Draft genome sequence of a *Serratia marcescens* strain (PIC 3611) proficient at recalcitrant polysaccharide utilization

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Running Title

S. marcescens recalcitrant polysaccharide utilization genome sequence

Keywords

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ABSTRACT

Serratia marcescens is a Gram-negative bacterium found in terrestrial and aquatic environments and studied for its polysaccharide utilization capabilities as part of larger efforts to discover novel carbohydrate active enzymes. Here we announce the genome sequence of an *S. marcescens* strain (PIC3611) able to utilize complex polysaccharide substrates.

ANNOUNCEMENT

Serratia marcescens is a Gram-negative bacterium known for its red pigmentation and potent degradation of marine polysaccharides, in particular chitin (1–3). The *S. marcescens* strain PIC3611, previously available at Presque Isle Cultures (PIC), has a robust ability to degrade various complex chitin-containing substrates (**Fig 1**). Despite the closure of PIC, *S. marcescens* strain PIC3611 is still used as a model system (4–6), which justifies genome sequencing.

S. marcescens PIC3611 was stored in 50% glycerol (w:v) at -80 ^{O}C. The strain was grown to full density (OD_{600} ≅ 1.5) in a MOPS-glucose (0.2% w:v) broth at 37 ^{O}C for 48 hours, and cell pellets were collected as previously described (7). The pellets were flash frozen in a dry ice and 95% ethanol bath, and then stored at -80 ^{O}C before DNA extraction and whole-genome sequencing at Azenta (South Plainfield, NJ). Genomic DNA was extracted with a PureLink™ Genomic DNA Mini-kit per manufacturer's instructions (Invitrogen, Waltham, MA). Extracted DNA was quantified using a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA). DNA library preparation used a NEBNext Ultra DNA Library Preparation kit per manufacturer's instructions (NEB, Inc., Ipswich, MA). The adaptor-ligated DNA library was cleaned and validated using an Agilent TapeStation (Santa Clara, CA) and quantified via a Qubit 2.0 Fluorometer with Real-Time PCR (Applied Biosystems, Carlsbad, CA) analysis as needed. The DNA library was added to a single flow cell and sequenced on an Illumina MiSeq instrument using a 2 x 250 bp paired-end read configuration (San Diego, CA). For all software referenced below, default parameters were used unless otherwise specified. MiSeq Control Software (v2.6) was used for base-calling. The raw sequence files (.bcl)

generated from MiSeq were converted to FASTQ files and demultiplexed via Illumina's bcl2fastq software (v2.17), and allowed one mismatch during index sequence identification (7). Reads were trimmed using Trimmomatic (v0.36) with the following parameters: LEADING:3, TRAILING:3, SLIDINGWINDOW:4:15, and MINLEN:30 (8). *De novo* genome assembly was completed with Spades *de novo* assembler (v3.10) with the following parameters: -k 21,33,55,77,99,127 --careful (9), which produced 299 contigs with an N_{50} value of 899,515, an average Q-score of 35.22, and a minimum length of 1000 bp (smaller contigs were manually filtered out). There were a total of 62,571,120 reads from the MiSeq, which corresponds to ~2,800x genome coverage. Sequencing and assembly found the *S. marcesens* PIC3611 genome was 5,531,323 bp and an average G+C content of 59%, both of which were in agreement with other sequenced *S. marcescens* strains (10, 11). A nucleotide BLAST (12) of the 16S rDNA gene returned *S. marcescens* strain JWCZ2 as the top hit with an E-value of 0.0 (100% coverage; 100% identity) as further confirmation. Quality assessment of the genome assembly used the getorf function in QUAST (v4.2) (13). Functional assessment used the NCBI Prokaryotic Genome Annotation pipeline (PGAP; v6.0) (14). PGAP found 5,429 genes, of which 5,246 coded for proteins, 103 tRNAs, 47 pseudogenes, 18 ncRNAs, and 15 rRNAs.

DATA AVABILABILITY

The NCBI BioProject number for this genome is PRJNA802829 and raw data files can be obtained from the NCBI SRA using ID SRX14024400. The genome sequence for *S. marcescens* PIC3611 can be found in the NCBI Genbank using accession number JAKQYC000000000 and assembly number ASM2260299v1.

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Author contributions

JKN generated cell pellets for whole genome sequencing, performed the growth and secretion assays, and contributed to writing the manuscript. **JGG** supervised all aspects of the work and contributed to writing the manuscript. All authors read and approved the final submitted version of the manuscript.

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Compliance with ethical standards

This article does not contain any studies using human participants or live vertebrate animals. In addition, the authors declare that they have no conflicts of interest.

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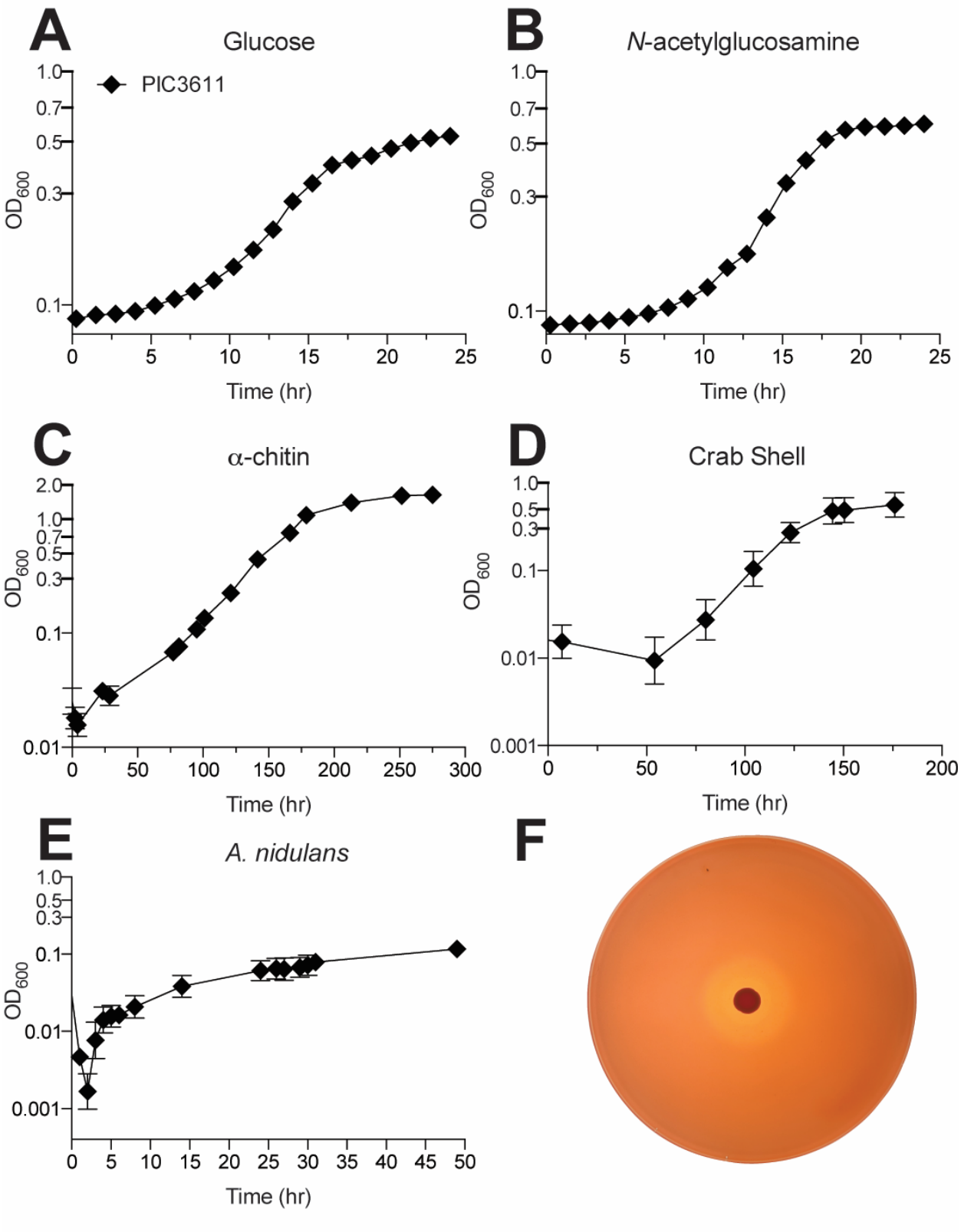
FIGURE LEGEND

Figure 1. Growth phenotypes of *S. marcescens* PIC3611 on 0.2% (w:v) glucose (**A**), 0.25% (w:v) *N*-acetylglucosamine (**B**), 1% (w:v) α-chitin (**C**), 5% (w:v) fungal biomass (*Apergillus nidulans*) (**D**), and 5% (w:v) crab shell (*Callinectes sapidus*) (**E**) as the sole carbon sources. Chitinase secretion of *S. marcescens* PIC3611 on 0.5% (w:v) colloidal chitin as the sole carbon source as shown by Congo Red staining (**F**). All media and plates were made with MOPS minimal medium and supplemented with the designated carbon source. All growth experiments were completed in biological triplicate with error bars representing standard deviation, though some are too small to be observed. Growth analyses on glucose and *N*-acetylglucosamine were completed in an EPOCH2 microplate reader (BioTek) while growth on α-chitin, fungal biomass, and crab shell were measured using test tubes and a spectrophotometer (Milton Roy Spec20D+)


A
Glucose
PIC3611
OD600
Time (hr)
B
N-acetylglucosamine
C
α-chitin
D
Crab Shell
E
A. nidulans
F

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