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ORIGINAL PAPER

Sinomonas cellulolyticus sp. nov., isolated from Loktak lake

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Abstract A novel cellulolytic strain JC656^T was isolated from the rhizosphere soil of *Alisma plantago-aquatica* of floating island (Phumdis) of Loktak lake, Manipur, India. The 16S rRNA gene sequence similarities between strain JC656^T and other *Sinomonas* type strains ranged between 98.5 and 97.3%, wherein strain JC656^T exhibited the highest sequence similarity (98.5%) to *Sinomonas notoginsengisoli* KCTC 29237^T. Colonies were yellow-colored and grew aerobically. Cells were gram-positive, rod-shaped and non-motile. The optimal growth of the strain JC656^T occured at 28 °C and pH 7. Strain JC656^T contained MK-9 as the predominant isoprenoid quinone and anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$ as the major fatty acids. Diphosphatidylglycerol, phosphatidylinositol, phosphatidylglycerol, phosphatidylmonomethylethanolamine and a glycolipid were the polar lipids. Strain JC656^T contained lysine, alanine, glutamine, diaminopimelic acid (DAP) and two unidentified amino acids as characteristic cell wall amino acids. The genome size of strain JC656^T was 3.9 Mb with a DNA G+C content of 69.9 mol %. For the affirmation of the strain's taxonomic status, a detailed phylogenomic study was done. Based on its phylogenetic position and morphological, physiological, and genomic features, strain JC656^T represents a new species of the genus *Sinomonas*, for which we propose the name *Sinomonas cellulolyticus* sp. nov. The type strain JC656^T = (KCTC 49339^T = NBRC 114142^T).

Keywords *Actinomycetota* · *Micrococaceae* · *Sinomonas* · Rhizosphere

Abbreviations

NCBI	National Centre for Biotechnology Information
*g*ANI	Genome average nucleotide identity
*d*DDH	Digital DNA–DNA hybridization
HPLC	High-pressure liquid chromatography
KCTC	Korean Collection for Type Cultures
NBRC	Biological Resource Centre, NITE

Introduction

The genus *Sinomonas*, is a member of the family *Micrococaceae* in the phylum *Actinomycetota* was first proposed by Zhou et al. (2009). At the time of writing, this genus comprised eleven species

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with ten valid names under the International Code of Nomenclature of Prokaryotes (ICNP), including *Sinomonas flava* (Zhou et al. 2009), *Sinomonas atrocyaneus* (Kuhn and Starr 1960; Zhou et al. 2009), *Sinomonas echigonensis* (Ding et al. 2009; Zhou et al. 2012), *Sinomonas albida* (Ding et al. 2009; Zhou et al. 2012), *Sinomonas soli* (Zhou et al. 2012), *Sinomonas notoginsengisoli* (Zhang et al. 2014), *Sinomonas mesophile* (Prabhu et al. 2015), *Sinomonas susongensis* (Bao et al. 2015), *Sinomonas humi* (Lee et al. 2015), and *Sinomonas halotolerans* (Guo et al. 2015) and "*Sinomonas gamaensis*" (Fu et al. 2019) (http://www.bacterio.net/sinomonas.html). The members of the genus *Sinomonas* were isolated from soil, volcanic rock and the surface of weathered biotite. All the members of the genus *Sinomonas* are aerobic, with rod-shaped cells; predominant respiratory quinone is MK-9; major fatty acids are iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteis*o*-$C_{17:0}$. A3α as the cell wall peptidoglycan type. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylmonomethylethanolamine and glycolipids are the major polar lipids.

Strain JC656^T was isolated from the rhizosphere soil of *Alisma plantago-aquatica* from the floating island (Phumdis) of Loktak lake, Manipur. Phumdis are heterogeneous masses of vegetation, soil, and organic matter at various decomposition stages (Reddy et al. 2005). This lake was declared a Ramsar site (a wetland site designated to be of importance) in 1990 as it is an ecological hotspot with a remarkable diversity of flora and fauna. Cellulolytic microorganisms play a significant role in the biosphere by recycling cellulose (Beguin and Aubert 1994). According to reports, members of the genus *Sinomonas* are involved in cellulose degradation, nitrogen fixation, aromatic compound degradation, antimicrobial activity, phosphate solubilization and desulphurization (Susilowati et al. 2015; Rao et al. 2018). In this study, strain JC656^T isolated from the rhizosphere soil of *Alisma plantago-aquatica*, which can hydrolyse cellulose has been reported. Based on the polyphasic taxonomic approach together with genomic information, strain JC656^T belongs to a new species of the genus *Sinomonas*, for which we propose the name *Sinomonas cellulolyticus* sp. nov.

Materials and methods

Habitat and isolation

Rhizsphere soil of *Alisma plantago-aquatica* of Phumdis (floating island) was collected from Loktak lake which is located in the north-eastern part of India, Manipur (24° 30′ 21″ N 93° 47′ 43″ E). One gram of the soil sample was serially diluted and plated on the mineral media containing pyruvate (22 mM) as the carbon source and incubated at 30 °C for 3 days. The mineral medium contained (g.L^{-1}): KH_2PO_4 (0.5), $MgSO_4.7H_2O$ (0.2), NaCl (0.4), NH_4Cl (0.6), $CaCl_2{\cdot}2H_2O$ (0.05), sodium pyruvate (3.0), yeast extract (0.3), ferric citrate (5 mL L^{-1} from a 0.1%, w/v, stock) and trace element solution SL 7 (1 mL L^{-1}). The SL7 solution contained (mg L^{-1}): HCl (25%, v/v; 1 mL), $ZnCl_2$ (70), $MnCl_2$ $4H_2O$ (100), H_3BO_3 (60), $CoCl_2{\cdot}6H_2O$ (200), $CuCl_2{\cdot}H_2O$ (20), $NiCl_2{\cdot}6H_2O$ (20), $NaMoO_4{\cdot}2H_2O$ (40) (Biebl and Pfennig 1981). Yellow-coloured colonies that appeared after 3 days of incubation were purified through repeated streaking on nutrient agar (NA; Himedia M002) and were assigned as strain JC656^T. The pure culture was preserved at − 80 °C as glycerol stocks.

DNA isolation, PCR, 16S rRNA gene sequencing and BLAST analysis

Genomic DNA was extracted using the commercial DNA isolation kit (Nucleo-pore gDNA Fungal Bacterial Mini Kit, from M/s. Genetix Biotech Asia Pvt. Ltd, India. The 16S rRNA gene was amplified using the primers F'-8 (5′–AGAGTTTGATCCTGG CTCAG–3′) and R'-1525 (5′–AAGGAGGTGATC CAGCC–3′) (Gandham et al. 2018). The amplified PCR products were sent to M/s. AgriGenomePvt. Ltd. (Kochi, India) for purification and 16S rRNA gene sequencing. Sequence was identified using BLAST search analysis on the EzBioCloud database (Yoon et al. 2017).

Genomic and in-silico analysis

Whole-genome sequencing (WGS) of strain JC656^T was outsourced at BGI (GCM 10 K type strain sequencing project) in China (Wu and Ma 2019). WGS was carried out using the Illumina XTen

platform. Paired end sequencing of (150×2 bp) fragment library resulted in about 2.96% quality filter reads. Assembly of the reads was carried out using the SOAPdenovo v1.05 software (Luo et al. 2012). ContEst service (Yoon et al. 2017) was used for any possible contamination. NCBI Prokaryotic Genome Annotation Pipeline and RAST server (http://rast.theseed.org/FIG/rast.cgi) (Aziz et al. 2008) was used for annotating the genomes. ANI score was calculated from the genome sequences using the online service in the EzBioCloud (https://www.ezbiocloud.net/tools/ani) (Yoon et al. 2017). Digital DNA–DNA hybridization (*d*DDH) values were calculated using the Genome-to-Genome Distance Calculator (GGDC 2.1) online service (https://ggdc.dsmz.de/distcalc2.php) (Auch et al. 2010). Carbohydrate active enzymes (CAZy) were determined using the *db*CAN meta server (http://bcb.unl.edu/dbCAN2/) by choosing default parameters (Zhang et al. 2023). Resistance Gene Identifier (RGI) is used to predict resistome genomic data based on homology and SNP models (Alcock et al. 2023). The orthologous gene comparisons were performed using orthovenn v2.0 (Xu et al. 2019). In silico metabolic characterisation and reconstruction of metabolic pathways were carried out on the basis of its genome information through the online tools Blastkoala (Kanehsia et al. 2016) and KEGG mapper (Kanehisa et al. 2022). The identification of gene clusters responsible for the biosynthesis of secondary metabolites was carried out using the online, freely available tool antiSMASH v 7.1 (Blin et al. 2023).

Phylogenetic analysis

The 16S rRNA gene sequence of strain JC656^T was extracted from its genome using ContEst16S (https://www.ezbiocloud.net/tools/contest16s) and analysis of identity was performed using NCBI BLAST (Johnson et al. 2008). 16S rRNA gene sequences of the closely related members were taken from the EZbioCloud database and aligned with the MUSCLE algorithm (Edgar 2004) of MEGA7 software (Kumar et al. 2016) for 16S rRNA gene phylogenetic analysis. The distance was calculated using the Kimura two-parameter model with uniform rates and pairwise deletion (Kimura 1980). Neighbour-joining (NJ), minimum evolution (ME), and maximum likelihood (ML) methods in the MEGA7 software were used to construct phylogenetic trees with bootstraps of 1000 replications (Felsenstein 1985). The phylogenomic tree was constructed using 92 core genes (retrieved using the UBCG tool as described by Na et al. 2018) from all the publicly available genomes of the genus *Sinomonas*, along with the outgroup. A concatenated sequence of 92 genes was used to construct the RAxML-based phylogenomic tree. The type strain *S. notoginsengisoli* KCTC 29237^T was used as a reference strain for further studies.

Cell morphology and physiological analysis

Colony morphology was studied in cultures grown on nutrient agar (aerobically at 28 °C for 3 days), whereas cell size, shape, and motility were examined in cultures grown on nutrient broth. Organic substrate utilization for growth was checked in mineral salt medium as described previously by Divyasree et al. (2017), supplemented with 2% NaCl, removing the carbon source pyruvate and adding different carbon sources (0.1% [w/v]). For nitrogen source utilization, NH_4Cl was removed and different nitrogen sources (0.1% [w/v]) were added in the same medium. NaCl tolerance (0–10% w/v with an interval of 1% w/v) was tested at 28˚C and pH 7. The pH range (4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) for growth was tested at 28 °C in buffered media at an interval of 1 pH unit; Citrate buffer [$C_6H_5Na_3O_7{\cdot}2H_2O$ and $C_6H_8O_7$] 0.1 M for pH 4.0–6.5; phosphate buffer [KH_2PO_4. and K_2HPO_4], 0.1 M for pH 6.5–9; carbonate buffer [$NaHCO_3$ and Na_2CO_3] 0.1 M for pH 9.5–11.0). The optimal temperature range required for growth (6, 12, 18, 24, 30, 35, 40, 45) was tested at pH 7. Hydrolysis of starch, casein, CMC, tween 20, 40, 80, gelatin, urea, oxidase, catalase, nitrate reduction, indole production, Voges-Proskauer, citrate utilization and other biochemical and carbohydrate tests were carried out as described previously (Divyasree et al 2017; Smibert and Krieg 1981).

The antibiotic susceptibility test was performed using the Icosa G-I Plus flat circular ring (Himedia) according to the manufacturer's instructions. Antimicrobials used and the amounts per disc (Oxoid) were as follows (µg/disk): ampicillin (10), gentamicin (10), erythromycin (15), tetracycline (30), cephalothin (30), chloramphenicol (30), clindamycin (2), co-trimoxazole (25), ofloxacin (5), vancomycin (30), oxacillin (1), linezolid (30), azithromycin (15), amikacin

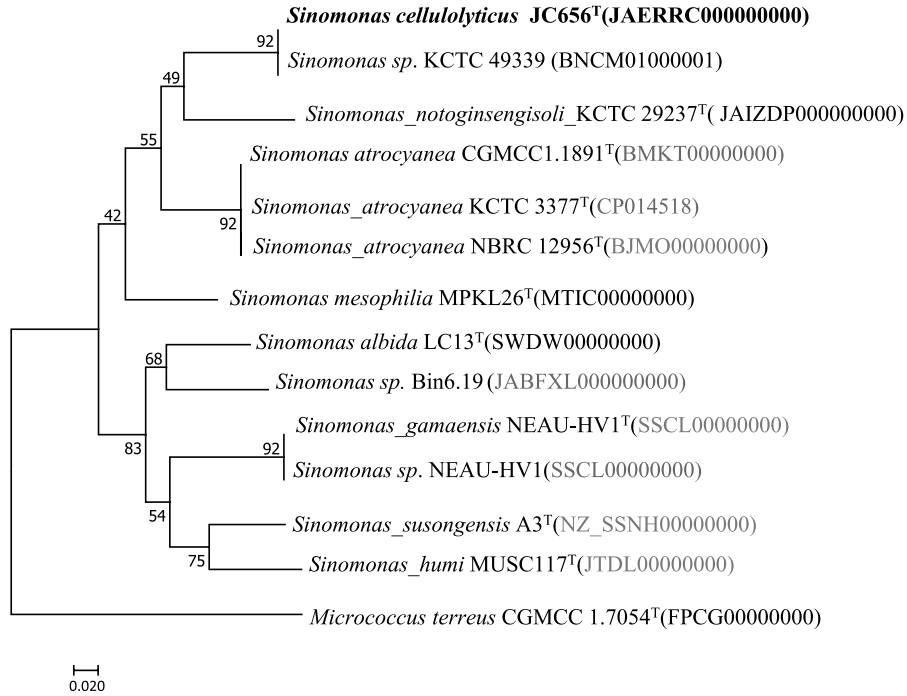

Fig. 1 RAxML based phylogenomic tree of strain JC656^T along with publicly available genome sequences of the genus *Sinomonas*. The tree was constructed using 92 core genes tool based on the Up-to-date Bacterial Core Gene (UBCG) AND rooted with *Micrococcus terreus* CGMCC1.7054^T as the outgroup. Bar, 0.02 nucleotide substitution per position

(30), clarithromycin (15), teicoplanin (10), methicillin (5), amoxyclav (30), novobiocin (5), and penicillin (10). Susceptibility or resistance was checked by the presence or absence of an inhibitory zone after 48 h of incubation at 30 °C. Enzyme activities were assayed using the API ZYM kit (Biomerieux, France) according to the manufacturer's protocol.

Chemotaxonomic characterization

Cultures grown in nutrient broth were harvested (10,000 g at 4 °C for 10 min) when the growth reached around 70% of the maximal optical density (late exponential growth phase). The pellet was used for the analysis of cellular fatty acid, polar lipid and quinone. Cellular fatty acids were methylated, separated, and identified according to instructions for the Microbial Identification System [Microbial ID; MIDI 6.0 version; method, RTSBA6 (Sasser 1990) and outsourced to Royal Life Sciences Pvt. Ltd, Secunderabad, India. For both strains, polar lipids were extracted, separated, and characterized as described previously (Kates 1972; Oren et al. 1996). Quinones were extracted with a chloroform/methanol (2:1, v/v) mixture, purified by TLC and analysed by HPLC (Imhoff 1984). Cell wall amino acids for both strains were extracted and determined as studied previously (McKerrow et al. 2000; Schleifer 1985; Schleifer and Kandler 1972).

Results and discussions

Phylogenetic inference and genomic characteristics

EzBioCloud BLAST analysis for taxonomic identification using the partial 16S rRNA gene (1344 bp) sequenced using PCR amplified product showed that strain JC656^T has the highest identity with the members of the genus *Sinomonas*; *S. notoginsengisoli* (98.58%) being the closest. The full-length 16S rRNA gene (retrieved from the genome) based phylogenetic tree with combined bootstrap values obtained from individual NJ, ME, and ML trees is shown in Fig. S1 and the ninety-two core gene-based phylogenomic tree (Fig. 1) confirmed the clustering of strain JC656^T with its closest related species of the genus *Sinomonas*.

The genome size of strain JC656^T was 3.9 Mb with N_{50} value 269,315 bp, while the genome of *S. notoginsengisoli* was 3.5 Mb with an N_{50} value of

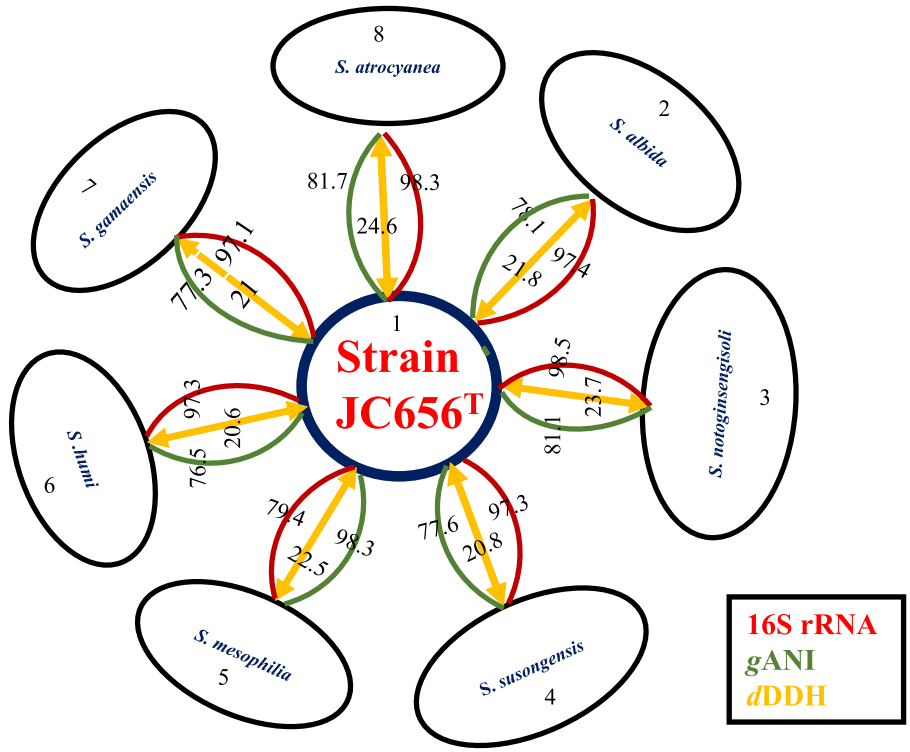

Fig. 2 Analysis of phylogenetic markers for delineation of the novel strain JC656^T along with the reference strains of the genus *Sinomonas*. Methods used:16S rRNA gene identity (16S), average nucleotide identity (ANI), DNA-DNA hybridization (dDDH). Strains: 1. JC656^T 2. *S. albida* LC13^T 3. *S. notoginsengisoli* KCTC 29237^T 4. *S. susongensis* A31^T 5. *S. mesophilia* MPKL 26^T 6. *S. humi* MUSC 117^T 7. *S. gamaensis* NEAU-HV1^T 8. *S. atrocyanea* KCTC 3377^T

261,769 bp. The genomic G+C content (mol%) of strain JC656^T and *S. notoginsengisoli* was 69.9% and 68.8%, respectively. The gANI and *d*DDH values (Fig. 2) of strain JC656^T with members of the genus *Sinomanas* fall below the recommended cut-off values i.e., 95–96% and 70% for prokaryotic species delineation (Rodriguez and Konstantinidis 2016; Meier-Kolthoff et al. 2014; Chun et al. 2018). The genome data analysis through RAST of strain JC656^T and other members of the genus *Sinomonas* is given in Table S1. The genome sequences of strain JC656^T and members of the genus *Sinomonas* were aligned using PATRIC software to identify the multiple maximal matches and local collinear blocks (LCBs; www.patricbrc.org). The genome sequence of strain JC656^T was used as a reference. The alignment of LCBs in the genus *Sinomonas* varied greatly from one another (Fig. S2). The genomic arrangement of strain JC656^T appeared to be rearranged and inverted when compared to the other genomes as a result of DNA rearrangements, recombination, and horizontal transfer (Fig. S2) (Davis et al. 2020). The comparison of the genome map based on protein sequence identity also revealed that the majority of the proteins of strain JC656^T shared 20–80% similarity with those of the clade members of *Sinomonas* (Fig. S3). This indicated a clear-cut dissimilarity between strain JC656^T and other *Sinomonas* strains. The whole-genome orthologous gene and protein comparisons among the species of the genus *Sinomonas* performed based on orthovenn2 show that they form 5043 clusters, among which 3693 orthologous clusters were present in at least two species and 1350 single-copy gene clusters. The genome of strain JC656^T showed 3457 orthologous and 33 single-ton copy gene clusters and showed differences in the distribution of orthologous gene clusters. Three clusters as shown in Fig. S4 do not have proteins from strain JC656^T.

Analysis of core and pan-genome

The distribution of genes based on USEARCH clustering for the genus *Sinomonas* as given by Bacterial Pangenome analysis pipeline (BPGA) tool (Chaudhari et al. 2016) is depicted in Table S1. Members of the genus *Sinomonas* share 1647 core genes (9.31%), 10,785 accessory genes (60.9%), and 5258 strain-specific genes (29.7%) (Fig. S5). The core-pan plot revealed that the genus *Sinomonas* has an open pan-genome since it did not plateau and expanded with the

increase in genomes (Fig. S6). Strain JC656^T showed differences in the number of unique genes (550), and the majority of the unique genes were associated with cellulose degradation and other metabolic process.

In-silico metabolic characterization

The CAZy annotation of the genome of strain JC656^T reveals a number of genes encoding carbohydrate-active enzymes (CAZymes). Strain JC656^T contains genes encoding glycoside hydrolases (GH), followed by glycosyl transferases (GT), and carbohydrate esterases (CE) (Fig. S7). The members of the genus *Sinomonas* were reported to be involved in cellulose degradation, nitrogen fixation, phosphate solubilization, aromatic compound degradation, antimicrobial activity and desulphurization (Rao et al. 2018). The *in-silico* metabolic annotation of strain JC656^T along with other members of the genus *Sinomonas* helps in exploring the metabolic potential of the strain and also helps in understanding its ecological significance. Genes encoding for enzymes like Endo β-1,4 gluconisidase (E.C.3.2.1.4) and β-glucosidase (E.C.3.2.1.21) involved in the degradation of cellulose, Pho regulon genes like phoB, phoU, and phoR, phosphate transporter genes, alkaline phosphatase (EC 3.1.3.1) and polyphosphate kinase (EC 2.7.4.1) involved in phosphate solubilization were predicted in the genome of strain JC656^T.The genes involved in the synthesis of phytochemicals like mugineic acid and tad genes located on wide spread colonization islands (WCI) which help in encoding the machinery required for the assembly of adhesive Flp (fimbrial low-molecular-weight protein) pili, were also predicted in the genome of strain JC656^T. These tad genes are essential for biofilm formation, colonization, and pathogenesis, and may also play an important role in enhancing plant growth (Tomich et al. 2007). The genes coding for enzymes like tryptophan synthase alpha chain (EC 4.2.1.20), aromatic-L-amino-acid decarboxylase (EC 4.1.1.28), anthranilate phosphoribosyltransferase (EC 2.4.2.18), tryptophan synthase beta chain (EC 4.2.1.20), monoamine oxidase (1.4.3.4), phosphoribosylanthranilate isomerase (EC 5.3.1.24) and excinuclease ABC subunit C responsible for auxin biosynthesis were, also predicted in the genome of strain JC656^T indicating the importance of the strain in promoting the proliferation of phumids. Genes responsible for allantoin utilization, nitrate and nitrite ammonification, ammonia assimilation and denitrification were predicted in genome of strain JC656^T which helps in nutrient cycling of Loktak lake (Singh and Kalamdhad 2014). The complete gene cluster responsible for the aromatization of cyclohexane carboxylate degradation pathway and benzoate degradation was predicted in the genome of strain JC656^T (Yamamoto et al. 2021). Incomplete pathways of benzoate, aminobenzoate, fluorobenzoate degradation, chloroalkane, chloroalkene, toluene, xylene, ethylbenzene degradation, naphthalene and polycyclic aromatic hydrocarbon degradation were also predicted in the genome of strain JC656^T indicating the importance of phumids in recycling not only plant-derived organic compounds but also other xenobiotics (Puranik et al. 2016). Antimicrobial resistance (AMR) genes like vanY of the vanA cluster, vanW of vanG and cluster APH (3′)-Ia aiding in the inactivation of the aminoglycoside class of antibiotics were also predicted in the genome of strain JC656^T.

A total of 10 putative secondary metabolite gene clusters were predicted among the members of the genus *Sinomonas*. The gene clusters responsible for the biosynthesis of betalactone, non-alpha polyamino acids like e-Polylysin (NAPAA), terpene and type III Polyketide synthase (T3PKS) were predicted in all the members (Fig. S9).

Morphological, physiological analysis and biochemical analysis

Strain JC656^T was found to be gram-positive, aerobic, non-motile and rod-shaped (2–4 L × 0.2–0.5 W μm; Fig. S10). Colonies were observed to be round, yellow, and convex on the nutrient agar medium. Strain JC656^T and *S. notoginsengisoli* SYP-B575^T could grow in nutrient broth at 28 °C for 3 days. Strain JC656^T grew well in the temperature range of 14–37 °C (optimum 28 °C), whereas *S. notoginsengisoli* SYP-B575^T could grow well in temperature range of 17–41 °C (optimum 30 °C). Both strains could grow well in the pH range of 6.0–8.0 (optimum pH 7). Strain JC656^T did not require NaCl for its growth; however it could tolerate up to 6.0% (w/v) whereas *S. notoginsengisoli* could tolerate up to 5.0% (w/v). Strain JC656^T hydrolysed tween 40 and cellulose, while tween 80, starch, gelatin, and casein were not

Table 1 Differentiating characteristics that distinguish strain JC656^T from its closely related species of the genus *Sinomonas*

Characteristics	1*	2*	3	4	5	6	7	8	9	10	11
Morphological characters											
Colony shape	Round, convex	Round	Round, convex	Circular, convex	Circular, convex	Circular, convex	Circular, convex	Circular, convex	Circular, convex	Circular, convex	Circular, convex
Colony colour	Yellow	Pale yellow	Whitish	Grey-white	Pale yellow	Yellowish to white	Creamy white	Pale yellow	Yellow	Creamy white	Pale yellow
Physiological characters											
Growth media	Biebl and Pfennig	YDC	Luria broth	TYB	TYB	TSB	TYB	PYES	Luria broth	Luria broth	Nutrient broth
pH range (optima)	6–8 (7)	6–8 (7)	6–10 (7)	4–8 (ND)	5–9 (6–8)	5–8 (6–7)	6–8 (7)	5–9 (8)	5–9 (6–8)	5–9 (7)	5–10 (8)
Temp range© (optima)	14–37 (28)	17–41 (28)	10–40 (30)	14–43 (ND)	15–42 (30–37)	24–40 (28–36)	2–40 (30)	15–37 (30)	15–42 (30–37)	10–37 (28)	10–45 (30)
NaCl Tolerance (w/v) %	0–6	0–5	3–7	ND	0–3	0–4	0–4	0–5	0–3	0–7	0–4
Hydrolysis of											
Casein	−	−	ND	ND	−	ND	ND	ND	−	−	−
Starch	−	−	ND	ND	ND	+	−	−	−	−	−
Tween 80	−	+	+	−	+		−	−	−	−	−
Gelatin	−	−	−	−	−	−	−	−	−	+	−
Biochemical characters											
Catalase	+	+	−	+	+	+	+	+	+	−	
Urease	−	−	−	−	−	−	ND	+	−	−	+
Voges Proskauer	+	+	ND	+	+	ND	+	−	+	−	+
Nitrate reduction	−	−	−	+	−	+	−	−	+	−	+
Carbon sources utilization											
D-Mannitol	+	+	w	+	+		+	+	+	+	−
D-lactose	+	w	+	w	w	−	−	−	−	+	−
D-raffinose	+	w	+	w	w	+	+	−	−	−	+
D-sorbitol	−	+	w	+	w	−	−	+	+	−	−
D-maltose	+	+	+	+	+	+	+	+	−	+	−
D-fructose	−	+	+	+	+	+	+	+	+	−	+
D-cellubiose	−	+	+	+	+	−	ND	+	+	+	−
D-galactose	+	+	+	w	+	+	+	−	w	W	+
Inositol	+	+	+	+	+		+	−	+	−	−
Citric acid	+	w	w	+	w	ND	ND	ND	+	−	+

Table 1 (continued)

Characteristics	1*	2*	3	4	5	6	7	8	9	10	11
Mannose	−	w	+	+	+	−	+	−	+	W	−
Glucose	+	+	+	+	−	+	+	+	+	+	−
Enzyme activities (API ZYM)											
Easterase (C4)	+	+	+	w	+	+	+	−	+	+	+
Easterase lipase (C8)	+	+	−	w	+	+	+	−	+	+	+
Lipase	−	−	−	−	−	+	−	−	−	−	+
Valine-arylamidase	−	−	−	−	+	+	−	−	+	−	+
Cysteine-arylamidase	−	−	−	−	−	+	+	−	−	−	−
α-Galactosidase	−	−	−	−	−	+	−	+	−	−	+
N-acetyl-β-glucosaminidase	−	−	+	−	−	+	−	−	−	−	+
α-Mannosidase	+	−	−	−	−	+	−	+	w	−	+
Antibiotic susceptibility											
Ampicillin	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methicillin	−	+	ND	ND	ND	ND	ND	ND	ND	ND	ND
Amoxyclav	+	−	ND	ND	ND	ND	ND	ND	ND	ND	ND
Vancomycin	+	W	ND	W	W	ND	+	ND	W	ND	ND
Rifamycin	−	W	ND	W	W	ND	+	ND	W	ND	ND

1. Strain JC656^T; 2. *S. notoginsengisoli* KCTC 29237^T (Zhang et al. 2014); 3. *S. albida* LC13^T (Ding et al. 2009); 4. *S. atrocyana* KCTC 3377^T (Ding et al. 2009) (Guo et al. 2015); 5. *S. soli* CW 59^T (Zhou et al. 2012); 6. *S. humi* MUSC 117 ^{T} (Lee et al. 2015); 7. *S. mesophilia* MPKL 26 ^{T} (Prabhu et al. 2015); 8. *S. susongensis* A31^T (Bao et al. 2015); 9. *S. flava* CW 108^T (Zhou et al. 2009); 10. *S. halotoelrans* CFH S0499^T (Guo et al. 2015); 11. S. *gamaensis* NEAU-HVI^T (Fu et al. 2019)

*Data presented were performed in the author's laboratory W: weakly positive and ND- Not Determined

hydrolysed. Strain JC656^T was positive for catalase, and Vogues-Proskauer, whereas urease, and nitrate reduction were negative. Both strains utilized the following carbon sources for their growth: glucose, D-mannitol, D-lactose, D-raffinose, D-maltose, D-galactose and citric acid. Mannose, D-sorbitol, D-fructose and D-cellubiose were solely utilized by *S. notoginsengisoli* SYP-B575^T (Table 1; Fig. S8).

Strain JC656^T showed positive results for easterase (C4), easterase lipase (C8) and α-monosidase, while *S. notoginsengisoli* showed positive for easterase (C4) and easterase lipase (C8). Both strains were negative for lipase, valine arylamidase, cystine arylamidase, α-galactosidase and *N*-acetyl-β-glucosaminidase (Table 1). Antibiotic susceptibility also revealed that strain JC656^T was resistant to ampicillin, vancomycin and amoxiclav, whereas *S. notoginsengisoli* SYP-B575^T was resistant to ampicillin and methicillin. The difference in growth and physiological characteristics between strain JC656^T and its closest relatives on different organic substrates are given in Table 1.

Chemotaxonomic characterization

The major fatty acids identified in strain JC656^T were anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$. The differences in fatty acid profile of strain JC656^T along with the type strains of the genus *Sinomonas* are given in Table S2. The polar lipids of strain JC656^T contained diphosphatidylglycerol, phosphatidylinositol, phosphatidylglycerol, phosphatidylmonomethylethanolamine, glycolipids (GL1-GL5), and an unidentified lipid (Fig. S11). Strain JC656^T contained menaquinone-9 (H2) as the predominant quinone. Lysine, alanine, glutamine, DAP, and two unidentified amino acids were detected as the diagnostic cell wall amino acids of strain JC656^T (Fig. S12; Table S3).

Proposal of strain JC656^T as the type strain of a new species in the genus ***Sinomonas***

The genome-based and phylogenetic delineation of strain JC656^T from its closest phylogenetic neighbour, *S. notoginsengisoli* KCTC 29237^T is well demonstrated by the differences in phylogenetic, chemotaxonomic, phenotypic, and genotypic properties presented in this study (Figs. 1, 2; S1; Tables 1, S1, S3). Therefore, we suggest the placement of strain JC656^T as a novel species in the genus *Sinomonas*, for which we propose the name *Sinomonas cellulolyticus* sp. nov.

Description of ***Sinomonas cellulolyticus*** sp. nov.

Sinomonas cellulolyticus (cel.lu.lo.ly'ti.cus. M. L. n. *cellulosum*, cellulose; Gr. adj. *lyticus*, dissolving; M. L. adj. *cellulolyticus*, decomposing cellulose).

Cells are gram-stain-positive, aerobic, non-motile and rod shaped. Colonies are yellow coloured, circular, convex and 2.0–3.0 mm in diameter after 3 days of cultivation at 28 °C on nutrient agar. Optimum temperature and pH for growth are 28 °C (range 14–37 °C) and 7.0 (6.0–8.0), respectively. NaCl is not essential for growth (tolerate up to 6% v/w). Hydrolysed tween 40 and cellulose, while tween 80, starch, gelatin, and casein are not hydrolysed. Catalase, and Vogues-Proskauer are positive; urease and nitrate reduction are negative. Glucose, inositol, citric acid, D-galactose, D-mannitol, D-lactose, D-lactose, D-raffinose and D-maltose are used as sole carbon sources. L-glutamic acid, L-alanine, and L-aspartic acid are utilized as nitrogen sources. Major fatty acids are anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$. The major cell wall amino acids are alanine, glutamine, lysine, DAP and two unidentified amino acids. Menaquinone-9 (H2) is the predominant respiratory quinone and the polar lipid consists of diphosphatidylglycerol, phosphatidylinositol, phosphatidylglycerol, phosphatidylmonomethylethanolamine, glycolipid (GL1-GL5) and an unidentified lipid. The genome size is 3.9 Mb with a genomic DNA G + C content of 69.9%.

The type strain JC656^T (= KCTC 49339 = NBRC 114142) was isolated from the rhizosphere soil of *Alisma plantago-aquatica* floating island (Phumdis) collected from Loktak lake, India. The GenBank accession number for the 16S rRNA gene sequence of strain JC656^T is ON908988 and the genome sequence has been deposited in GenBank under the accession number JAERRC010000000.

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Author contributions KL performed sample collection, isolated the strain, performed the initial cultivation, strain deposition and strain characterization; KL, JU, SC and CVR performed the genomic and phylogenetic analysis; and KL and SA performed the cell wall analysis. All authors participated in the

data finalization, wrote up and approved the final version of the manuscript.

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Data availability The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the strain JC656^T is ON908988. The GenBank/EMBL/DDBJ accession number for the whole genome shotgun sequence for strain JC656^T is NZ_JAERRC01000000.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical statement Not applicable.

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