TITLE: Identification of a plasmidial gene related to expression of an antimicrobial substance active against multidrug-resistant *Acinetobacter baumannii* clinical isolates

AUTHORS: Guimarães, N. R.[1]; Vieira, L. S.[1]; Batista, T. M. [2]; Tagliaferri, T. L.[1,3]; Santos, S. G.[1]; Farias, L. M.[1]; Magalhães, P. P.[1]

INSTITUTION: [1]Laboratório de Microbiologia Oral e Anaeróbios, Departamento de Microbiologia, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, MG, Brasil. [2]Centro de Formação em Ciências Ambientais, Universidade Federal do Sul da Bahia. [3]Institute of Medical Microbiology, RWTH, Aachen University, Germany. E-mail: natyroguiman@yahoo.com.br

ABSTRACT:
Acinetobacter baumannii, included in the ESKAPE group, is a bacterium of unquestionable clinical relevance. Recently, the World Health Organization has published the "Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics". This list identifies the most important antimicrobial resistant bacteria at a global level for which there is an urgent need for development of new treatments. Among them, *A. baumannii* resistant to carbapenems holds the first place in the group defined as "Priority 1: critical". Bearing in mind the urgency in finding new antimicrobial molecules, this study aimed at the identification of a plasmid gene that codes for a new antimicrobial substance using NGS and cloning. For plasmid sequencing, DNA was extracted, quantified, and its integrity was evaluated by gel electrophoresis. Paired-end libraries were constructed with Nextera XT DNA Library Preparation Kit. Generated reads with a mean length of 301 bp were sequenced using the Illumina MiSeq. The *de novo* assembly was performed employing the software SPAdes. Sequence similarity was assessed using the Blastx tool. The ORFs encoding proteins of unknown function were selected. The sequences were amplified by PCR and ligated into TOPO vector. The insert was cleaved, subcloned into the *NdeI* and *XhoI* sites of pET28a (TEV) vector, generating pET28a (TEV)+ORFs. Gene insertion was confirmed by colony PCR using T7 primers. The recombinant proteins were expressed in *E. coli* BL21 (DE3) induced with 1M IPTG at an OD_{600} of approximately 0.8, for 4 h. The antimicrobial activity of the transformed colonies was evaluated by the double-layer diffusion method. The bacterial cells were harvested by centrifugation followed by ten cycles of 40 s on/off of sonication in an ice-water bath. The antibacterial activity of the intracellular and extracellular fractions was checked using the spread plate technique. The assembly of the plasmid DNA generated a contig of 11,060 bp, composed of twelve ORFs. The sequence similarity analysis demonstrated that the small plasmid should be included in the Rep-3 superfamily, according to a RepB replication protein. Moreover, the presence of three ORFs encoding proteins of unknown function was observed. The expression data and the antibacterial activity allowed the identification of the antimicrobial gene at ORF 3. The structural and functional characterization of the substance is the next step of this study.

Keywords: *Acinetobacter baumannii*, antimicrobial peptides, bacterial resistance, sequencing and cloning.
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