In silico comparative analysis of antibiotics RND-efflux pumps in Burkholderia genus

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Motivation

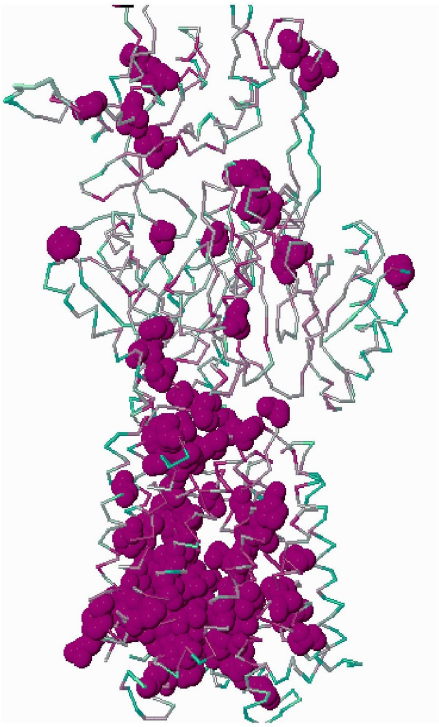
The genus Burkholderia includes a variety of species inhabiting different ecological niches, with strains able to promote plant growth and/or to degrade pollutants, as well as opportunistic human pathogen, such as representatives of the so-called B. cepacia complex (Bcc). Burkholderia human infections are usually treated with antibiotics in order to improve disease control and patient survivance. The increasing global resistance to antibiotics has become a public health problem. In this context a major role is played by multidrug efflux pumps that allow bacterial cells to extrude a wide range of different substrate, including antibiotics. This class of proteins includes a very interesting family, the RND (Resistance-Nodulation-Cell Division) family that is found ubiquitously in Bacteria, Archaea and Eukaryotes, being mainly involved in drug resistance of Gram-negative bacteria. In these microorganisms RND pumps can capture various structurally unrelated substrates from the periplasm and/or from the cytoplasm and push them out directly into the external media using proton-motive force. Although these proteins have been deeply analyzed from an experimental viewpoint in organisms belonging to other genera (mainly .-proteobacteria), very little is known in the genus Burkholderia ; indeed, only few works about the B. pseudomallei and B. cenocepacia RND proteins have been published. Hence, given their importance a large scale bioinformatic analysis was performed aiming to provide a deeper understanding of RND proteins in this genus, including their phylogenetic relationships and their specificity prediction (based on the conservation of key residues whose importance has been experimentally validated).

Methods

All the 21 available Burkholderia spp. genomes were retrieved from NCBI and preliminary analyses were conduced using ad hoc written Perl codes. Multiple sequence alignments and phylogenetic tests were performed using Muscle and PhyML packages, respectevely. Residues conservation on protein structure was determined using the Consurf web server (consurf.tau.ac.il).

Results

BLAST probing of the 21 Burkholderia sequenced genomes, using experimentally characterized ceoB sequences (one of the RND family counterpart in the genus Burkholderia) as seeds, allowed the assembly of a dataset embedding 254 RND proteins. An extensive phylogenetic analysis of these sequences revealed the occurrence of several independent events of gene loss and duplication across the different lineages of the genus Burkholderia. We found notable differences in the number of ceoB-paralogs between genome, ranging from 6 (in B. mallei NCTC 10247, NCTC10229 and SAVP1) to 18 (in B. cenocepacia HI2424 and MC0-3). This finding prompted us to investigate the correlation between the copy-number of RND related proteins and some phenotypic traits of bacteria possessing them. Interestingly, we found no correlation between the ecological environment, the lifestyle or the genome size of Burkholderia strains with the number of efflux pumps they possessed, suggesting that other evolutionary forces might have been responsible for the patchy phylogenetic distribution and copy number pattern of this protein family. Useful hints on this issue might be obtained from the study of the substrate specificity of each of the multiple ceoB copies retrieved in the Burkholderia genomes. Thus, in order to better define and predict the specificity of each retrieved sequence, a comparison of structure and genetic data coming from experimentally characterized homologous sequences was performed (see Figure). This analysis revealed the presence of several key-residues that are probably responsible for substrate recognition and expulsion of toxic compounds from the cell. This finding, in turn, may serve as a basis for generating hypotheses on the biological role of each retrieved efflux pump sequence (antibiotic and/or heavy metal resistance) and may lead to future experimental tests.



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