TITLE: PROSPECTION AND DRAFT GENOME OF AROMATIC DEGRADING BACTERIA IN DRIP WATER SAMPLES FROM CAVES

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ABSTRACT:

Drip water in caves is a unique source of nutrients to microbes. However, few organic compounds are not totally used by surface organisms and can be transported by percolating water. Aromatic compounds are one of these molecules that can be transported by percolation water and reach the cave as drip water. However, no study isolated potential aromatic degradation bacteria from drip water. The present study was carried out to isolate, identify, and analyze the draft genome of a bacterium isolated from cave drip water. Drip water samples were collected in two caves in Paripiranga, Bahia (Gruta do Bom Pastor, and Furna do Fim do Morro do Parafuso). Samples were plated in Petri dishes containing minimal medium agar supplemented with 10 mM aromatic compound. Three aromatic compounds were used separated: phenol, naphthalene, and toluene. Plates were incubated for 7 days at 28°C. Isolated colonies were also tested the ability to grow in all aromatic sources. Colonies were isolated and identified by sequencing the 16S rDNA. A PCR with (GTG)₅ primer followed by agarose gel electrophoresis was performed with identified isolates. The genome of one of the isolates was sequenced in Illumina Miseq. Sequences were analyzed in MaSuRCA follow by RAST. Among the 6 bacterial morphotypes that grown in each media, three was selected because they grow in all aromatic compounds tested. All three were identified as Acinetobacter nosocomialis. A (GTG)₅ molecular typing was performed, and distinct profiles were observed for each isolated. One of then was selected for genome sequencing and a draft genome with 378 contigs that we were able to identify genes related with several aromatic compounds including toluene tolerance, phenol hydroxylase genes, a general aromatic-ring and ortho-cleavage of catechol (catechol 1,2-dioxygenase). Several mobile elements and metal resistance genes were identified. Some of those genes, such as the phenol hydroxylase is not commonly related to Acinetobacter nosocomialis as most of those genes are commonly identified in A. calcoaceticus. The findings support the idea that those bacteria can survive in the drip water environment in caves using aromatic compounds as energy or carbon source.

Keywords: Acinetobacter, aromatic degradation, phenol

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