

TITLE: LACTIC ACID BACTERIA ALONG METASANDSTONE AND LIMESTONE CAVES OF CHAPADA DIAMANTINA, BRAZIL.

AUTHORS: MARQUES, E.L.S.; SILVA, A.B.C.; GROSS, E.; DIAS, J.C.T; REZENDE, R.P.

INSTITUTION: UNIVERSIDADE ESTADUAL DE SANTA CRUZ, ILHÉUS, BA (ROD. JORGE AMADO, KM 16, S/N, CEP 45.662-900, ILHÉUS – BA, BRAZIL)

ABSTRACT:

Lactic acid bacteria (LAB) are widespread microorganisms commonly found in food-related samples, especially carbohydrate, nitrogen, and mineral-rich environments. As an important microbial group for the food industry, it is rarely studied in environments non-related with this type of application, despite another biotechnological potential such as antimicrobial production. In that context, caves represent a unique environment, usually characterized by oligotrophy; however, a mineral-rich environment. We decided to evaluate the presence of LAB in two caves, a metasandstone (Gruta do Lapão) and a limestone cave (Gruta de Manoel Ioiô) from Chapada Diamantina. Samples were collected in the entrance of both caves and three samples along the cave at 75 (S1), 200 (S2), and 500m (S3) from the entrance. The DNA of each sample was extracted and amplified with Lac1/Lac2 primers and Lac3/Lac2 primers; each pair amplified different groups of LAB. The amplification was applied in agarose gel to evaluate the presence of bands. Following this, a semi-nested PCR was performed using GC-clamped primers for DGGE for both primers' pairs, separately. A DGGE analysis was performed. Bands were excised, re-amplified with non-GC-clamped primers and sequenced. Energy-Dispersive X-ray spectroscopy (EDX) analysis was performed for each sample in triplicate. The primers pairs Lac1/Lac2 do not amplify any samples in agarose gel or DGGE analysis (repeated 2x), but the *Lactobacillus* used as control amplified in both tests. It is plausible to infer that *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella* are not present in those cave samples. However, Lac3/Lac2 primers amplification was detected in one sample in agarose gel (limestone inner sample 3), and in DGGE analysis it was detected in all samples with faint bands in the entrance, S1 and S2 of limestone cave and S2 and S3 of metasandstone cave. Lac3/Lac2 primers amplified for *Lactococcus*, *Streptococcus*, *Enterococcus*, *Tetragenococcus*, and *Vagococcus* and all 5 sequenced bands were identified as *Streptococcus* with similarity ranged from 95 to 97% with GenBank database. The EDX analysis showed considerable high amount of Mg in the samples with highest diversity of LAB bands (limestone S2 and S3) and presence of others mineral-composing nutrients such as Ca, P, Fe and Mn in many samples. The present study showed that LAB can be found in the oligotrophic environment, such as caves and may represent potentially new lactic acid bacteria.

Keywords: DGGE, LAB, *Streptococcus*

Development Agency: Conselho Nacional de Pesquisa, Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior e Fundação de Amparo à Pesquisa do Estado da Bahia.