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Draft genome sequence of Acinetobacter sp. neg1 capable of degrading ochratoxin A

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ABSTRACT

Ochratoxin A (OTA) is a nephrotoxic and potentially carcinogenic mycotoxin produced by several species of Aspergillus and Penicillium. It is one of the major mycotoxins contaminating grain, grapes and a variety of food products, and the development of methods for reducing pre- and post-harvest contamination has drawn considerable attention. In the current study, we isolated and sequenced the genome of a novel free-living Acinetobacter strain able to degrade OTA. Biochemical studies suggest that the degradation reaction proceeds via peptide bond hydrolysis.

Keywords: ochratoxin A; degradation; Acinetobacter

INTRODUCTION

The genus Acinetobacter was proposed by Brisou and Prevot (1954) and to date comprises 34 species (http://www.bacterio.net/acinetobacter.html) of Gram-negative, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase positive, oxidase-negative organisms with a G+C content of 38–47% (Chan et al., 2012). The genus Acinetobacter belongs to the gamma subgroup of Proteobacteria, and is widespread in nature, being isolated from soil and water. Some species are also recognized as significant pathogens in hospital environment (Juni 1978; Munoz-Price and Weinstein 2008). To date, the only species of Acinetobacter known to be capable of degrading OTA is A. calcoaceticus (Hwang and Draughon 1994). Distinct from the OTA-degrading A. calcoaceticus strain, which was isolated from poultry carcasses (Thornley 1960), Acinetobacter sp. neg1 was isolated from soil in vineyards contaminated...
by high level of OTA (Somma, Perrone and Logrieco 2012), suggesting possible applications of this species in mycotoxin biodegradation.

METHODS AND RESULTS

Preliminary analyses suggested that a bacterial strain isolated in October 2013 from a soil sample in a Negroamaro vineyard in Cellino S. Marco (BR, Italy)—during a study of microbial biodiversity in vineyards reported as contaminated by high levels of OTA (Somma, Perrone and Logrieco 2012)—was capable of degrading OTA. The 16S rRNA gene sequence of the strain showed a closest match (98.6%) to Acinetobacter gyllenbergii. The strain was assigned the name Acinetobacter sp. neg1 and entered in the ISPA ITEM Culture Collection (http://server.ispa.cnr.it/ITEM/Collection) under the accession ITEM 17016.

The capacity of Acinetobacter sp. neg1 to degrade OTA was confirmed by growth in MMP medium (2.5 g l⁻¹ K₂HPO₄, 1.0 g l⁻¹ (NH₄)₂HPO₄, 0.2 g l⁻¹ MgSO₄·7H₂O, 0.5% Bacto Peptone) supplemented with OTA (1 μg ml⁻¹) at 28°C and 120 rpm. ITEM 17016 was able to degrade more than 70% of OTA in 144 h and comparable levels of the catabolic product O'Talpaha (O'Ta) were detected (fig. 1), suggesting that degradation proceeds via hydrolysis of the amide bond that links the L-β-phenylalanine moiety to the O'Ta moiety. Since O'Ta and L-β-phenylalanine are not toxic, this mechanism can be considered to be a true detoxification pathway, distinct from the adsorption of OTA to cells walls which is the predominant removing mechanism reported for lactic acid bacteria and yeast (Abrunhosa, Paterson and Venancio 2010).

DNA was extracted from a single colony of Acinetobacter sp. neg1 and subjected to whole-genome shotgun sequencing using the Nextera XT library preparation workflow (Illumina, San Diego, CA, USA). 2 x 250-nucleotide paired-end reads were generated on an Illumina MiSeq instrument and quality trimmed using the sliding window mode of the Trimmomatic (Bolger, Lohse and Usadel 2014) program, with a cut-off value of 20. De novo genome assembly was performed using SPAdes v 3.11 (Nurk et al., 2013), and scaffolding using the SSPACE program (Boetzer et al., 2011).

The draft genome sequence is 3.92 Mbp in length (c. 100x coverage, 42.2% G+C) and consists of 28 contigs (27 scaffolds). The overall continuity of the assembly is good, with a N50 of 298 Kbp—the longest assembled contig is 1.22 Mb in length. Genome annotation was performed using xbase (Chaudhuri et al., 2008) using A. gyllenbergii CIP 110306 (ATGG00000000.1) as a reference, resulting in the prediction of 3521 protein-coding genes and 183 tRNAs. Consideration of contig coverage suggests the presence of six to seven copies of the ribosomal operon.

1436 clusters of orthologous genes identified by best reciprocal BLASTP searches of inferred protein sequences were represented in all sequenced Acinetobacter genomes. Protein sequence for each COG were aligned independently with MUSCLE (Edgar 2004) and unambiguously aligned regions were selected with GBLOCKS (Castresana 2000). The ProtTest software (Darriba et al., 2011) selected the WAG substitution model (Whelan and Goldman 2001) with invariable and four gamma-distributed site rate categories as the best fit to the concatenated alignment. Maximum likelihood (ML) bootstrap analysis using PhyML (Guindon et al., 2010) recovered a well-supported tree topology, consistent with a recent phylogenomic analysis of the genus Acinetobacter (Toucheon et al., 2014) and which places Acinetobacter sp. neg1 as sister to A. gyllenbergii, (fig. 2). Pairwise nucleotide identity of BLASTN-aligned genomic regions (ANIb) scores (Richter and Rossello-Mora 2009) for Acinetobacter sp. neg1 were ≤86% with A. gyllenbergii and other Acinetobacter isolates suggesting that Acinetobacter sp. neg1 represents a novel species.

For 173 predicted genes, reciprocal best BLASTP analyses recovered no orthologs in the genomes of recognized Acinetobacter species, 115 of these gave significant matches against the NCBI non-redundant protein database. 66% of these matches were within Acinetobacter (suggesting that these genes result from recent gene duplications), 33% of genes giving best matches outside Acinetobacter (candidates for acquisition by recent lateral transfer) showed highest identity to Pseudomonas or Pseudoalteromonas homologs.

Several enzyme activities have been shown to be capable of degrading OTA to produce O'Ta and phenylalanine. Acinetobacter sp. neg1 lacks homologs of recently characterized ochratoxinase from Aspergillus niger (Dobritzsch et al., 2014). Several
carboxypeptidase activities have also been shown to be capable of degrading OTA in this way (Stander et al., 2001). Numerous potential carboxypeptidase-encoding genes are present in the genome of Acinetobacter sp. neg1, many of these have likely orthology with genes in at least several other Acinetobacter species. However, in the absence of a comprehensive evaluation of the capacity of members of the genus to degrade OTA, and given that the A. calcoaceticus strain shown to degrade OTA (NRRL B-1596) has apparently not been subjected to whole-genome sequencing, the identification of candidate genes through comparative genomic approaches remains difficult.

These considerations notwithstanding, we note the presence, in the genome of Acinetobacter sp. neg1, of a cluster of genes (PJ15_0898 to PJ15_0903) which include a β-metallo-lactamase, a major facilitator superfamily gene an ATPase and a reverse transcriptase. This cluster, within the genus Acinetobacter at least, is shared with only a limited number of A. baumannii and A. calcoaceticus genomes.

The Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank as Acinetobacter sp. strain neg1 under the accession JSZD00000000. The version described in this paper is version JSZD01000000.

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Conflict of interest. None declared.

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