Draft Genome Sequence of *Raoultella terrigena* strain Ech2A causing soft rot on sweeth pepper (*Capsicum annuum)*

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ABSTRACT

We report the draft genome sequence of *Raoultella terrigen*a strain Ech2A causing Soft Rot on pepper. To verify pathogenicity, Koch's postulates were performed on sweet pepper. Genes encoding pectinolytic enzymes were found in the genome.

KEYWORDS: phytopathogen, pectinolytic enzymes, Enterobacterales

ANNOUNCEMENT

Bacterial soft rot is caused by pathogens that can degrade pectin a component of the cell wall of plant tissues, including crops such as sweet pepper (*Capsicum annuum*). While *Dickeya* and *Pectobacterium* are the most widely studied soft-rot bacterial pathogens, other genus species also cause soft-rot on plant organs, yet little information is available regarding these pathogens (1). Sweet peppers exhibiting symptoms of soft rot were collected from plants between three and four months of maturity in Oreamuno, Cartago, Costa Rica (9.86916667, -83.90333333). The disease symptoms observed on the pepper fruits and the details of the bacterial isolation process were reported by Cubero-Agüero and colleagues (2). The symptomatic tissue edges were excised and macerated in 10 mL of 0.85% NaCl solution. The resulting suspension was streaked onto MacConkey agar and incubated at 30 ºC for 24 hours. Subsequently different morphotypes were tested for pectinolytic activity on Crystal violet pectate medium (3). DNA was purified using Macherey-Nagel™ NucleoSpin™ Microbial DNA extraction kit according to the manufacturer's instructions. The isolate with the highest pectolytic activity was identified by polymerase chain reaction (PCR) amplification of the region 16S rRNA, using the universal primer set of 27F/907R (4). Strain Ech2A shown 98.13% similarity to *Raoultella terrigena* JCM1687 (type strain) (5), as described in (2). To verify the pathogenicity, Ech2A was cultivated on nutrient agar (OXOID) at 30 °C for 24 hours. Aditionally, Koch’s postulates were tested by injecting 50 µL of bacterial suspension (9 × 108 CFU/mL) into three different sections of healthy pepper fruits, which were then incubated at 30°C for 5 days, until soft rot symptoms were observed. Sterile distilled water was used as negative control (2).

For genomic analysis, total DNA was processed by using a NexteraXT DNALibrary PrepKit and MiSeq paired-end sequencing (MiSeq Reagent Kit v2, 2×250 bp Illumina, Inc) in the Center for Research in Cellular and Molecular Biology (CIBCM) of the Universidad de Costa Rica. Bioinformatic analyses were performed using the Kabré Supercomputer from National High Technology Center (CeNAT), Costa Rica. Default

parameters were used for all software unless otherwise specified. The sequenced reads were filtered using Trimmomatic v0.36 (6), SLIDINGWINDOW:4:15 and MINLEN of 100 bp, resulting in 426,968 reads. De novo genome assembly was performed using SPAdes v3.11.0 (7). Prokka v.1.12 (8) was used to automatically generate annotations. The publicly accessible genome at Genbank was annotated using NCBI's Prokaryotic Genome Annotation Pipeline (PGAP) (9). To assess the average nucleotide identity (ANI), we employed ANI calculator to comparefor genomes (10). To estimate the completeness and redundancy we used CheckM (11). The draft genome of *Raoultella terrigena* strain Ech2A into 452 contigs totaling 5,504,929 bp, with an N50 value of 23,847, GC content of 58% and genome coverage of 10X. The genome contains 5,229, 70 tRNA and 187 coding and non-coding sequences respectively. The completeness is 98.9% and the redundancy 0.83%. The genomic similarity between *Raoultella terrigena* NBRC 14941 and Ech2A is 97.10%. The following genes were found in the prokka annotation: endo-polygalacturonase (*pehA*), and exo-pectate lyase (*pelX*) genes were found using prokka annotation, which are involved in the degradation of pectin and present in bacterial plant pathogens as *Pectobacterium* genus (12).

Data availability

Amplified product was sequenced and deposited in NCBI (GenBank accession MN386196). The genome sequence of Raoultella terrigena strain Ech2A was deposited in DDBJ/ENA/GenBank under accession number GCA_030078455.1, respectively, and BioProject accession number PRJNA898399. The raw data are available under the SRA accession number SRR29857959.

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