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Draft Genome of an AmpC-β-Lactamase Producing *Serratia marcescens* Isolate from Fresh farm Tomatoes in South Africa

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Here we report essential features of the draft genome of an AmpC-β-lactamase-producing bacterial isolate obtained from farm tomatoes in South Africa. The isolate designated strain Tom1 featured a genome of 4950426 bp with a G+C% of 59.83. It was identified as *Serratia marcescens* by ribosomal multilocus sequence typing (rMLST), digital DNA-DNA hybridization (dDDH), average nucleotide identity (ANI), and phylogenetic analysis using reference genomes. Its genome encoded an AmpC-β-lactamase (*bla*$_{SST-1}$), an efflux pump providing tetracycline resistance (*tet*(*41*)), and an aminoglycoside acetyltransferase (*aac*(6')-*Ic*). Additionally, genes encoding proteins involved in prodigiosin biosynthesis and associated with adherence, biofilm formation, virulence, and pathogenicity were detected.

Keywords: ***Serratia marcescens,*** **draft genome, AmpC-β-lactamase, antibiotic-resistance, tomato, South Africa**

Tomatoes are a typical example of fresh produce frequently consumed raw. Consumption of raw or minimally processed fresh produce is an integral part of a healthy modern diet as it provides vitamins, minerals, fiber, and antioxidants [1]. However, ready-to-eat fresh produce contaminated with antibiotic-resistant pathogenic bacteria is a potential health risk, as such bacteria, upon entering the gut, might render antibiotic therapy ineffective and can contribute to undesirable antibiotic-resistance gene transfer [2, 3]. Antibiotic-resistant bacteria might contaminate fresh produce at various stages along the farm-to-fork production chain; contamination can occur via irrigation water, soil, fresh manure, or the handling and transportation of fresh produce [2]. Antibiotic-resistant opportunistic pathogens such as *Serratia marcescens,* a species known to be present in various environmental habitats, can cause severe invasive infections [3, 4]. This paper describes the genome-based characterization and classification of a multidrug-resistant strain of *S. marcescens* isolated from the surface of fresh farm tomatoes in South Africa.

The isolated strain designated Tom1 was originally obtained when assessing the microbial burden of fresh farm tomatoes via establishing aerobic plate counts, with a fraction of plates showing the presence of distinct red colonies after incubation. Using standard methods, a representative isolate was characterized microscopically, phenotypically, and by 16S rRNA gene amplicon Sanger sequencing (Genbank accession number ON630377.1). Shotgun genome sequencing (paired-end 2 × 150 bp, Illumina Nova Seq) was done by Zymo Research (USA) using extracted genomic DNA (ZymoBIOMICS-96 MagBead kit, Zymo, USA) to create a library (Nextera DNA Flex Library Prep Kit, Illumina, USA). The reads

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obtained were quality checked, trimmed, *de novo* assembled and analyzed using FastQC, Trimgalore (V0.6.5), Cutadapt (V2.2), Unicycler (V 0.4.8), Quast (V5.02), and additional bioinformatics tools available from the BV-BRC [5]. Assembly completeness was analyzed by benchmarking universal single-copy orthologs

Table 1. Genome features and representative genes associated with antibiotic resistance, virulence, and pathogenicity of *Serratia marcescens* strain Tom1.

Feature	Detail
Identity and origin	*Serratia marcescens* strain Tom1, fresh farm tomato surface, South Africa
Genbank Accession no.	JANBMM010000000
Coverage, number of contigs, N_{50}, N_{75}, G+C content, total length	300 x, 22, 1349891 bp, 327775 bp, 59.83%, 4950426 bp
No. of CDS, tRNAs, rRNAs	4553, 82, 4
Plasmid[1]	Not detected
Completeness (BUSCO)	99.32%
rMLST profile best match	*Serratia marcescens* (100%)
Digital DDH best match	*Serratia marcescens* ATCC 13880 (95.20%)
Fast ANI best match	*Serratia marcescens* ATCC 13880 (99.31%)
Human pathogen probability[2]	0.744
Antibiotic resistance genes[3]	$bla_{SST-1;}$ *tet(41); aac(6')-Ic*
Antibiotic resistance phenotype	AMP-AMC-CL-FOX-CPT-TET-TGC
Detected stress response genes	*czcD* (Co/Cd/Zn antiport) *zntA* (Zn/Cd/Pb export) *sodA-C* (superoxide dismutase) *katA, G* (catalase/peroxidase)
Detected genes associated with virulence and pathogenicity[4]	Secretion, adherence, and biofilm formation *fimA, D* (type I fimbriae) *smfA* (fimbria A protein) *pilE, Q* (type IV pili) *bsmA* (lipoprotein A) *pgaA-C* (PGA biosynthesis) *clpV1* (T6SS) *ompA, X* (porins) *shlA-shlB* (T5SS) Iron acquisition *efeB, O, U* (iron recovery and uptake) *fepA-D, G* (ferric enterobactin transport) *entB, E, F, S* (enterobactin biosynthesis and export) *fecA, R* (iron citrate transport) *hasA* (hemophore) Exoenzymes and exotoxins *phlA* (phospholipase A, EC:3.1.1.32) *prtA* (serralysin, EC:3.4.24.40) *shlA* (hemolysin)

[1] Established using Plasmidfinder 2.1.
[2] Established using Pathogenfinder 1.1.
[3] Established using Resfinder 4.1. and CARD 6.02
[4] Established using Pathogenfinder 1.1., BV-BRC, and VFDB.
AMP-ampicillin, AMC-amoxicillin-clavulanate, CL-cephalexin, FOX-cefoxitin, CPT-ceftaroline, TET-tetracycline, TGC-tigecycline

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(http://cab.cc.spbu.ru), while genome annotation, average nucleotide analysis of related genomes, and resistance gene prediction were done using the tools Prokka (1.14.6), FastANI (1.3.3), and CARD (6.02) [6]. Additional genome features were analyzed using bioinformatics tools available from the center of genomic epidemiology (https://cge.cbs.dtu.dk) and by employing the bacterial virulence factor database (VFDB, http://www.mgc.ac.cn/VFs/main.htm). Unless stated otherwise, default parameters were used for all bioinformatics tools. Furthermore, the genome was analyzed by ribosomal Multilocus Sequence Typing (rMLST) (https://pubmlst.org/) and classified by digital DNA-DNA hybridization (dDDH) and phylogenetic analysis using reference genomes and the TYGS-LPSN system [7].

Antibiotic susceptibility testing was done following the latest EUCAST disk diffusion procedure (www.eucast.org, 2023) employing 6-mm disks (Oxoid, UK) and the quality control strain *E. coli* ATCC 25922. The presence of β-lactamase activity was verified using nitrocefin, a chromogenic cephalosporin (Oxoid, UK). In addition, the production of an inducible AmpC-β-lactamase was confirmed using the Mast D69C AmpC detection kit, while the production of extended-spectrum-β-lactamase was assessed using the matching Mast D63C kit (Mast Group, UK) for the detection of ESBL in AmpC-producing *Enterobacterales*, according to manufacturer's instructions.

The Gram- and oxidase-negative strain Tom1 formed motile rods (~2 × 0.9 µm), was β-galactosidase- and catalase-positive, hydrolyzed gelatin, and formed typical red colonies evidencing the production of prodigiosin (Fig. 1A and B). The assembled genome yielded 22 contiguous sequences (> 300 bp), was 4950426 bp long, and had a G+C content of 59.83%. The corresponding N_{50}, N_{75}, L_{50}, and L_{75} values were 1349891 bp, 327775 bp, 2, and 5, and the longest contig measured 1399301 bp. The genome was 99.32% complete based on universal single-copy ortholog analysis, with a total of 4639 protein-coding (CDS), tRNA and rRNA-coding sequences identified by Prokka (Table 1).

Ribosomal MLST identified the isolated strain Tom1 at the species level as *Serratia marcescens* (100% match), while the highest average nucleotide identity (ANI) was established for the genome of *S. marcescens* ATCC 13880 (GCA_000735445.1, 99.31%). Comparison

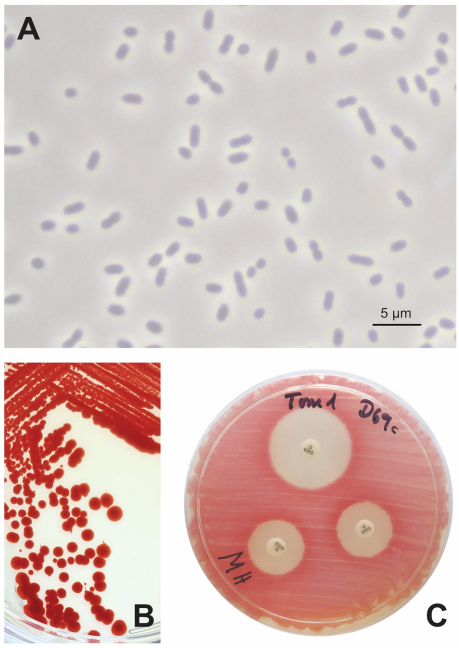

Fig. 1. Basic microscopic and macroscopic features of *Serratia marcescens* strain Tom1. (A) Microscopic (phase contrast) appearance of typical mid-exponential cells, (B) Typical red coloration of overnight colonies on plate count agar, (C) Phenotypic demonstration of an inducible AmpC-β-lactamase using the MAST 69C kit.

of the assembled genome against related reference genomes confirmed the identity of strain Tom1 as *S. marcescens* via digital DDH (95.20% match with *S. marcescens* ATCC 13880, GCA_000735445.1) and pairwise comparisons with type strain genomes using TYGS (Table 1, Fig. 2). The genome-based phylogenetic analysis highlighted that the isolated strain Tom1 clustered most closely with *S. marcescens* ATCC 13880 (Fig. 2).

The following resistance genes, bla_{SST1} (encoding an inducible AmpC-β-lactamase), *tet(41)* (encoding a tetracycline efflux pump), and *aac*(6')-*Ic* (encoding an aminoglycoside acetyltransferase), were detected on the draft genome of *S. marcescens* Tom1. Antibiotic susceptibility testing demonstrated that *S. marcescens* Tom1 was multidrug-resistant, with resistance to ampicillin (AMP, 10 µg), amoxicillin-clavulanate (AMC, 20+10 µg), cephalexin (CL, 30 µg), cefoxitin (FOX, 30 µg), ceftaroline (CPT, 5 µg), tetracycline (TET, 30 µg), and tigecycline (TGC, 15 µg) detected. The chromogenic cephalosporin, nitrocefin, was readily hydrolyzed by *S. marcescens*

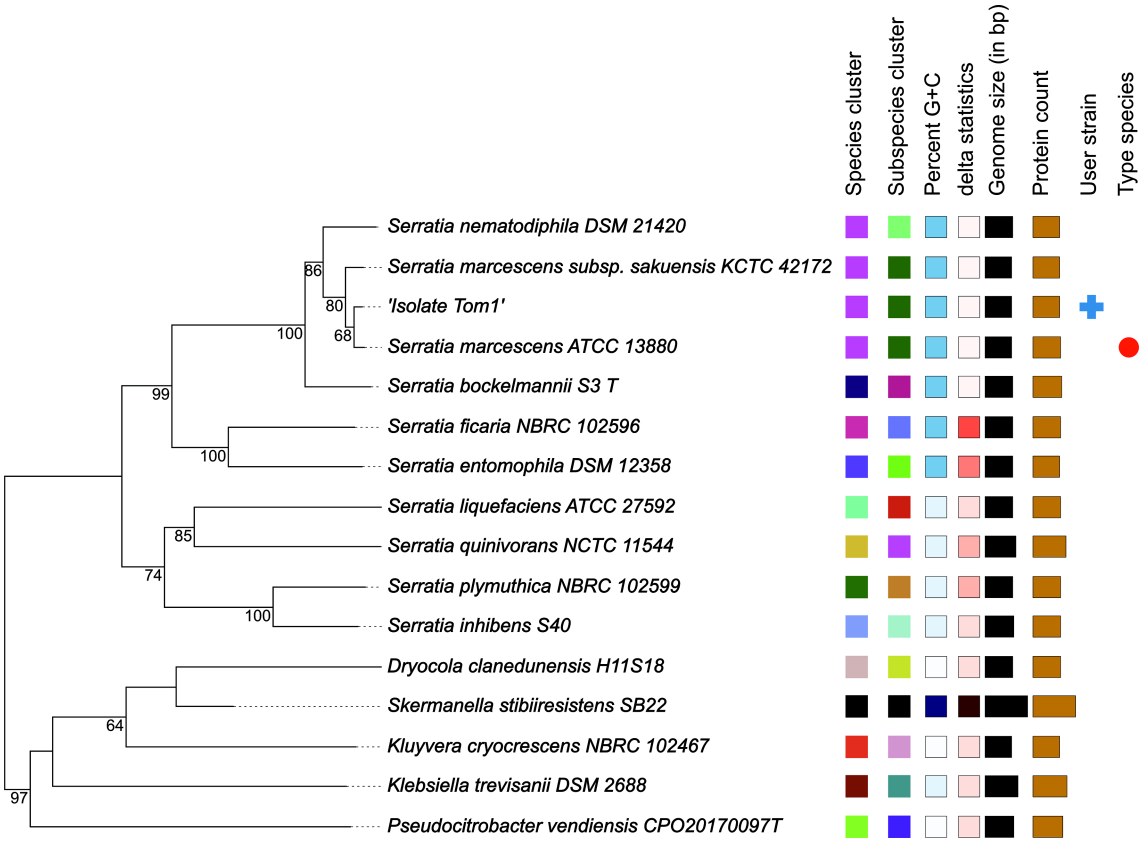

Fig. 2. Midpoint-rooted phylogenetic tree inferred with FastME (2.1.6.1) from GBDP distances calculated from type strain genome sequences using the TYGS server. Branch lengths are scaled based on the GBDP distance formula d_5; numbers shown are GBDP pseudo-bootstrap support values > 60% (100 replications, average branch support of 80.2%).

strain Tom1 to form the red-colored reaction product absorbing at 486 nm, confirming β-lactamase activity. Furthermore, the presence of an inducible AmpC-β-lactamase was demonstrated as the strain showed resistance against cefpodoxime in the presence of an AmpC inducer and a combination of an AmpC inducer and an ESBL inhibitor but not in the presence of an AmpC inhibitor (Mast D69C, Fig. 1C). In addition, the negative result obtained using the Mast D63C extended-spectrum-β-lactamase detection kit for AmpC-producing *Enterobacterales* matched the absence of known ESBL genes on the draft genome of *S. marcescens* strain Tom1. Thus, the phenotypic resistance profile of *S. marcescens* Tom1 confirmed its genome-based prediction as a multidrug-resistant strain with an inducible AmpC-β-lactamase. Vegetable-associated antibiotic-resistant strains of *S. marcescens* have been reported [2, 3], and similar antibiotic-resistance gene profiles were reported for the genomes of clinical and environmental *S. marcescens* isolates from various countries [4] and, most recently, for the genome of a *S. marcescens* strain isolated in South Korea associated with lettuce production [8].

Notably, along with stress response encoding genes (e.g., *katA, sodA*), the recently described gene *ydg*H, encoding a modifier of cephalosporin and detergent susceptibility in *S. marcescens* [9], was detected within the draft genome of *S. marcescens* strain Tom1, highlighting the potential of this strain to alter its susceptibility against antimicrobial agents further.

The ability of strain Tom1 to synthesize the pigment prodigiosin, yielding the typical red colonies (Fig. 1B), matched the presence of the prodigiosin biosynthesis gene cluster detected on the genome. The red pigment prodigiosin originating from *Serratia* species was reported to inhibit the growth of various Gram-negative and Gram-positive bacteria [10] and, additionally, to exhibit immunosuppressive properties [3, 10].

As expected on microbiological grounds, given the motile nature of this species, genes associated with the flagellar apparatus and chemotaxis (e.g., *fliC-J*, *fliM-R*, *flgA-I*, *cheABYZ*) were detected within the genome of *S. marcescens* strain Tom1. Notably, many of these genes have been associated with *S. marcescens* virulence [4]. In addition, genes encoding proteins associated with the pathogenicity of *S. marcescens,* such as serralysin (*prtA*), hemolysin (*shlA*), and phospholipase A (*phlA*), as well as genes encoding factors associated with biofilm formation (*bsmA*), cell adherence (*fimAD*), secretion (T5SS) and iron acquisition systems (*fepABCDG*) were detected (Table 1). Thus, this strain was categorized by Pathogenfinder 1.1 with a probability of 0.744 as a human pathogen (Table 1).

This is the first report of a multidrug-resistant *S. marcescens* isolate obtained from fresh farm tomatoes in South Africa, confirming the presence of this opportunistic pathogen within the fresh produce farm-to-fork chain. Ready-to-eat fresh produce carrying potentially pathogenic *Enterobacterales* expressing antibiotic resistance enzymes such as AmpC-β-lactamase is concerning, as especially the most vulnerable members of society might be affected when consuming such vegetables.

Nucleotide Sequence Accession Number

This whole genome shotgun project has been deposited at GenBank under the accession JANBMM000000000. The version described in this paper is version JANBMM010000000.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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