

TITLE: FERMENTATION OF GLYCEROL INTO ACETOIN BY BACTERIAL SPECIES ISOLATED FROM BRAZILIAN COLD SEEPS

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ABSTRACT:

More than twenty thousand natural products have been identified from marine environments, but less than 2% derive from deep-sea regions. In recent years, the deep-sea has been considered a hot spot for the discovery of new molecules in places such as hydrothermal vents and cold seeps, which harbor rich and poorly studied microbial chemosynthetic communities. These islands of biodiversity strongly contrast with the less biodiverse surrounding environments, receiving attention of the scientific community in the search for biological novelty. Between 2011-2013, four oceanographic cruises from the CONEGAS project (PETROBRAS-PUCRS) identified cold seeps associated with gas hydrate occurrences in the Rio Grande Cone (Brazilian western South Atlantic). During these cruises, a piston corer was used to collect sediment within the gas hydrate province at water depths ranging from 400 to 2000 meters. Sediment samples were appropriately collected and submitted to microbial isolation procedures. With the purpose of identifying valuable molecules synthesized by 100 obtained isolates, the Voges-Proskauer test was used to screen for acetoin producing microorganisms. Acetoin is a valuable (US\$41.0/Kg) fermentation product with many applications such as: flavor enhancer, precursor in the synthesis of pyrazines, amino nitriles and 4,5-dimethylimidazole, chelating agent and others. Glycerol is a low value (US\$0.33/Kg) by-product generated during biodiesel production. It is known that certain bacterial species are capable of fermenting glycerol into acetoin, turning a cheap residue into a profitable commodity. So far, ten isolates were grown over night at 28°C, under 100 RPM agitation, in 3 mL minimal media NMS containing 1% purified glycerol as sole carbon source. Three isolates were capable of reaching a high titer of acetoin during pure glycerol fermentation. Sanger sequencing the 16S rRNA gene fragment of the isolates allowed the taxonomic classification of two strains as *Enterobacter* sp. and one as *Pseudomonas* sp. The acetoin titers were determined by Voges-Proskauer test being 0.27 and 0.18 mM, for the two *Enterobacter* sp. isolates; and 0.14 mM for the *Pseudomonas* sp. Tests to evaluate growth and fermentation capacity of the isolates on crude glycerol are underway. These results indicate that cold seeps may hold new microbial species with great biotechnological potential.

Keywords: Glycerol, Acetoin, Cold seep, Deep-sea, *Pseudomonas* sp.

Development Agencies: PUCRS, PETROBRAS