difco), R2A (Difco) and MacConkey agars. The pH range for growth was determined on AMS agar. Growth temperature (4, 10, 20, 25, 30, 37 and 45 °C) and NaCl tolerance [0, 1, 2, 3, 4, 5 and 10 % (w/v)] were tested on AMS medium. Catalase activity was determined by assessing bubble production in 3 % (v/v) H₂O₂, and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine using Difco BBL catalase and oxidase reagent droppers according to the manufacturer's instructions (Difco, Becton Dickinson). Tests for hydrolysis of starch, casein, CM-cellulose, gelatin, aesculin and pectin were performed and evaluated after 5 days (Atlas, 1993; Kouker & Jaeger, 1987; Ten *et al.*, 2004). Lipolytic and proteolytic activities were investigated on plates containing tributyrin agar base and 1 % glycerol tributyrinate (Sigma) and 10 % TSA with 1.5 % skimmed milk powder, respectively. The plates were incubated for 3–5 days and examined for the presence of clearance zones around growing colonies. Nutritional features were determined using Biolog GN2 Microplates (Madhaiyan *et al.*, 2007a); carbon-source utilization tests other than those included in the Biolog plate were performed using a standard protocol (Green & Bousfield, 1982). Urease activity was tested using urea broth according to the manufacturer's instructions (Difco). Scanning electron microscope observations were performed as described by Bozzola & Russell (1998) using a Hitachi S-2500C instrument with GEMINI column (Hitachi Co.) equipped with a field-emission electron source. Quinones were extracted and analysed according to Kroppenstedt (1982). Flexirubin-like pigments were

observed by flooding the plates with 20 % (w/v) potassium hydroxide (Fautz & Reichenbach, 1980). Pigments were extracted from cultures grown in R2A liquid medium for 5 days using methanol and the resultant supernatant was scanned for absorbance at wavelengths of 190–1100 nm at room temperature on a UV-1601 spectrophotometer (Shimadzu). The antibiotic resistance patterns of the strains were determined and the detection and quantification of EPS were carried out as described previously (Chanprame *et al.*, 1996; Urai *et al.*, 2008).

Both strains were aerobic and Gram-negative and produced large amounts of an EPS. The cells were non-endospore-forming, non-motile, irregular short rods, 0.18–0.29 µm wide and 0.51–1.25 µm long, in chains when cultured on AMS agar plates at 25 °C for 2 days (Supplementary Fig. S1, available in IJSEM Online). Growth was observed on AMS or R2A medium supplemented with methanol, while no growth was observed on TSA or MacConkey agar. The strains showed good growth in the presence of 0.5 % (w/v) NaCl in AMS, but little or no growth occurred in the presence of 1 % (w/v) NaCl or higher. The nutritional and physiological characteristics of strains Gh-67^T and Gh-48^T are detailed in the species descriptions and differentiating characteristics from their closest relatives are given in Table 1.

A quantitative assay for the estimation of indole-3-acetic acid and plate assays for detecting the presence of siderophore production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity and sulfur oxidation were carried out as described previously (Poonguzhali *et al.*, 2006). A gnotobiotic assay using growth pouches was performed to measure root elongation induced by ACC deaminase. Tomato and canola seeds were used for this assay, since they are better representative of ethylene-sensitive plants (Glick *et al.*, 1994; Li *et al.*, 2000; Penrose & Glick, 2003; Ghosh *et al.*, 2003; Madhaiyan *et al.*, 2006, 2007b). The minimal inhibitory concentrations of heavy metals for the strains were determined as described by Madhaiyan *et al.* (2007b). Strains Gh-67^T and Gh-48^T were able to tolerate up to 1 mM NiCl₂/CdCl₂ and showed growth on plates, and no growth was observed above this concentration. From gnotobiotic growth pouch assays, the root length of tomato (23.3 and 32.9 % increase over controls, respectively) and canola (10.4 and 17.2 %) seedlings treated with *Mucilaginibacter* strains Gh-67^T and Gh-48^T was comparatively greater when compared with uninoculated controls. Both strains possessed ACC deaminase activity and oxidized sulfur when examined through plate assays but were negative in the siderophore assay. Strains Gh-67^T and Gh-48^T produced ACC deaminase activity of 5.32 and 12.32 nmol α-ketobutyrate (mg protein)⁻¹ h⁻¹. Both strains showed the presence of the *acdS* gene that encodes ACC deaminase activity when DNA extracts was analysed through PCR using the primer pair F1936/F1939 (Blaha *et al.*, 2006); a product of the expected size (558 bp) was detected, similar to the *acdS* genes of *Methylobacterium* species (Madhaiyan *et al.*, 2009).

Table 1. Physiological characteristics that allow differentiation among members of the genus *Mucilaginibacter*

Strains: 1, *Mucilaginibacter gossypii* sp. nov. Gh-67^T; 2, *Mucilaginibacter gossypicola* sp. nov. Gh-48^T; 3, *M. kameinonensis* KCTC 22227^T; 4, *M. gracilis* KACC 13451^T; 5, *M. paludis* KACC 13450^T; 6, *M. daejeonensis* Jip 10^T; 7, *M. ximonensis* XM-003^T; 8, *M. oryzae* B9^T. Data in columns 1–5 were obtained in this study and those in columns 6–8 were taken from An *et al.* (2009); Luo *et al.* (2009) and Jeon *et al.* (2009). +, Growth; –, no growth; w, weak growth; ND, no data available/not determined. All strains are positive for catalase production and assimilation of cellobiose, D-galactose, D-fructose, maltose, N-acetyl-D-glucosamine and lactose. All strains are negative for growth at 45 °C, utilization of acetate and indole production.

Characteristic	1	2	3	4	5	6	7	8
Colony colour*	PP	PP	PP	CR–LOR	LP–RH	O	PY	PP
Flexirubin-type pigments	+	+	+	–	–	ND	–	–
Cell shape†	R	SR	IR	R	R	R	IR	R
Cell width (µm)	0.18–0.2	0.2–0.3	0.3–0.7	0.4–0.5	0.5–0.8	0.6–0.8	0.3–0.5	0.5–0.8
Cell length (µm)	0.8–1.3	0.5–0.8	0.8–3.5	ND	ND	1.3–1.6	0.8–2.1	1.5–2.8
Oxidase	+	+	–	+	+	+	+	+
Urease	–	–	–	–	–	+	–	ND
Hydrolysis of:								
Aesculin	+	+	+	–	–	+	+	+
CM-cellulose	–	w	w	–	–	ND	–	+
Pectin	+	–	w	+	+	ND	–	w
Starch	–	–	–	+	+	+	–	–
pH range for growth	4–7	2–8	4–9	4.2–8.2	4.2–8.2	4.5–8.5	5–8	5–8
Growth at 37 °C	–	–	–	–	–	+	–	+
Growth at >1 % NaCl	–	–	–	–	–	+	–	+
Acid production from:								
L-Rhamnose	+	–	–	+	–	+	–	–
D-Mannose	+	+	+	–	+	+	+	+
Melezitose	+	+	+	–	–	–	–	–
Melibiose	+	w	+	–	–	–	w	+
Sucrose	+	+	+	+	+	+	–	+
D-Glucose	+	+	w	+	+	+	–	+
Trehalose	+	+	+	–	+	+	+	+
D-Xylose	w	+	+	+	+	–	+	–
Assimilation of:								
Melibiose	+	w	+	–	–	–	+	+
Raffinose	+	+	+	+	–	–	+	+
myo-Inositol	–	–	–	–	+	–	–	–
Acetic acid	w	+	–	–	–	–	+	+
Gluconate	+	w	–	–	+	–	–	+
Salicin	w	+	+	+	–	+	ND	+
Quinone(s)	MK-7	MK-7	MK-7	MK-6, MK-7	MK-6, MK-7	MK-7	MK-7	MK-7
DNA G + C content (mol%) (T _m)	46.7	44.2	43.7	42.4	46.1	40.4	43.4	44.4

*CR, Cream; LOR, light orange-reddish; LP, light pink; O, orange; PP, pale pink; PY, pale yellow; RH, reddish.

†IR, Irregular rods; R, rods; SR, short rods.

Chromosomal DNA was extracted according to the kit instructions (QIAamp DNA mini kit; Qiagen). The 16S rRNA gene was amplified using universal primers 27F and 1492R as described by Madhaiyan *et al.* (2009) and the partial gene sequence was determined by the fluorescent dye terminator method using an ABI Prism Big Dye terminator cycle sequencing ready reaction kit version 3.1; products were run on an ABI 3730XL capillary DNA sequencer (ABI Prism 310 Genetic Analyzer; Applied Biosystems). The resultant 16S rRNA gene sequence was

compared with representative organisms from the same and related genera from GenBank and was aligned by using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic relationships were determined by the neighbour-joining method (Saitou & Nei, 1987) with the program package MEGA 4.1 (Tamura *et al.*, 2007). Bootstrap confidence values were obtained using 1000 resamplings. DNA–DNA hybridization was carried out following the filter hybridization method (Seldin & Dubnau, 1985). The hybridization temperature was 65 °C and DNA–DNA relatedness was

quantified by using a densitometer (Bio-Rad). Probe labelling was conducted by using the non-radioactive DIG-High prime system (Roche Diagnostics) and hybridized DNA was visualized using the DIG luminescent detection kit according to the manufacturer's instructions (Roche Diagnostics). Fatty acid methyl ester analysis was carried out for a culture grown on R2A agar (Difco) at 28 °C for 48 h using the Microbial Identification System (Microbial ID; MIDI Inc.), according to standard protocols (Sasser, 1990). The G+C content of genomic DNA was determined by HPLC analysis using a reversed-phase column (Supelcosil LC-18S; Supelco) of individual nucleosides as described by Mesbah *et al.* (1989).

The phylogenetic tree obtained using selected 16S rRNA gene sequences showed that the strains were related to members of the *Sphingobacteriaceae*, having high similarity to members of the genus *Mucilaginibacter*. The 16S rRNA gene sequence of strain Gh-48^T had 97.5, 96.9, 94.3 and 95.2 % pairwise similarity, respectively, to those from *M. kameinonensis* SCK^T, *M. oryzae* B9^T, *M. gracilis* TPT18^T and *M. paludis* TPT56^T. Strain Gh-67^T grouped with Gh-48^T with 98.2 % similarity, while it showed lower pairwise similarity to *M. oryzae* B9^T (96.7 %), *M. kameinonensis* SCK^T (96.5 %), *M. gracilis* TPT18^T (93.9 %) and *M. paludis* TPT56^T (94.7 %) (Fig. 1). Since strain Gh-67^T showed relatively low 16S rRNA gene sequence similarity (<97 %) to its closest relatives, DNA–DNA hybridization was carried out for strain Gh-48^T only. It showed a low level of DNA–DNA relatedness to strain Gh-67^T (18 %) and to *M. kameinonensis* KCTC 22227^T (11 %). The DNA G+C contents of strains Gh-67^T and Gh-48^T were 46.7 and 44.2 mol%, respectively. The fatty acid profiles of strains Gh-67^T and Gh-48^T (summarized in Table 2) consisted mainly of C_{16:0}, iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), which are normal components of other members of *Mucilaginibacter* (Pankratov *et al.*, 2007; Urai *et al.*, 2008). Based on the data presented, on the recommendation of Wayne *et al.* (1987) and on the suggestion that a low degree of 16S rRNA gene sequence similarity (<97 %) is sufficient for differentiating at the species level (Stackebrandt & Goebel, 1994), strains Gh-67^T and Gh-48^T represent novel species of the genus *Mucilaginibacter*, for which we propose the names *Mucilaginibacter gossypii* sp. nov. and *Mucilaginibacter gossypicola* sp. nov., respectively.

Description of *Mucilaginibacter gossypii* sp. nov.

Mucilaginibacter gossypii (gos.sy'pi.i. N.L. gen. n. *gossypii* of *Gossypium*, the generic name of cotton, referring to the isolation of the type strain from the cotton rhizosphere).

Gram-negative, aerobic, non-endospore-forming, non-motile, irregular rods, 0.18–0.20 µm wide and 0.80–1.25 µm long (Supplementary Fig. S1). Growth occurs on AMS agar, R2A agar and NA, but not on TSA or MacConkey agar. Colonies are pale-pink, mucoid, convex and round with entire margins on R2A agar and AMS agar. Growth occurs at 20–30 °C with an optimum at 28 °C. The pH range for growth is pH 4–7, with optimum growth at pH 6–7. The pale-pink pigment is water-insoluble and has absorption maxima at 208, 263 and 478 nm in methanol. Growth occurs in the presence of 0.5 % (w/v) NaCl, but not in the presence of 1 % (w/v) NaCl or higher. Flexirubin-type pigments are present. Tests for catalase, oxidase and pectinase are positive but urease and cellulase activities are absent. Proteolytic and lipolytic activities are present. Positive for hydrolysis of aesculin and pectin but negative for hydrolysis of gelatin, CM-cellulose and starch. Nitrate reduction is positive but negative for indole production. Methanol, dichloromethane, succinate and ethanol are utilized as sole carbon sources but trimethylamine, methylamine, diethanolamine, formaldehyde, acetate, formate, dimethylamine and methane are not. Ammonium chloride, potassium nitrate, sodium nitrate, L-alanine, L-glutamine, glycine, potassium thiocyanate, triethylamine and diethylamine are utilized as nitrogen sources but ACC, ammonium sulfate, L-glutamate, L-aspartic acid, L-tryptophan, urea, methylamine, potassium cyanate and diphenylamine are not. The type strain is highly resistant (up to 500 µg ml⁻¹) to spectinomycin, carbenicillin, gentamicin, kanamycin, nalidixic acid, bacitracin, cefotaxime, chloramphenicol, erythromycin, penicillin G, cephalosporin and trimethoprim, but sensitive to (µg ml⁻¹) tetracycline (500), ampicillin (500), doxycycline (50), polymyxin B (300), rifampicin (100), novobiocin (500) and vancomycin (500). Positive for utilization of α-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, tura-

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence comparison showing the positions of strains Gh-67^T and Gh-48^T and related members of the genus *Mucilaginibacter*. Numbers at nodes indicate percentages of occurrence in 1000 bootstrapped trees; values below 70 % are not shown. Bar, 0.01 substitutions per site.

Table 2. Cellular fatty acid compositions (%) of *Mucilaginibacter* strains

Strains: 1, *M. gossypii* sp. nov. Gh-67^T; 2, *M. gossypicola* sp. nov. Gh-48^T; 3, *M. kameinonensis* KCTC 22227^T; 4, *M. gracilis* KACC 13451^T; 5, *M. paludis* KACC 13450^T; 6, *M. daejeonensis* Jip 10^T; 7, *M. ximonensis* XM-003^T; 8, *M. oryzae* B9^T. Data in columns 1–5 were obtained in this study; data in columns 6–8 were taken from An *et al.* (2009); Luo *et al.* (2009) and Jeon *et al.* (2009). Values are percentages of total fatty acids. Fatty acids representing less than 0.1 % in all strains were omitted. ECL, Equivalent chain-length; –, not detected/not reported.

Fatty acid	1	2	3	4	5	6	7	8
Saturated								
C _{14:0}	1.2	1.7	1.4	1.2	1.3	—	1.3	1.7
C _{15:0}	—	—	—	3.5	2.9	—	—	1.6
C _{16:0}	15.0	17.0	14.8	1.4	2.5	9.3	6.8	8.5
C _{17:0}	0.2	0.3	0.7	0.3	—	—	—	—
C _{18:0}	1.6	—	—	—	—	1.7	1.4	—
Hydroxy								
iso-C _{11:0} 3-OH	0.3	—	—	—	—	—	—	—
C _{14:0} 2-OH	0.3	0.6	0.5	0.4	0.2	—	—	0.6
iso-C _{14:0} 3-OH	—	—	—	—	—	—	—	0.2
iso-C _{15:0} 3-OH	1.9	4.5	8.7	3.2	1.5	2.2	1.9	2.2
C _{15:0} 2-OH	—	0.6	0.4	0.2	0.2	—	—	0.3
iso-C _{16:0} 3-OH	0.3	0.5	0.6	0.4	0.2	—	—	0.2
C _{16:0} 2-OH	—	—	0.3	—	—	—	—	0.2
C _{16:0} 3-OH	5.1	4.6	3.2	1.4	1.6	2.3	1.5	4.6
iso-C _{17:0} 3-OH	6.5	6.1	3.1	12.5	7.5	7.1	10.0	5.4
Branched								
iso-C _{11:0}	0.2	—	—	—	—	—	—	0.1
iso-C _{13:0}	0.2	—	0.2	0.1	0.2	—	—	—
iso-C _{15:0}	25.8	20.2	19.3	29.5	17.5	26.5	26.2	23.9
anteiso-C _{15:0}	—	—	—	6.8	15.2	1.8	—	0.2
iso-C _{16:0}	—	—	—	—	1.4	—	—	—
iso-C _{17:0}	0.8	—	0.3	0.21	—	—	2.0	0.3
anteiso-C _{17:0}	—	0.5	—	—	3.8	—	—	—
Unsaturated								
C _{15:1} ω6c	0.7	0.6	0.5	1.3	1.2	—	—	0.4
C _{16:1} ω5c	2.8	1.3	2.1	2.7	6.5	5.9	3.1	1.4
C _{17:1} ω5c	1.5	—	—	—	—	—	—	—
C _{17:1} ω8c	0.3	0.5	0.4	0.8	0.7	—	—	0.3
iso-C _{17:1} ω9c	1.1	0.5	0.6	2.1	0.2	3.0	6.2	1.9
C _{18:1} ω7c	0.2	—	—	—	—	—	—	—
C _{18:1} ω9c	0.5	—	—	—	0.3	—	—	0.1
C _{18:3} ω6c	—	—	—	—	—	1.3	—	—
Summed features*								
2	—	0.5	0.3	—	0.2	—	—	0.4
3	33.0	39.6	42.6	31.6	34.8	39.1	26.7	43.7
4	0.5	0.6	—	—	0.1	—	—	—
Unknowns								
ECL 13.565	—	—	—	—	—	—	—	0.7
ECL 16.582	—	—	—	—	—	—	—	0.3

*Summed features are groups of two or three fatty acids that cannot be separated using the MIDI system. Summed feature 2 contains unknown ECL 10.928 and/or C_{12:0} aldehyde?; summed feature 3 contains C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; summed feature 4 contains iso-C_{17:1} I and/or anteiso-C_{17:1} B.

nose, xylitol, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, *p*-hydroxyphenyl-acetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, DL-lactic acid, succinic acid, bromosuccinic acid, succinamic acid, L-alaninamide, D- and L-alanine, L-alanyl

glycine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-ornithine, L-proline, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, 2,3-butanediol, glycerol and DL-α-glycerol phosphate. Other organic substrates included in the Biolog GN2 MicroPlate are not oxidized. The

predominant respiratory quinone is MK-7. The main fatty acids are iso-C_{15:0}, C_{16:0} and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH).

The type strain, Gh-67^T (=NCIMB 14470^T =KCTC 22380^T), was isolated from rhizosphere soil of cotton (*Gossypium hirsutum* L. 'MCU-12'), the sample being collected at the flowering stage from an experimental field at the Cotton Breeding Station, TNAU, India. The DNA G+C content of the type strain is 46.7 mol%.

Description of *Mucilaginibacter gossypiicola* sp. nov.

Mucilaginibacter gossypiicola (gos.sy.pi.i.co'la. N.L. n. *Gossypium* a scientific botanical genus name; L. suff. -cola inhabitant, dweller; N.L. n. *gossypiicola* *Gossypium*-dweller, referring to the isolation of the type strain from the cotton rhizosphere).

Gram-negative, aerobic, non-endospore-forming, non-motile, irregular rods, 0.2–0.3 µm wide and 0.5–0.8 µm long (Supplementary Fig. S1). Growth occurs on AMS agar, R2A agar and NA, but not on TSA agar or MacConkey agar. Colonies are pale-pink, mucoid, convex and round with entire margins on R2A agar and AMS agar. Growth occurs at 20–30 °C with an optimum at 28 °C. The pH range for growth is pH 2–8, with optimum growth at pH 6–7. The pale-pink pigment is water-insoluble and has absorption maxima at 213, 262, 329, 346 and 479 nm in methanol. Growth occurs in the absence of NaCl and in the presence of 0.5% (w/v) NaCl, but not in the presence of 1% (w/v) NaCl or higher. Flexirubin-type pigments are present. Tests for catalase, oxidase and cellulase are positive but pectinase and urease activities are absent. Proteolytic and lipolytic activities are present. Positive for hydrolysis of aesculin and CM-cellulose but negative for hydrolysis of pectin, gelatin and starch. Nitrate reduction is positive but negative for indole production. Methanol, dichloromethane, succinate and ethanol are utilized as sole carbon sources but trimethylamine, methylamine, diethanolamine, formaldehyde, acetate, formate, dimethylamine and methane are not. Ammonium chloride, potassium nitrate, ACC, L-alanine, L-glutamine, glycine, potassium thiocyanate, triethylamine and diethylamine are utilized as nitrogen sources but sodium nitrate, ammonium sulfate, L-glutamate, L-tryptophan, urea, methylamine, potassium cyanate and diphenylamine are not. The type strain is highly resistant (up to 500 µg ml⁻¹) to spectinomycin, ampicillin, carbenicillin, gentamicin, kanamycin, nalidixic acid, bacitracin, cefotaxime, chloramphenicol, erythromycin, penicillin G, polymyxin B, novobiocin, vancomycin and trimethoprim, but sensitive to (µg ml⁻¹) tetracycline (300), doxycycline (300), rifampicin (25) and cephalosporin (500). Positive for utilization of α-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, D-arabitol, cellobiose, i-erythritol, L-fucose, D-galactose, gentiobiose, α-D-glucose, lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl

β-D-glucoside, D-psicose, raffinose, D-sorbitol, sucrose, trehalose, turanose, xylitol, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, DL-lactic acid, succinic acid, bromosuccinic acid, succinamic acid, L-alaninamide, D- and L-alanine, L-alanyl glycine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-ornithine, L-proline, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, 2,3-butanediol, glycerol and DL-α-glycerol phosphate. Other organic substrates included in the Biolog GN2 MicroPlate are not oxidized. The predominant respiratory quinone is MK-7. The main fatty acids are iso-C_{15:0}, C_{16:0} and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH).

The type strain, Gh-48^T (=NCIMB 14471^T =KCTC 22379^T), was isolated from rhizosphere soil of cotton (*G. hirsutum* L. 'MCU-12'), the sample being collected at the flowering stage from an experimental field at the Cotton Breeding Station, TNAU, Coimbatore, Tamilnadu, India. The DNA G+C content of the type strain is 44.2 mol%.

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