

Título: CULTURE OF AMMONIA OXIDIZER ARCHAEA FROM A CERRADO STREAM SEDIMENT

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Resumo:

The classification of living organisms in three domains, proposed by Woese and collaborators in 1990, revealed the importance of the *Archaea* as widely distributed organisms, with important roles in many ecological processes including the nitrogen and carbon cycles. Results from our research group revealed that *Archaea* are abundant in soils and sediments of the Cerrado biome but one of the major difficulties in studying these mesophilic archaea consists in their cultivation in artificial media. This work describes the cultivation and characterization of mesophilic archaea from a stream sediment of the IBGE ecological reserve located in Brasília-DF. Samples of sediments, collected near the border of the stream, at 5 cm deep, were inoculated in solid and liquid media prepared with sterilized water collected from the stream. In order to enhance growth of ammonia oxidizers, ammonium chloride was added to the media. Antimicrobial agents were also added to prevent bacterial and fungal growth. The samples were incubated at 28°C and analyzed weekly for growth. The cultures in liquid media revealed an incipient growth, but on solid media, we were able to obtain different colony morphologies, which were submitted to DNA extraction procedures and subjected to PCR assays with primers directed to the 16S rRNA gene of *Archaea* and *Bacteria*, as well as the archaeal *amoA* gene. The amplicons were submitted to automatic DNA sequencing procedures, and the results revealed that all colonies consisted in co-cultures of *Archaea* and *Bacteria*. All archaeal members of these co-cultures were classified as ammonia oxidizing Thaumarchaeota, preliminarily affiliated to *Nitrosotalea* and *Nitrososphaera*, while the bacterial members were classified in four different genres of the Brucellaceae and Burkholderiaceae families, which are known for their resistance to antimicrobial agents. Optical microscopy analyses revealed small gram negative cells. Due to the size, it was not possible to distinguish different types of microorganisms. Scanning electronic microscopy showed bacillary shaped cells, of approximately 1 µm of diameter.

Palavras-chaves: Archaea, Cerrado, In-vitro culture, Thaumarchaeota

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