Curtobacterium salicis name sp. nov., isolated from willow tree stems in Washington state

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Abstract

Curtobacterium sp. strain WW7 is a Gram-positive, non-motile, orange rod-shaped bacterium isolated from branches of wild willow (Salix sitchensis) trees on nitrogen-free media. The WW7 strain shows growth in the temperature range between 4 and 30°C, a pH range of 6–7.7, and tolerates up to 5.5% (w/v) of NaCl. The genome sequencing of strain WW7 revealed a genome size of approximately 3.8 Mbp and a G + C content of 71.3 mol%. The phylogenomic analyses support the WW7 affiliation to a novel *Curtobacterium* lineage, with *C. herbarum* being the closest type-strain. Chemotaxonomic analysis indicates that WW7 capacity to assimilate carbohydrates was similar to the type strains, i.e. *C. luteum*, *C. albidum*, and *C. flaccumfaciens*, while no assimilation of the organic acids succinate, alpha-Ketobutyrate, mono methyl-succinate, and lactate was observed. Finally, fatty acid methyl ester (FAME) analysis identifies anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ major cellular fatty acids (FAs) which is a common feature for members of the *Curtobacterium* genus. Based on the results of phylogenomic and chemotaxonomic analyses, strain WW7 represents a novel *Curtobacterium* lineage, for which the name *Curtobacterium salicis* sp. nov. is proposed. The type strain is WW7^{PP}(DSM 34805^{PP} - NRRL B-68078^{PP}).

Introduction

The genus *Curtobacterium* comprises Gram-positive aerobic corynebacteria of the *Microbacteriaceae* family. Currently, *Curtobacterium* includes 9 well-defined species: *C. flaccumfaciens*, *C. albidum*, *C. ammoniigenes*, *C. citreum*, *C. herbarum*, *C. luteum*, *C. oceanosedimentum*, *C. allii*, and *C. pusillum* all having a genome size between 3.4 and 3.8 Mbp and an average GC content of ~ 70%. *Curtobacterium* are commonly isolated from terrestrial environments such as soil and plants, as epiphytic and endophytic bacteria (Chase et al. 2016), and, only in one documented apparently rare case, from humans (Francis et al. 2011). Although most of the *Curtobacterium* species are soil inhabitants, most of the information we have about this genus comes from *C. flaccumfaciens*, a plant pathogen of economically important crops (i.e., dry beans and sugar beet), and ornamental plants (i.e., poinsettia and tulip) (Osdaghi et al. 2018). However, according to the v214 of the Genome Taxonomy Database (GTDB) (Parks et al. 2022a), the *Curtobacterium* genus currently accounts for 52 species 28 of which are singletons, i.e., currently represented only by one single type-strain genome. Therefore, despite being commonly referenced as a phytopathogen, there is evidence suggesting that several, as yet uncharacterized, *Curtobacterium* species may play other important ecological roles (Chase et al. 2016; South et al. 2021; Scales et al. 2022; Chandel et al. 2022).

Here we describe one such example wherein *Curtobacterium* strain WW7, was isolated from a healthy wild willow (*Salix sitchensis*) tree growing in a native environment for the *Salicis* genera, *Populus* and *Salix*, in 2005 in the state of Washington (Doty et al. 2009). The genome of WW7 was sequenced in 2014 within a cooperation project founded by the Joint Genome Institute, which aimed to characterize diazotrophic aboveground endophytes in native pines, poplar, and willow (https://www.osti.gov/award-doi-service/biblio/10.46936/10.25585/60000936). Phylogenomic analysis supports the identification of strain WW7 as representative of a new *Curtobacterium* taxonomic lineage. Compared to other

Curtobacterium species, strain WW7 shows broader capacities to assimilate carbon sources, especially monosaccharides and amino acids, while its lipid content was remarkably similar to other *Curtobacterium* type-strains and, more in general, to members of the *Microbacteriaceae* family. Based on both phylogenomic and chemotaxonomic analyses, we propose strain WW7 as a member of *Curtobacterium salicis* sp. *nov*.

Methods

Genome sequencing, assembly, and functional annotation

The whole-genome sequencing of the strain WW7 was performed at the Joint Genome Institute (JGI, U.S. Department of Energy), using an Illumina NovaSeq in paired-end mode (PE x 150) achieving a sequencing coverage of 430×. High-quality Illumina reads were assembled with the SPAdes v. 3.13.0 genome assembler (Bankevich et al. 2012), and the resulting scaffolds were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Li et al. 2021).

Phylogenomic analysis

A phylogenomic analysis was performed using the Type Strain Genome Server (TYGS) database and the Genome Taxonomy Database (GTDB-tk) (Meier-Kolthoff and Göker 2019; Parks et al. 2022b). TYGS was used to calculate pairwise digital DNA-DNA hybridization (dDDH) values using the *d4* formula described in Meier-Kolthoff et al. 2013 (Meier-Kolthoff et al. 2013). The *de novo workflow* pipeline implemented in GTDB-tk was used to identify, align, and concatenate 120 phylogenetically informative markers in WW7 and in the representative members of the *Curtobacterium* genus according to the GTDB-tk database *v214*. The resulting alignment file was finally used to compute a maximum likelihood phylogenetic tree in IQ-TREE (Nguyen et al. 2015) under the WAG model with 1000 bootstrap replicates.

Physiology and chemotaxonomic analysis

The colorimetric assay described in Varga et al. (Varga et al. 2020) was used to test the capacity of WW7 to solubilize the insoluble phosphate salts aluminum [$AlPO_4$], tri-calcium [$Ca_3(PO4)_2$] and iron phosphate [$FePO_4$]. Assimilation of carbon sources and tolerance to osmotic stresses was performed using the Biolog PM1 and PM9 plates, respectively, according to manufacturer instructions. The Fatty Acid Methyl Ester (FAME) analysis was performed following the MIDI Microbial Identification System guidelines.

Results and discussion

Isolation and ecology

WW7 strain was isolated in 2005 from willow tree cuttings inhabiting the riparian zone of the Snoqualmie River (+ 47° 31' 14.30", -121° 46' 28.32") (Three Forks Park, King County; WA). This area is characterized by a moist-cool (mesic) climate (Firrincieli et al. 2020). The collection site is typically subjected to regular

floodings, exposing bare mineral soils and gravel bars on which the pioneer plant genera, Populus and Salix, commonly grow (Doty et al. 2009). The river water and the rocky substrate are also characterized by low levels of nitrogen, which makes these trees interesting for the study of plant-associated bacteria capable of improving plant adaptation to low-nutrient soil (Doty et al. 2009). The isolation strategy used for *Curtobacterium* strain WW7 is described in Doty et al. 2009 (Doty et al. 2009). Briefly, tree branch cuttings were allowed to sprout in nitrogen-free medium, and branches of the new growth were collected, surface sterilized, and placed on Murashige and Skoog (MS) agar plates. Morphologically distinct colonies forming on MS plates were streak-purified on Yeast Mannitol Agar, and the resulting isolates were finally stored in rich-media glycerol stock at – 80 ºC.

16S RNA phylogeny and phylogenomic analysis

As reported in another study (Scales et al. 2022), phylogenetic analysis on 16S rRNA provides very limited information regarding the intra-species variability within the *Curtobacterium* genus. Despite this, 16S rRNA phylogenies show a clear separation of the WW7 strain from the representative members of the *Curtobacterium* genus (Fig. 1).

Pairwise digital DNA-DNA hybridization values (dDDH, *d4* formula) calculated through TYGS were below the species delineation threshold of 70% (Table 1) (Meier-Kolthoff and Göker 2019), supporting the lack of affiliation of WW7 to other *Curtobcaterium* type-strain, and with *C. herbarum* DSM 14013 being the closest representative genome which was isolated in Germany from the grass leaf phyllo-sphere (Behrendt et al. 2002).

Table 1
dDDH values of strain WW7 vs. *Curtobacterium* type strains

Type strain	dDDH (d4, in %)[1]	C.I. (d4, in %)
C. herbarum DSM 14013	43.1	[40.6–45.7]
C. flaccumfaciens LMG 3645	24.7	[22.4–27.2]
C. flaccumfaciens CFBP 3418	24.7	[22.4–27.2]
C. albidum DSM 20512	24.5	[22.1–26.9]
C. pusillum ATCC 19096	24.5	[22.2–27.0]
C. citreum DSM 20528	24.4	[22.1–26.9]
C. citreum JCM 1345	24.4	[22.1–26.8]

[1] The *d4* formula was chosen because it is independent of genome length and therefore, robust against the use of draft genomes (Meier-Kolthoff et al. 2013).

In agreement with TYGS, GTDB identifies WW7 as the representative strain of a novel *Curtobacterium* species cluster i.e., *Curtobacterium flaccumfacies_C*, (https://gtdb.ecogenomic.org/species?id=Curtobacterium%20flaccumfaciens_C). The phylogenetic consensus tree calculated from the concatenated alignment of 120 ubiquitous bacterial genes places the WW7 strain within a branch including mostly uncharacterized *Curtobacterium* species and *C. herbarum*. This cluster includes isolates obtained from leaf litter associated soil samples, stratosphere aerosol dust collected at 38 km above sea-level, and inside the grass leaf phyllo-sphere (**Table S1**).

Genome features

The whole-genome sequencing of the strain WW7 was performed at the Joint Genome Institute (JGI, U.S. Department of Energy), using an Illumina NovaSeq in paired-end mode (PE x 150) with a sequencing coverage of 430×. High-quality sequencing reads were assembled using SPAdes v. 3.13.0, and an assembly of 7 scaffolds with an overall length of 3,486,446 bp and a G + C content of 71.3% was generated. The WW7 genome is available in the NCBI Assembly database under the accession number GCF_011759505.1. According to the NCBI Prokaryotic Genome Annotation Pipeline (PGAP), the WW7 strain possesses 3,365 genes, 3,025 of which are protein-coding genes. The remaining genes are tRNA (49), rRNA (16S, 23S, and 5S rRNA), ncRNA (4), and pseudogenes (170). A blast search analysis indicates that WW7 lacks major virulence factors located in the plasmid pCff1 (CP045288) of the *C. flaccumfaciens pv. flaccumfaciens* strain P990, and also conserved in other *C. flaccumfaciens pv. flaccumfaciens* strains with a confirmed plant pathogenicity phenotype (Table S1) (Evseev et al. 2022). These virulence genes commonly found in several *C. flaccumfaciens pv flaccumfaciens* strains but were not present in WW7 are: the expansin EXLX1 family cellulose-binding protein (https://www.ncbi.nlm.nih.gov/ipg/QIH95666.1), trypsin-like serine protease https://www.ncbi.nlm.nih.gov/ipg/QIH95653.1; https://www.ncbi.nlm.nih.gov/ipg/QIH95654.1; https://www.ncbi.nlm.nih.gov/ipg/QIH95655.1), pectate lyases (https://www.ncbi.nlm.nih.gov/ipg/QFS80865.3), glycosyl hydrolases (https://www.ncbi.nlm.nih.gov/ipg/?term=QFS80892.1; https://www.ncbi.nlm.nih.gov/protein/QIH95652.1) (Chen et al. 2021).

Physiology and chemotaxonomy

C. salicis showed significant increases in phosphate solubilization for the insoluble forms $AlPO_4$ and $Ca_3(PO4)_2$, but not for $FePO_4$ (**Figure S1**). On average, phosphate levels in liquid culture were increased by 29 and 100% in the presence of $AlPO_4$ and $Ca_3(PO4)_2$, respectively.

The assimilation of carbohydrates, chemicals, amino acids, and organic acids was tested with the Phenotype microarray (PM) plates PM01 (Biolog, Hayward, CA, USA). Phenotypic characterization on the PM01 plate was carried out under aerobic conditions at 30°C using the inoculation fluid IF-0 and the tetrazolium dye Dye-G. Of the 95 tested carbon sources, strain WW7 showed metabolic activity towards 39 of them. Compared to the closest type strains *C. herbarum* and *C. ammoniigenes*, WW7 showed a

greater capacity to assimilate di- and monosaccharides, organic acids, and amino acids (Table 2), while the overall assimilation profile of WW7 was like those observed in the type-strain of the species *C. flaccumfaciens*, especially concerning the utilization of monosaccharides and amino acids.

Table 2
Comparison of the Phenotype Microarray results of the strain WW7 and the phenotypic traits of type strains representative of the *Curtobacterium* genus

Substrate[1]	Type	1	2	3	4	5	6	7	8
L-proline	Amino acid	+	-	-	-	-	-	+	ND
L-glutamic acid	Amino acid	+	-	+	-	-	-	+	-
L-alanine	Amino acid	+	-	-	-	-	W	+	ND
L-alanyl glycine	Amino acid	-	-	-	-	-	+	+	-
Tween 40	Chemical	+	-	+	-	+	+	+	-
Tween 80	Chemical	+	-	+	-	+	+	-	-
α-d-lactose	Disaccharide	-	+	+	-	+	+	+	-
Lactulose	Disaccharide	-	-	+	-	+	+	+	-
D-cellobiose	Disaccharide	+	-	+	-	+	+	+	-
D-melibiose	Disaccharide	-	+	+	+	+	+	+	+
D-mannitol	Monosaccharide	+	ND	+	+	+	+	+	+
D-ribose	Monosaccharide	+	ND	+	+	+	+	+	+
L-rhamnose	Monosaccharide	-	-	+	+	+	+	+	+
L-arabinose	Monosaccharide	+	-	-	-	-	-	+	+
N-acetyl-d-glucosamine	Monosaccharide	+	-	+	-	+	W	+	ND
D-sorbitol	Monosaccharide	+	+	-	+	-	+	+	-
L-fucose	Monosaccharide	-	-	W	-	+	-	W	ND
D-glucuronic acid	Monosaccharide	-	-	-	-	-	-	+	-
D-gluconic acid	Monosaccharide	+	+	-	+	-	+	+	ND
Uridine	Monosaccharide	+	-	-	-	-	-	+	-
ß-methyl-D- glucoside	Monosaccharide	+	-	-	+	-	+	+	-
Adonitol	Monosaccharide	-	-	-	+	-	-	+	-
Myo-inositol	Monosaccharide	+	-	-	-	-	-	+	-
Succinic acid	Organic acid	-	-	+	-	+	-	+	-
α-keto-butyric acid	Organic acid	-	+	-	+	-	+	-	-
Bromo succinic acid	Organic acid	-	-	+	-	-	-	+	-

Substrate[1]	Type	1	2	3	4	5	6	7	8
Mono methyl succinate	Organic acid	-	-	-	-	-	-	+	-
D-lactic acid	Organic acid	-	+	+	-	W	-	+	ND

[1]Phenotypic data were collected from Kim et al. 2008 (7); Aizawa et al., 2007 (8); Behrendt et al., 2002 (9); ND, No data available; W, weak; +, growth; -, no growth. 1, *C. salicis* WW7; 2, *C. ammonigenes*; 3, *C. citreum*; 4, *C. luteum*, 5; *C. pusillum*; 6, *C. albium*; 7, *C. flaccumfaciens*; 8, *C. hermarum*

WW7 lipid composition was like that observed in other *Curtobacterium* type-strains, with the anteiso forms $C_{15:0}$ and $C_{17:0}$ accounting for 85% of the total FAs content (Table 3). On the other hand, FAs detected in WW7 but missing in other *Curtobacterium* type-strains were iso-$C_{12:0}$ (1.47%), C_{12} (1.57%), and C_{11} (1.98%).

Table 3
Fatty acid methyl ester profile of *C. salicis* WW7 and *Curtobacterium* type-strains

Fatty acid %	1	2	3	4	5	6	7	8	9
C_{11}	1.98	ND	ND	ND	ND	ND	ND	ND	ND
C_{12}	1.57	ND	ND	ND	ND	ND	ND	ND	ND
$C_{14:0}$	–	0.4	0.3	–	–	–	ND	ND	0.2
$C_{16:0}$	2.67	1	0.8	0.4	0.2	0.4	ND	ND	0.9
$C_{18:0}$	0.4	0.1	0.3	–	–	0.4	ND	ND	–
$C_{20:0}$	ND	0.1	0.4	ND	ND	ND	ND	ND	ND
iso-$C_{12:0}$	1.47	–	–	–	–	–	–	–	–
iso-$C_{14:0}$	–	0.3	0.5	–	0.4	3.2	ND	ND	–
iso-$C_{15:0}$	–	6.5	5.8	2.8	1.6	7.9	3.1	1.6	0.1
iso-$C_{16:0}$	6.96	3.5	5.4	3.9	11.8	24	9.9	17.5	0.4
iso-$C_{17:0}$	–	1.1	1.4	1.9	1.2	2.8	ND	ND	0.1
$C_{17:1}$ iso ω9c	0.39	ND	ND	ND	ND	ND	ND	ND	ND
iso-$C_{19:0}$	–	0.1	–	ND	ND	ND	ND	ND	ND
anteiso-$C_{13:0}$	–	0.1	–	ND	ND	ND	ND	ND	ND
anteiso-$C_{14:0}$	–	0.1	–	–	0.1	–	ND	ND	–
anteiso-$C_{15:0}$	40.95	52.9	48	19.6	37.2	39.5	58.2	43.4	1.3
anteiso-$C_{17:0}$	44.15	28.5	32.1	18.6	36.6	18.4	25.3	30.4	2.4
C18:3 ω6c	0.93	ND	ND	ND	ND	ND	ND	ND	ND

[1]FAME data were collected from Khanal et al. (Khanal et al. 2023); ND, no data available; –, not detected. 1, *C. salicis* WW7; 2, *C. allii*; 3, *C. flaccumfaciens*; 4, *C. pusillum*; 5, *C. citreum*; 6, *C. luteum*; 7, *C. albidum*; 8, *C. herbarum*; 9, *C. ammoniigenes*.

Description of Curtobacterium salicis name sp. nov.

Curtobacterium salicis (lat. salix -ĭcis, referring to the source of isolation of the species).

The colonies of this novel species in rich media are orange-pigmented, smooth, and shiny. The cells of *C. salicis* are rod-shaped, gram-positive, and non-motile. Growth is detected under aerobic conditions between 4 and 30°C, with an optimal temperature observed in the range between 25 and 30°C. No growth is observed at 37°C. *C. salicis* tolerates pH values between 6 to 7.7 and grows up to 5.5% (v/w) NaCl. Positive growth was observed for the utilization of D-cellobiose, maltotriose, N-acetyl-D-glucosamine, sucrose, glycerol, D-trehalose, D-mannitol, β-methyl-D-glucoside, D-galactose, D-mannose, Myo-inositol, maltose, uridine, L-arabinose, D-fructose, D-gluconic acid, D-ribose, D-xylose, α-D-glucose, adenosine, inosine, L-alanine, L-asparagine, L-glutamine, L-glutamic acid, D-sorbitol, glycyl-L-proline, D-glucose-1-phosphate, tween 40, L-proline, acetoacetic acid, D-psicose, L-lyxose, α-methyl-D-galactoside, tween 80, L-aspartic acid, and pyruvic acid. Weak growth was observed in the presence of dulcitol, acetic acid, and thymidine. Similar to other *Curtobacterium* type-strains, *C. salicis* major FAs were anteiso-$C_{15:0}$ (40.9%) and anteiso-$C_{17:0}$ (44.1%). *C. salicis* showed capacity to solubilize P and siderophore activity on CAS plates.

Abbreviations

dDDH
digital DNA-DNA hybridization
FA
Fatty acid
FAME
Fatty acid methyl ester analysis
GTDB-tk
Genome Taxonomy database
MS
Murashige and Skoog
TYGS
Type-strain genome server
PM
Phenotype microarray.

Declarations

Author contribution.

JF conceived the experiments with help from AF. AF conducted the genomic and, bioinformatics analyses. DB conducted chemotaxonomic analyses. SLD isolated the strain. All authors contributed to writing the manuscript.

Conflict of interest.

Intrinsyx Bio is developing agricultural products based on the microbial strain in this study.

Funding information

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Ethical approval

Not applicable

Data availability

WW7 genome sequence and annotation have been submitted to the NCBI Assembly database under the accession number GCF_011759505.1. Partial 16S rRNA sequence of the WW7 strain has been deposited to the NCBI Nucleotide database with the accession number KU523564.1.

References

1. Bankevich A, Nurk S, Antipov D, et al (2012) SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. Journal of Computational Biology 19:455. https://doi.org/10.1089/CMB.2012.0021
2. Behrendt U, Ulrich A, Schumann P, et al (2002) Diversity of grass-associated Microbacteriaceae isolated from the phyllosphere and litter layer after mulching the sward; polyphasic characterization of Subtercola pratensis sp. nov., Curtobacterium herbarum sp. nov. and Plantibacter flavus gen. nov., sp. nov. Int J Syst Evol Microbiol 52:1441–1454. https://doi.org/10.1099/00207713-52-5-1441
3. Chandel A, Mann R, Kaur J, et al (2022) Australian native Glycine clandestina seed microbiota hosts a more diverse bacterial community than the domesticated soybean Glycine max. Environ Microbiome 17:. https://doi.org/10.1186/S40793-022-00452-Y
4. Chase AB, Arevalo P, Polz MF, et al (2016) Evidence for ecological flexibility in the cosmopolitan genus Curtobacterium. Front Microbiol 7:1874. https://doi.org/10.3389/FMICB.2016.01874/BIBTEX
5. Chen G, Khojasteh M, Taheri-Dehkordi A, et al (2021) Complete Genome Sequencing Provides Novel Insight Into the Virulence Repertories and Phylogenetic Position of Dry Beans Pathogen Curtobacterium flaccumfaciens pv. flaccumfaciens. Phytopathology 111:268–280. https://doi.org/10.1094/PHYTO-06-20-0243-R
6. Doty SL, Oakley B, Xin G, et al (2009) Diazotrophic endophytes of native black cottonwood and willow. Symbiosis 47:23–33. https://doi.org/10.1007/BF03179967/METRICS
7. Evseev P, Lukianova A, Tarakanov R, et al (2022) Curtobacterium spp. and Curtobacterium flaccumfaciens: Phylogeny, Genomics-Based Taxonomy, Pathogenicity, and Diagnostics. Curr Issues Mol Biol 44:889–927. https://doi.org/10.3390/CIMB44020060/S1
8. Firrincieli A, Khorasani M, Frank AC, Doty SL (2020) Influences of Climate on Phyllosphere Endophytic Bacterial Communities of Wild Poplar. Front Plant Sci 11:.

https://doi.org/10.3389/FPLS.2020.00203

9. Francis MJ, Doherty RR, Patel M, et al (2011) Curtobacterium flaccumfaciens septic arthritis following puncture with a Coxspur Hawthorn thorn. J Clin Microbiol 49:2759–2760. https://doi.org/10.1128/JCM.00340-11
10. Kalyaanamoorthy S, Minh BQ, Wong TKF, et al (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 2017 14:6 14:587–589. https://doi.org/10.1038/nmeth.4285
11. Khanal M, Bhatta BP, Timilsina S, et al (2023) Curtobacterium allii sp. nov., the actinobacterial pathogen causing onion bulb rot. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology 116:83–96. https://doi.org/10.1007/S10482-022-01775-Z/TABLES/3
12. Li W, O'Neill KR, Haft DH, et al (2021) RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. Nucleic Acids Res 49:D1020–D1028. https://doi.org/10.1093/NAR/GKAA1105
13. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:. https://doi.org/10.1186/1471-2105-14-60
14. Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nature Communications 2019 10:1 10:1–10. https://doi.org/10.1038/s41467-019-10210-3
15. Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/MOLBEV/MSU300
16. Osdaghi E, Taghavi SM, Calamai S, et al (2018) Phenotypic and Molecular-Phylogenetic Analysis Provide Novel Insights into the Diversity of Curtobacterium flaccumfaciens. Phytopathology 108:1154–1164. https://doi.org/10.1094/PHYTO-12-17-0420-R
17. Parks DH, Chuvochina M, Rinke C, et al (2022a) GTDB: an ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. Nucleic Acids Res 50:D785–D794. https://doi.org/10.1093/NAR/GKAB776
18. Parks DH, Chuvochina M, Rinke C, et al (2022b) GTDB: an ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. Nucleic Acids Res 50:D785–D794. https://doi.org/10.1093/NAR/GKAB776
19. Scales NC, Chase AB, Finks SS, et al (2022) Differential Response of Bacterial Microdiversity to Simulated Global Change. Appl Environ Microbiol 88:. https://doi.org/10.1128/AEM.02429-21/SUPPL_FILE/AEM.02429-21-S0001.PDF
20. South KA, Nordstedt NP, Jones ML (2021) Identification of Plant Growth Promoting Rhizobacteria That Improve the Performance of Greenhouse-Grown Petunias under Low Fertility Conditions. Plants 10:. https://doi.org/10.3390/PLANTS10071410

21. Varga T, Hixson KK, Ahkami AH, et al (2020) Endophyte-Promoted Phosphorus Solubilization in Populus. Front Plant Sci 11:1585. https://doi.org/10.3389/FPLS.2020.567918/BIBTEX

Figures

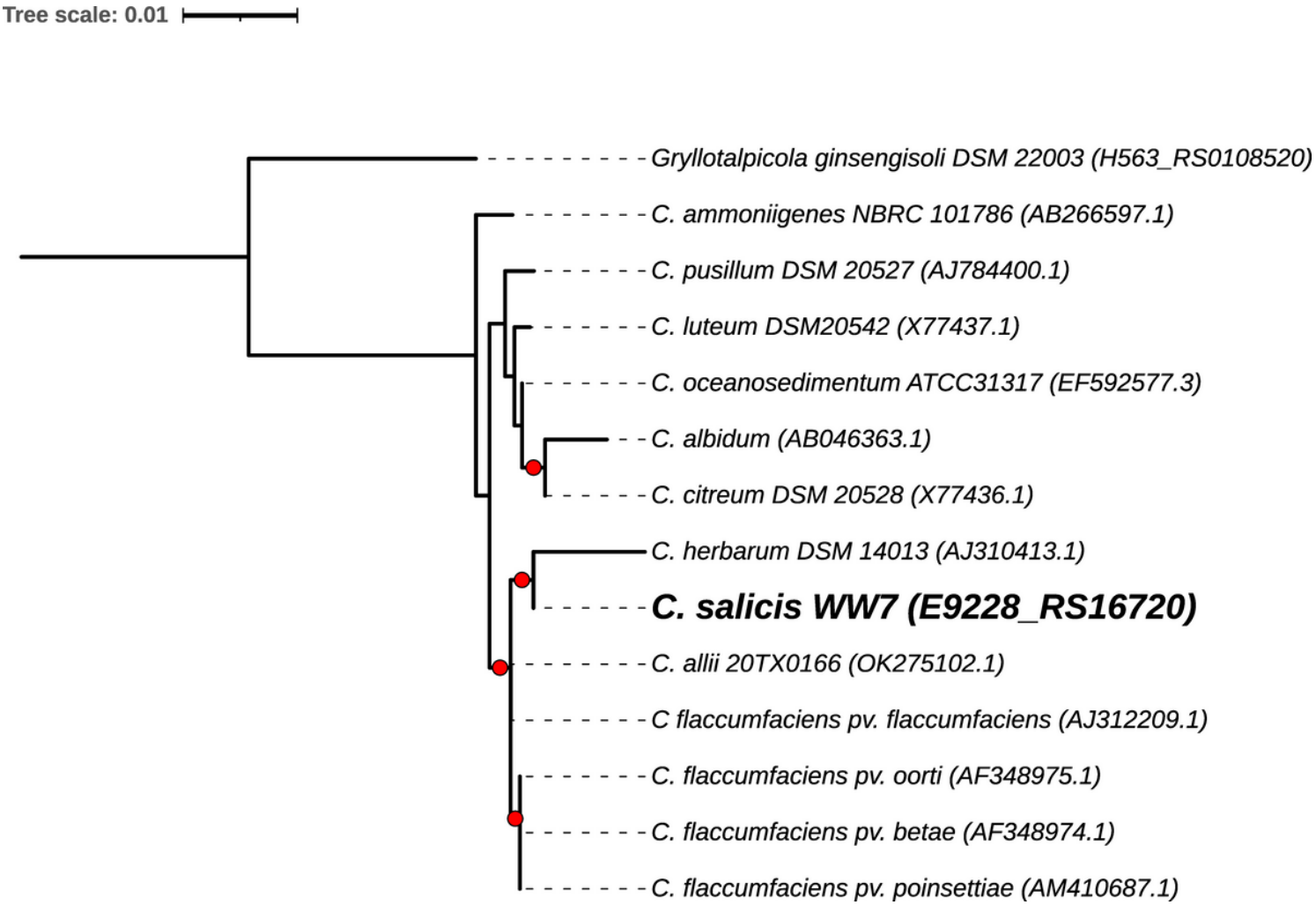

Figure 1

Maximum Likelihood phylogenetic tree of 16S rRNA gene from *Curtobacterium* type strains. The tree was inferred using IQTREE (model HKY+F+I) (Nguyen et al. 2015; Kalyaanamoorthy et al. 2017) using 16S rDNA gene sequences retrieved from the Type-Strain Genome Server (TYGS) (Meier-Kolthoff and Göker 2019). Bootstrap values were calculated based on 1000 replications and only nodes with support value > 80% are shown (red circles). The tree was rooted at *Gryllotalpicola ginsengisoli* DSM 22003

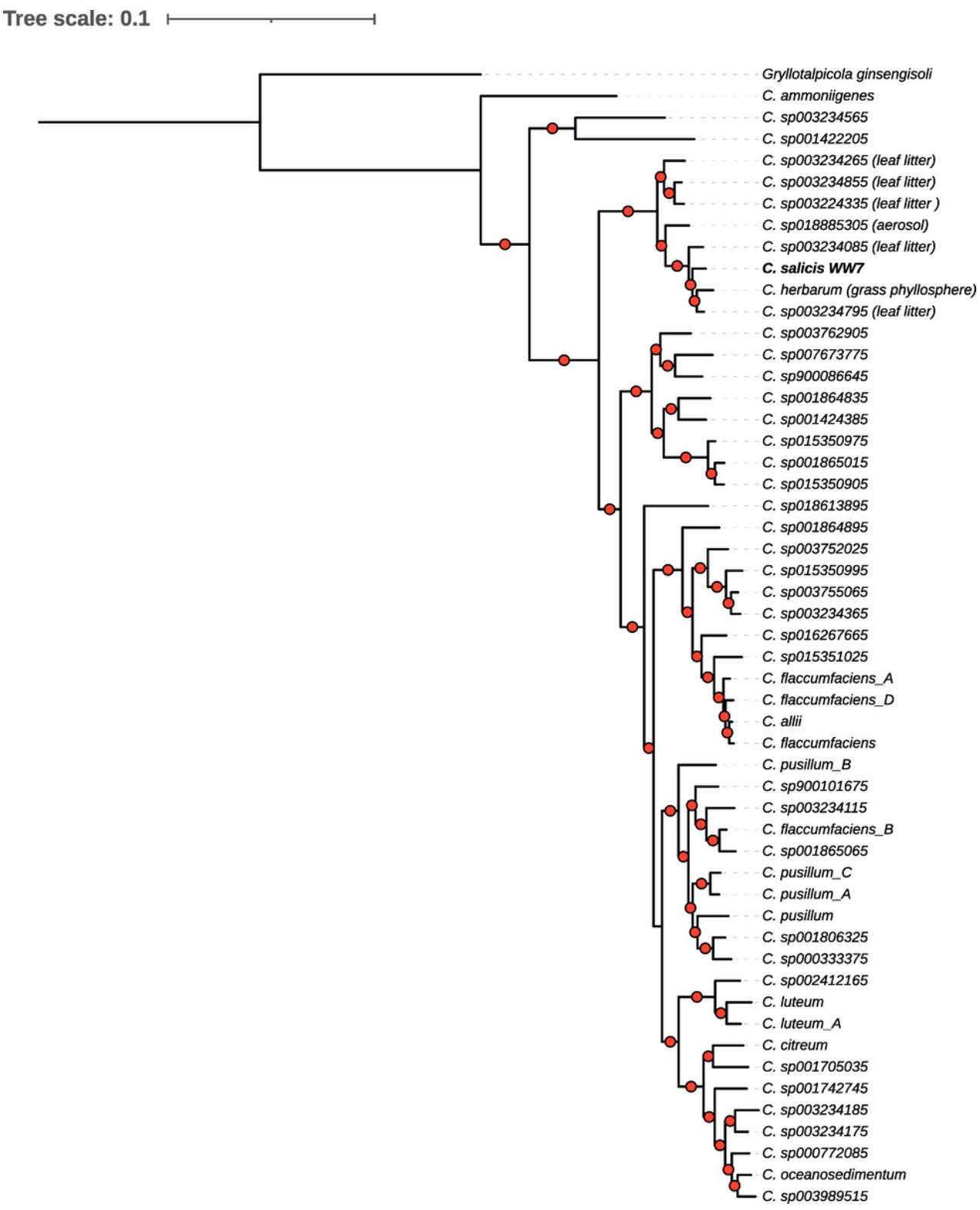

Figure 2

Maximum likelihood phylogenetic consensus tree based on the concatenated alignment of 120 ubiquitous bacterial genes. The concatenated alignment of the 120 bacterial genes was created using the GTDB-tk de novo workflow. The resulting alignment file was analyzed in IQTREE under the WAG model. Bootstrap values were calculated based on 1000 replications and only nodes with a support value > 80% are shown. *G. ginsengisioli* was used as the outgroup.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.pdf
- TableS1.xlsx