***Curtobacterium allii* sp. nov., the actinobacterial pathogen causing onion bulb rot**

Manzeal Khanal1,2, Bed Prakash Bhatta1,2, Sujan Timilsina3,+, Sudeep Ghimire4, Kimberly Cochran2,5, and Subas Malla1,2\*

1Department of Horticultural Sciences, Texas A&M University, College Station, Texas, 77843, USA;

2Texas A&M AgriLife Research and Extension Centre, Uvalde, Texas, 78801, USA;

3 Department of Plant Pathology, University of Florida, Gainesville, Florida, 32611, USA;

4Department of Pathology, University of Iowa, Iowa City, Iowa, 54442, USA;

5Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, 77843, USA;

+ Current address: Charles River Laboratories, Newark, DE, USA.

**Corresponding author**

\*Subas Malla, [subas.malla@ag.tamu.edu](mailto:subas.malla@ag.tamu.edu)

**Supplementary figures and table**

**Fig. S1.** A symptomatic rotting onion bulb from Texas, USA, from which the pathogenic strain 20TX0166T of *Curtobacterium* was isolated.

A picture containing cup, indoor, orange, several

Description automatically generated

**Fig. S2.** Graphic visualization of the 20TX0166T genome of a *Curtobacterium* isolate obtained from a rotting onion bulb in Texas, USA, constructed using DNAplotter v18.1.0. Progressing from the outer ring inwards, brown, green, and blue coloured circles represent the total coding sequences (CDSs) present in the double strand, forward strand, and reverse strand, respectively, of the bacterial strain. The fourth circle comprised of red marks denotes the positions of tRNAs in the genome. The fifth circle represents the average G+C content, and the innermost circle represents the GC skewness. Teal and purple colours represent GC content above and below the average, respectively.

Circle

Description automatically generated with medium confidence

**Fig. S3.** Necrotic rotting observed on red onion scales 10 d post-inoculation with strain 20TX0166T of *Curtobacterium* isolated from a rotten onion bulb in Texas, USA. A suspension of the bacterium (10 µl of 108 CFU/ml) was placed on the scale (~3 cm x 4 cm) after making a wound at the centre of the scale with a sterile needle. The inoculated scales were incubated at 25 °C.

A picture containing text, indoor, container, plastic

Description automatically generated

**Fig. S4.** Phylogenetic tree based on whole genome sequences of *Curtobacterium allii* 20TX0166T, *C. flaccumfaciens* LMG 3645T, and 35 strains deposited as *C. flaccumfaciens* in the NCBI Genome database. The tree was constructed using the TYGS webserver. The type strains of the species used are indicated in parenthesis.

A picture containing chart

Description automatically generated

**Fig. S5.** Gram stain of strain 20TX0166T of *Curtobacterium* isolated from a rotten onion bulb in Texas, USA. The purple color resulting from the stain indicates the bacterium to be Gram positive.

A picture containing nature

Description automatically generated

**Fig. S6.** Endospore staining of strain 20TX0166T of *Curtobacterium* isolated from a rotten onion bulb in Texas, USA, to determine if the strain can form endospores. Bacterial cells stained red (absence of green), which confirmed the bacterium does not form endospores.

Map

Description automatically generated

**Fig. S7.** A 3-d-old culture of strain 20TX0166T of *Curtobacterium* isolated from a rotten onion bulb in Texas, USA, on YPGA medium (A) and NBYA medium (B) grown at 25 °C.

A close-up of a petri dish

Description automatically generated with medium confidence 

**B**

**A**

**Table S1.** Comparison of selected physiological and phenotypic characteristics of strain 20TX0166T of *Curtobacterium* isolated from a rotten onion bulb in Texas, USA, and closely related *Curtobacterium* type strains.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Reactions** | **Strains of *Curtobacterium*†** | | | | | | |
| **1‡** | **2‡** | **3§** | **4§** | **5§** | **6§** | **7§** |
| **Biolog:** |  |  |  |  |  |  |  |
| Negative control | -¶ | - | ND | ND | ND | ND | ND |
| Dextrin | + | + | - | + | + | - | - |
| D-Maltose | + | + | + | + | + | + | ND |
| D-Trehalose | + | + | ND | ND | ND | ND | ND |
| D-Cellobiose | + | + | - | + | + | + | - |
| Gentiobiose | + | + | ND | ND | ND | ND | ND |
| Sucrose | + | + | ND | ND | ND | ND | ND |
| D-Turanose | + | + | + | - | - | + | + |
| Stachyose | + | + | ND | ND | ND | ND | ND |
| P. control | + | + | ND | ND | ND | ND | ND |
| pH 6 | + | + | ND | ND | ND | ND | ND |
| pH 5 | + | + | ND | ND | ND | ND | ND |
| D-Raffinose | + | + | - | - | - | + | - |
| alpha-D-Lactose | + | + | - | + | + | + | + |
| D-Melibiose | + | + | - | + | + | w | + |
| beta-Methyl-D-Glucoside | + | + | + | - | - | w | - |
| D-Salicin | + | + | ND | ND | ND | ND | ND |
| N-Acetyl-DGlucosamine | + | + | - | + | + | w | - |
| N-Acetyl-beta-D-Mannosamine | w | w | ND | ND | ND | ND | ND |
| N-Acetyl-DGalactosamine | + | + | - | - | - | - | - |
| N-Acetyl Neuraminic Acid | w | - | ND | ND | ND | ND | ND |
| 1% NaCl | + | + | ND | ND | ND | ND | ND |
| 4% NaCl | + | + | ND | ND | ND | ND | ND |
| 8% NaCl | + | + | ND | ND | ND | ND | ND |
| alpha-D-Glucose | + | + | ND | ND | ND | ND | ND |
| D-Mannose | + | + | ND | ND | ND | ND | ND |
| D-Fructose | + | + | ND | ND | ND | ND | ND |
| D-Galactose | + | + | ND | ND | ND | ND | ND |
| 3-Methyl Glucose | + | + | ND | ND | ND | ND | ND |
| D-Fucose | + | + | ND | ND | ND | ND | ND |
| L-Fucose | + | + | - | + | + | - | - |
| L-Rhamnose | + | + | + | + | + | + | ND |
| Inosine | + | + | ND | ND | ND | ND | ND |
| 1% Sodium Lactate | + | + | ND | ND | ND | ND | ND |
| Fusidic Acid | - | - | ND | ND | ND | ND | ND |
| D-Serine | - | - | ND | ND | ND | ND | ND |
| D-Sorbitol | + | + | + | - | - | + | + |
| D-Mannitol | + | + | + | - | - | w | + |
| D-Arabitol | + | + | w | - | - | - | - |
| myo-Inositol | + | + | - | - | - | - | - |
| Glycerol | + | + | ND | ND | ND | ND | ND |
| D-Glucose6-PO4 | + | w | ND | ND | ND | ND | ND |
| D-Fructose6-PO4 | + | w | ND | ND | ND | ND | ND |
| D-Aspartic Acid | w | w | ND | ND | ND | ND | ND |
| D-Serine | - | - | ND | ND | ND | ND | ND |
| Troleandomycin | - | - | ND | ND | ND | ND | ND |
| Rifamycin SV | - | - | ND | ND | ND | ND | ND |
| Minocycline | - | - | ND | ND | ND | ND | ND |
| Gelatin | + | w | ND | ND | ND | ND | ND |
| Glycyl-L-Proline | + | + | ND | ND | ND | ND | ND |
| L-Alanine | + | + | - | - | - | w | - |
| L-Arginine | + | + | ND | ND | ND | ND | ND |
| L-Aspartic Acid | + | + | ND | ND | ND | ND | ND |
| L-Glutamic Acid | + | + | - | + | - | - | - |
| L-Histidine | - | - | ND | ND | ND | ND | ND |
| L-Pyroglutamic Acid | + | w | ND | ND | ND | ND | ND |
| L-Serine | + | + | ND | ND | ND | ND | ND |
| Lincomycin | - | - | ND | ND | ND | ND | ND |
| Guanidine HCl | + | + | ND | ND | ND | ND | ND |
| Niaproof 4 | - | - | ND | ND | ND | ND | ND |
| Pectin | + | + | ND | ND | ND | ND | ND |
| D-Galacturonic Acid | w | w | ND | ND | ND | ND | ND |
| L-Galactonic Acid Lactone | + | w | ND | ND | ND | ND | ND |
| D-Gluconic Acid | + | + | + | - | - | + | + |
| D-Glucuronic Acid | + | + | - | - | - | - | - |
| Glucuronamide | + | + | ND | ND | ND | ND | ND |
| Mucic Acid | - | - | ND | ND | ND | ND | ND |
| Quinic Acid | - | - | ND | ND | ND | ND | ND |
| D-Saccharic Acid | - | - | ND | ND | ND | ND | ND |
| Vancomycin | - | - | ND | ND | ND | ND | ND |
| Tetrazolium Violet | - | - | ND | ND | ND | ND | ND |
| Tetrazolium Blue | - | - | ND | ND | ND | ND | ND |
| p-HydroxyPhenylacetic Acid | - | - | ND | ND | ND | ND | ND |
| Methyl Pyruvate | w | - | ND | ND | ND | ND | ND |
| D-Lactic Acid Methyl Ester | - | - | ND | ND | ND | ND | ND |
| L-Lactic Acid | w | w | - | w | w | - | + |
| Citric Acid | + | + | ND | ND | ND | ND | ND |
| alpha-Keto-Glutaric Acid | - | - | ND | ND | ND | ND | ND |
| D-Malic Acid | - | w | ND | ND | ND | ND | ND |
| L-Malic Acid | - | + | ND | ND | ND | ND | ND |
| Bromo-Succinic Acid | - | + | - | w | - | - | - |
| Nalidixic Acid | + | + | ND | ND | ND | ND | ND |
| Lithium Chloride | + | + | ND | ND | ND | ND | ND |
| Potassium Tellurite | + | + | ND | ND | ND | ND | ND |
| Tween 40 | + | + | - | w | w | + | - |
| gamma-Amino-Butryric Acid | - | w | ND | ND | ND | ND | ND |
| alpha-HydroxyButyric Acid | - | w | ND | ND | ND | ND | ND |
| beta-Hydroxy-D,LButyric Acid | - | - | ND | ND | ND | ND | ND |
| alpha-Keto-Butyric Acid | w | w | ND | ND | ND | ND | ND |
| Acetoacetic Acid | + | + | ND | ND | ND | ND | ND |
| Propionic Acid | w | w | ND | ND | ND | ND | ND |
| Acetic Acid | + | + | ND | ND | ND | ND | ND |
| Formic Acid | - | - | ND | ND | ND | ND | ND |
| Aztreonam | + | + | ND | ND | ND | ND | ND |
| Sodium Butyrate | + | + | ND | ND | ND | ND | ND |
| Sodium Bromate | + | + | ND | ND | ND | ND | ND |
|  |  |  |  |  |  |  |  |
| **API Coryne:** |  |  |  |  |  |  |  |
| Nitrate Reduction | - | - | ND | ND | ND | ND | - |
| Pyrazinamidase | + | + | ND | - | - | ND | ND |
| Pyrolidonyl arylamidase | + | + | ND | ND | ND | ND | ND |
| Alkaline Phosphatase | + | - | ND | ND | ND | ND | ND |
| β-Glucuronidase | - | - | ND | ND | ND | ND | ND |
| β-Galactosidase | + | + | ND | ND | ND | ND | ND |
| α-Glucosidase | + | + | ND | ND | ND | ND | ND |
| N-Acetyl-β-Glucosaminidase | + | + | ND | ND | ND | ND | ND |
| Esculin/β-glucosidase | + | + | + | + | - | + | + |
| Urease | - | - | ND | ND | ND | ND | - |
| Gelatin hydrolysis/protease | - | - | + | - | - | + | - |
| Negative control (Fermentation) | + | - | ND | ND | ND | ND | ND |
| D-glucose (Fermentation) | - | - | ND | ND | ND | ND | ND |
| L-ribose (Fermentation) | + | - | + | + | + | - | + |
| D-xylose (Fermentation) | - | - | ND | ND | ND | ND | ND |
| D-mannitol (Fermentation) | + | - | ND | ND | ND | ND | ND |
| D-maltose (Fermentation) | - | - | + | + | + | + | ND |
| D-lactose (Fermentation) | + | - | ND | ND | ND | ND | ND |
| D-saccharose (Fermentation) | - | - | ND | ND | ND | ND | ND |
| glycogen (Fermentation) | + | - | - | + | + | - | - |
|  |  |  |  |  |  |  |  |
| **API 20NE:** |  |  |  |  |  |  |  |
| Nitrate Reduction | - | - | ND | ND | ND | ND | ND |
| L-tryptophane | - | - | ND | ND | ND | ND | ND |
| D-glucose (fermentation) | - | - | ND | ND | ND | ND | ND |
| Arginine DiHydrolase | - | - | ND | ND | ND | ND | ND |
| Urease | - | - | ND | ND | ND | ND | ND |
| Esculin/β-glucosidase | + | + | + | - | + | + | + |
| Gelatin hydrolysis/protease | - | - | + | - | - | + | - |
| β-galactosidase | + | + | ND | ND | ND | ND | ND |
| D-glucose (Assimilation) | + | + | ND | ND | ND | ND | ND |
| L-arabinose (Assimilation) | + | + | ND | ND | ND | ND | ND |
| D-mannose (Assimilation) | + | + | ND | ND | ND | ND | ND |
| D-mannitol (Assimilation) | - | + | ND | + | - | ND | ND |
| N-acetyl-glucosamine (Assimilation) | - | + | + | + | - | - | - |
| D-maltose (Assimilation) | + | + | + | + | + | + | ND |
| Potassium gluconate (Assimilation) | + | + | - | - | - | - | + |
| Capric acid (Assimilation) | - | - | ND | ND | ND | ND | ND |
| Adipic acid (Assimilation) | - | - | ND | ND | ND | ND | ND |
| Malic acid/Malate (Assimilation) | - | + | ND | + | + | ND | ND |
| Trisodium citrate (Assimilation) | - | w | ND | ND | ND | ND | ND |
| Phenylacetic acid (Assimilation) | - | - | ND | ND | ND | ND | ND |

† Strains: 1, 20TX0166T; 2, *C. flaccumfaciens* LMG 3645T; 3, *C. pusillum* JCM 1350T; 4, *C. citreum* JCM 1345T; 5, *C. luteum* JCM 1480T; 6, C. albidum NBRC 15078T; 7, *C. ammoniigenes* B55T.

**‡** Data are from this study.

**§** Data are from Aizawa et al.2007.

¶ +, Positive reaction; –, negative reaction; w, weakly positive reaction; ND, No data available.