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Draft Genome Sequence of the Agarase-Producing *Sphingomonas* sp. MCT13

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INTRODUCTION

The genus *Sphingomonas*, originally proposed by Yabuuchi et al., was subsequently amended and is now subdivided into four genera: *Sphingomonas sensu stricto*, *Sphingobium*, *Novosphingobium,* and *Sphingopyxis* (Yabuuchi et al., 1990; Takeuchi et al., 2001). Sphingomonads have gained particular attention for their unique abilities to degrade a variety of compounds, including pollutants produced by industrial processes, polycyclic aromatic hydrocarbons (PAH) and dibenzofurans, and other toxic chemicals such as insecticides, herbicides, and heavy metals (Leys et al., 2004). Due to these features, sphingomonads are of great interest for bioremediation purposes, and have been previously exploited for the decontamination of groundwater systems from chemical pollutants of anthropogenic nature such as herbicides (Samuelsen et al., 2017), pentachlorophenol (Yang and Lee, 2008), and heavy metals (Vílchez et al., 2007). Agarose is a neutral linear polymer constituting the main fraction of agar produced by red macroalgae (Kim et al., 2016), which is widely used as a starting compound for the production of agar-derived oligosaccharides at industrial level (Yun et al., 2016). Enzymatic saccharification of agarose has a number of remarkable advantages over traditional acid degradation methods, such as the obtainment of well-defined chemical products, lower environmental pollution, and low energy costs (Fu and Kim, 2010).

Sphingomonas sp. MCT13 was isolated from a water sample collected in April 2002 at an artificial basin at the Experimental Ecology and Acquaculture Laboratories (Tor Vergata, Rome, Italy). The strain was able to form evident pitting on agar plates, suggesting the ability to degrade agar by the production of agarase activity, a feature that has not been previously reported for sphingomonads, and has till now been reported only twice in α-Proteobacteria isolated from marine environments (Hosoda and Sakai, 2006; Kang and Lee, 2009). Here, we report on the draft genome sequence of the putative novel species *Sphingomonas* sp. MCT13, providing also the results of preliminary bioinformatic analyses that suggest gene candidates of potential interest in either bioremediation or for industrial applications in the field of complex carbohydrates degradation.

MATERIALS AND METHODS

Isolation of *Sphigomonas* sp. MCT13

A water sample (1.2 L) was collected from a drainage ditch within a disused system of constructed wetlands. The ditch flows through uncultivated land, within the Experimental Ecology and Aquaculture Laboratories area of the University of Rome Tor Vergata, at the south eastern outskirts of the town. Aliquots (10 μL each) of this water sample have been plated on Tryptic-Soy, ZoBell,

and water agar plates and incubated at room temperature until the appearance of colonies. An isolated colony grown on the ZoBell plate was picked up, identified at the genus level by 16S rDNA sequencing as *Sphingomonas* sp., and subjected to further analysis.

DNA Extraction and Sequencing

Sphigomonas sp. MCT13 was grown overnight at 35°C in Tryptic-Soy agar plates. A single colony was inoculated in LB broth and grown overnight at 35°C. Bacterial DNA was extracted using the phenol–chloroform method (Sambrook and Russell, 2001) and then subjected to whole-genome sequencing with a MiSeq platform (Illumina Inc., San Diego, CA), using a 2 × 250 paired-end approach.

Genome Assembly and Annotation

De novo assembly was performed by using SPAdes 3.8 software (Bankevich et al., 2012) using default parameters. Scaffolds characterized by a length ≤200 bp were removed. The quality of genome assembly was checked by read mapping performed with SAMtools (Li et al., 2009) and by BLASTn comparison with genomes of other members of the *Sphingomonadaceae* family to assess the collinearity of selected gene clusters. Automated annotation of the draft genome sequence has then been performed with NCBI Prokaryotic Genome Annotation Pipeline (PGAP) web-service available at NCBI (http://www.ncbi.nlm.nih.gov/genome/annotation_prok).

Bioinformatic Analysis

Analysis of the 16S rDNA sequences were performed as previously described by using a custom database of reference 16S rDNA sequences (Leys et al., 2004). Phylogenetic relationships were assessed by using the ANIb method implemented in the JSpecies software V1.2.1 (Richter and Rossello-Mora, 2009). Detection of acquired antimicrobial resistance genes was carried out with ResFinder V2.1 (Zankari et al., 2012), while plasmid replicons were searched by using PlasmidFinder (Carattoli et al., 2014). The presence of prophages was investigated with the PHAST web-service (Zhou et al., 2011).

RESULTS

In total, 1,787,804 reads were obtained and assembled into 102 scaffolds (>200 bp in size) having a total length of 4,108,924 bp and characterized by a N_{50} of 147,599 bp and an L_{50} of 11. Genome raw coverage was ≈215X. The average GC content was 65.3%. A total of 3,851 CDS, 48 tRNAs, and 3 complete rRNAs were annotated by the PGAP web-service. Phylogenetic analysis performed as previously described (Leys et al., 2004) using a set of 40 reference 16S rDNA sequences from *Sphingomonas* sp., revealed that *Sphingomonas* sp. MCT13 shows the highest identity (98%) with *Sphingomonas* sp. B101/7, representative of a putative new species.

Analysis of the whole genome of *Sphingomonas* sp. MCT13 in comparison with those of other *Sphingomonas* sp. deposited in the INSDC databases, by using the ANI (Average Nucleotide Identity) method, showed that *Sphingomonas koreensis* NBRC 16723 is the closest homolog, with an ANIb value of 78.3. This result supports the hypothesis that *Sphingomonas* sp. MCT13 belongs to a novel species, given the fact that cut-off values of ANIb for species delineation are <95% (Goris et al., 2007).

ResFinder revealed the absence in *Sphingomonas* sp. MCT13 of any acquired resistance gene, while PlasmidFinder did not detect the presence of known plasmid replicon type. PHAST revealed the presence of two incomplete and one putative prophages. Detailed analysis of the PGAP annotation of the MCT13 draft genome revealed the presence of three hypothetical agarase-encoding genes (Accession numbers: ODP38961.1, ODP36587.1, and ODP36570.1), coding for enzymes displaying 60% identity with a hypothetical agarase from *Cellvibrio* sp. BR, 48% identity with a hypothetical beta-agarase from *Pseudoalteromonas* sp. BSi20429, and 53% identity with an hypothetical agarase from *Gilvimarinus agarilyticus* (Lee et al., 2015), respectively. Interestingly, ODP38961.1 and ODP36570.1 displayed also 48 and 53% protein identity, respectively, with a previously characterized exo-beta-agarase (Accession number 4BQ2_A) from the marine bacterium *Saccharophagus degradans* (Pluvinage et al., 2013), while ODP36587.1 displayed also a 31% identity with a beta-porphyranase identified in the gut bacterium *Bacteroides plebeius* (Hehemann et al., 2012). Further work is currently ongoing to characterize these enzymes. Results of annotation from PGAP revealed also the presence in *Sphingomonas* sp. MCT13 of several gene clusters putatively associated to the degradation of PAH. In particular the presence of genes encoding homologs of *fldZAB*/*fldYXWVUT* and *flnA1A2* gene products of *Sphingomonas* sp. LB126 (Accession No. AJ277295.1 and EU024110.1, respectively; Wattiau et al., 2001; Schuler et al., 2008), suggests the ability of *Sphingomonas* sp. MCT13 to degrade fluorene.

The capability of *Sphingomonas* sp. MCT13 to degrade agar and possibly toxic PAH, together with the lack of any known antibiotic resistance gene, make this strain potentially interesting for industrial applications in the field of complex carbohydrates degradation or for bioremediation purposes.

The complete genome sequence of *Sphingomonas* sp. MCT13 was deposited at DDBJ/EMBL/GenBank databases under the accession no. MDDS00000000. The version described in this paper is the version MDDS01000000. Read data were deposited in the NCBI SRA database under BioProject ID PRJNA338394 (experiment SRR5139223).

AUTHOR CONTRIBUTIONS

NC performed DNA extraction. DNA sequencing has been performed by VDP. Data analysis has been performed by MD, NC, and MT. MD, GR, and MT contributed to the writing and the editing of manuscript.

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