

Title: The environmental diversity of antibiotic resistance genes indicated by the resistance screening of two soil metagenomic libraries – impact on microbial ecology and bioremediation

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Abstract:

The environmental diversity of antibiotic resistance genes (ARGs) is under evaluation and is still an unknown universe, whereas the research on clinical ARGs has been a priority. Hence, accessing the collection of ARGs from natural environments may identify new genes and mechanisms for antibiotic resistance outside the clinical setting. Metagenomics rise as a potential tool, since the majority of microorganisms in environmental samples, such as soil, are not yet cultivable and, as a consequence, are still unknown. In the screening of two Brazilian Cerrado soil metagenomic libraries with beta-lactamic antibiotics, 62 resistant clones were isolated and some selected for sequencing and phenotypic analysis. Ten clones, from the small (~8Kb) and large-insert libraries (~35 Kb), comprised a 25 Mb dataset that revealed an unexpected diversity of ORFs not yet related to antibiotic resistance, such as dioxygenases. These enzymes act in the disruption of aromatic rings, producing substrates for energy producing cycles. They appear once, in average, in the metagenomic resistant clones. This finding stimulated the construction of a subclone containing a putative dioxygenase ORF for further phenotypic assays. These showed not only the role of this specific enzyme on both antibiotic and aromatic resistance, but also were initially characterized as the first metagenomic gentisate 1,2-dioxygenase. Moreover, no betalactamases were identified, which shows that the classical resistance mechanism for beta-lactamic antibiotics in clinical isolates may not follow the same pattern in the environment. These findings are a strong evidence that the environmental ARG diversity is much broader than it appears and that accessing these uncharted genes may reveal new mechanisms of antibiotic resistance, improve the study on microbial ecology and aid the research on the biotechnology industry, such as in bioremediation.

Key-words: antibiotic resistance, metagenome, dioxygenase, soil

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