RESISTANCE TO TOXIC COMPOUNDS IN METAGENOMIC FOSMID LIBRARY FROM MANGROVE SEDIMENTS IN SÃO PAULO STATE, BRAZIL.
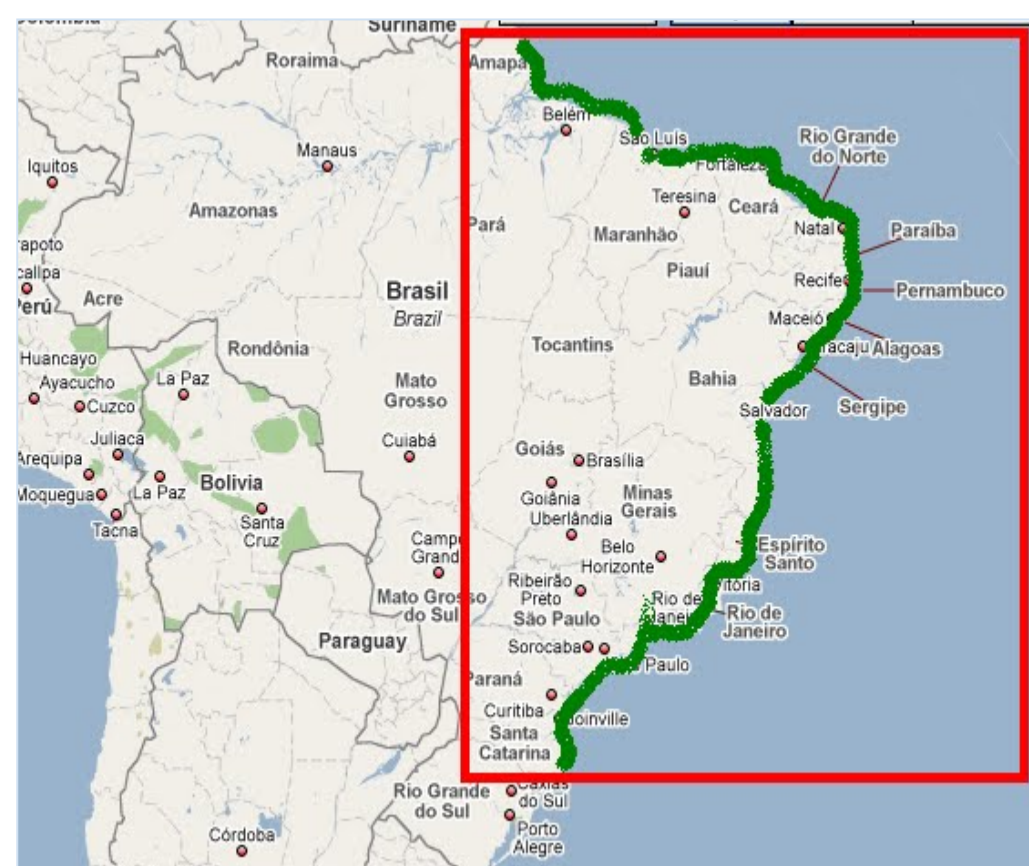
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Introduction

Brazil holds one of the largest mangrove extensions in the world, an ecosystem rich in biodiversity, including aquatic animals, birds, reptiles, mammals and microorganisms. In Brazil, mangroves are areas of permanent environmental protection. Despite of this, mangroves have been highly exposed to anthropic activities, including oil spills and industrial wastes that carry heavy metals (Figure 1).

http://wwwmanguemeioambiente.blogspot.com
http://www.nature-education.org/water-pollution.html

Figure 1. The mangrove extension in Brazilian coast **(a)**; Pollution source of mangroves in the world **(b)**.

Microorganisms have developed mechanisms to adapt to the presence of contaminants (Silver and Phung 2005). The biochemical diversity and adaptability of microorganisms allow biodegradation or transformation of xenobiotics via the production of enzymes (Gadd 2004). Detoxification of heavy metals also includes mechanisms such as biosorption, bioleaching, bioaccumulation and biomineralization and production of a variety of products that can be used in traditional and biotechnological manipulations (Bhattacharya et al. 2006).

Material and Methods

Fosmid library (13,960 clones):
◇ Sampling of sediments in Bertioga Mangrove;
◇ Total DNA extraction (Groβkopf et al., 1998 and Neria-González et al., 2006, with modifications);
◇ Pulsed-field gel electrophoresis for DNA size selection;
◇ Metagenomic library construction - CopyControl™ HTP Fosmid Library Production Kit (Epicentre);
◇ 454 sequencing;

Data processing
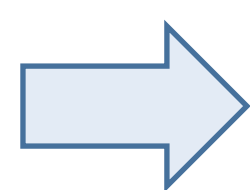
MG-RAST: Functional classification was conducted using MG RAST version 3.3.9, sequences associated to antibiotics resistance and toxic compounds were analyzed following the parameters: 1) Annotation sources = Subsystems (Max. e-value cutoff $1e^{-5}$, Min. % Identity cutoff 60%, Min. alignment length cutoff 15); 2) Level 1 = Virulence, disease and defense; 3) Level 2 = Resistance to antibiotics and toxic compounds; 4) Function; 5 e 6) Abundance and #Hits.

Results

✓ The data were normalized in MG- RAST and almost 0.7% of the data are involved with Virulence, Disease and Defense (Figure 2).
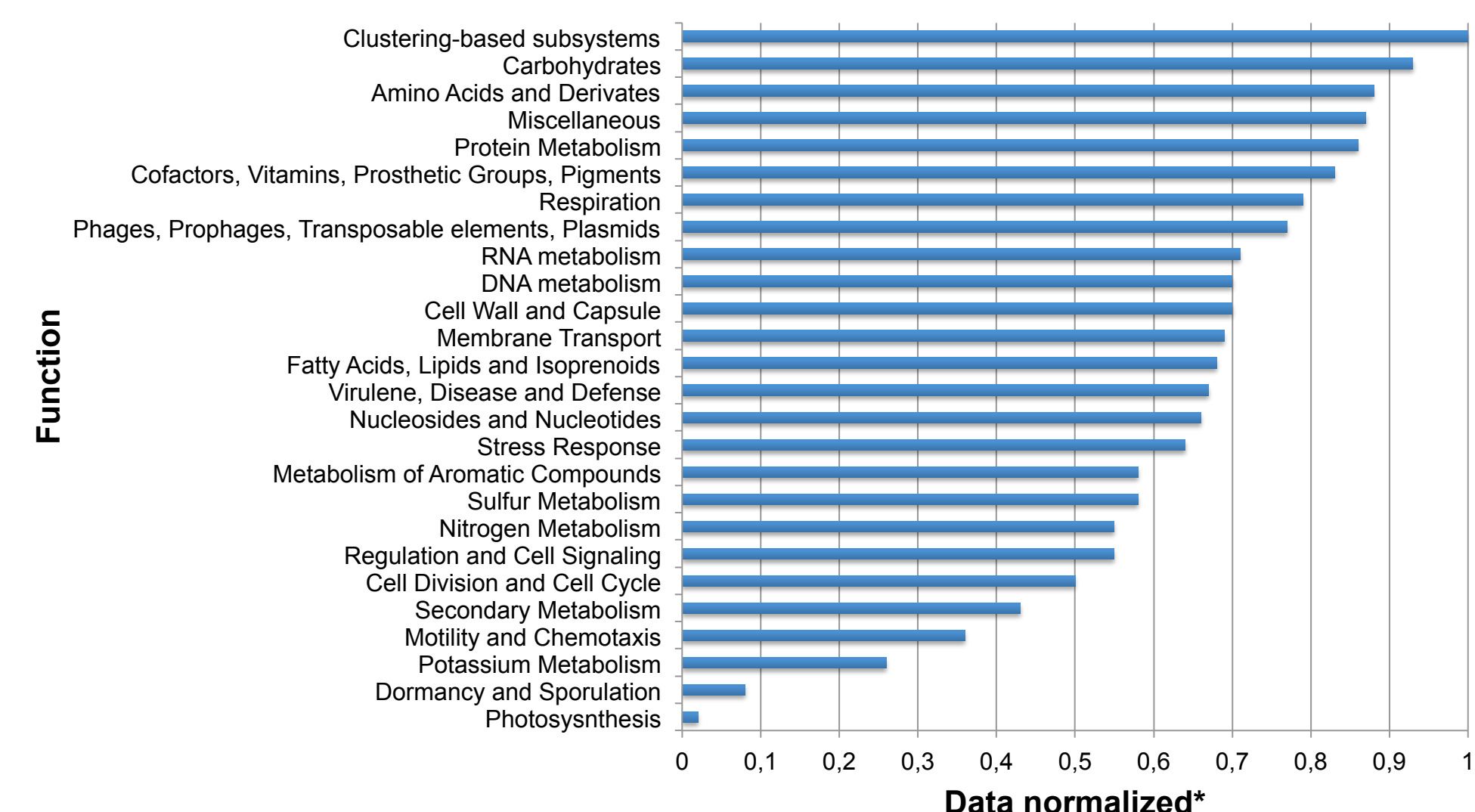

Figure 2. Function analysis was performed in MG-RAST V3.3.7.3. * The displayed data have been normalized to values between 0 and 1 to allow for comparison of differently sized samples.

✓ The most abundant sequences involved in metal resistance in the dataset were cobalt-zinc-cadmium resistance detected by the presence of Cobalt-zinc-cadmium resistance protein and Cobalt-zinc-cadmium resistance protein CzcA (489 and 346 hits, respectively). Sequences related with copper and silver resistance were detected by the presence of cation efflux system protein CusA (330 hits). Also, sequences related with resistance of dangerous compounds (arsenate and mercury) were detected in the dataset by the presence of Arsenate reductase (EC 1.20.4.1), Arsenical-resistance protein ACR3, and mercuric ion reductase (EC 1.16.1.1) (115, 98 and 42 hits, respectively) (Figure 3).
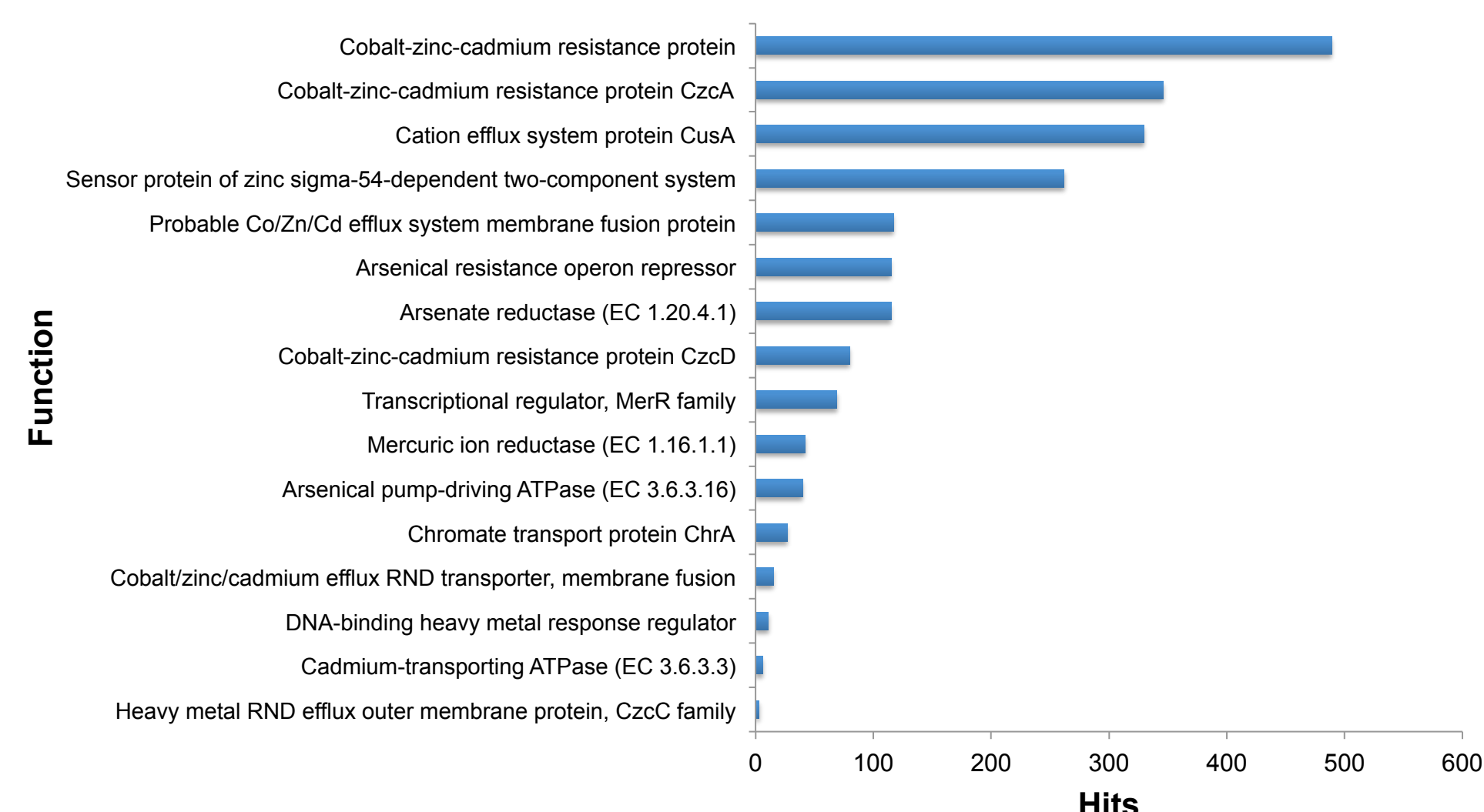

Figure 3. Resistance to toxic compounds. Level 3 Subsystems were used for annotation in MG-RAST V3.3.7.3.

Further Analysis

✓ Functional screening of the fosmid library was performed and the positive clones were selected for further studies on metal tolerance and degradation.
✓ Structural characterization of operons and bioinformatics analysis are currently being undertaken.

References

- Silver S, Phung L T (2005) A bacterial view of the Periodic Table: Genes and proteins for toxic inorganic ions. J Indust Microbiol Biotechnol 32:587-605
- Gadd G M (2004) Microbial influence on meal mobility and application for bioremediation. Geoderma, 122:109-119.
- Bhattacharya J, Islam M, Cheong Y (2006). Microbial growth and action: Implications for passive bioremediation of acid mine drainage. Mine Water Env 25: 233-240
- Groβkopf, R., Stubner, S., Liesack, W. (1998) Novel Euryarchaeita lineages detected on rice roots and in the anoxic bulk soil of flooded rice microcosms. Appl Environ Microbiol 64: 960-969.
- Meyer, F., Paarmann, D, D'Souza, M., Olson, R., Glass, E.M., et al. (2008) The Metagenomics RAST server - A public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics, 9: 386.