

**TITLE:** CULTURABLE BACTERIA AND ITS ENZYMATIC REPERTOIRE IN FROG FOAM NESTS

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**ABSTRACT:**

Frog foam nests are one of the various reproductive mode found in amphibians, in which some species deposit their eggs in stable foams. This curious biostructure is mainly composed of proteins and carbohydrates, which are responsible for their stability. Besides that, studies have also pointed out the presence of a microbial community in these biological foams. However, these microorganisms and the roles they perform are still unknown. In addition, the fact that there are very few studies concerning the foam nests, make them a valuable source for prospecting novel microorganisms with biotechnological potential. The aim of the current study was to screen for enzymatic activities from the bacterial isolates from the foam nests of *Leptodactylus vastus* and *Physalaemus curvieri*. The foam nests were collected in Monte Alegre Reserve, Pacatuba-CE, Brazil. For the viable count of *L. vastus* and *P. curvieri* nests, serial dilutions were performed in saline, with subsequent cultivation on ATGE plates at room temperature for 48 h and isolation of the different morphotypes. For enzymatic activities, isolates were transferred to ATGE supplemented with starch (0.1% w/v) to evaluate amylases production; with tributyrin (0.1% v/v) to verify lipase production; with skim milk (1% w/v) and gelatin (3% w/v), for the detection of proteases. The foam nests samples presented  $2.8 \times 10^7$  cfu/g and  $2.7 \times 10^7$  cfu/g for *P. curvieri* and *L. vastus*, respectively. The sample of water from which the *P. curvieri* nest was removed showed  $3.3 \times 10^4$  cfu/mL and the soil sample from which the *L. vastus* nest was removed presented  $1.4 \times 10^6$  cfu/g. Counts of foam nest bacteria at least 10 to 1000 times greater than the surrounding environment support its frogs' origin. Among the 66 different bacterial isolates from *L. vastus* foam nest, 25% were able to produce amylases, 36% caseinases, 44% gelatinases and 79% lipases. The nests of *P. curvieri* yielded 115 isolates in which 34.02% presented amylases, 35.05% caseinases, 37.2% gelatinases and 49% lipases. Our study starts to unravel the origin and number of culturable bacteria in frogs foam nests as well as its enzyme diversity. Further studies are in progress to identify the composition and function of the frogs' foam nest microbiome.

**Keywords:** Hydrolases activity, Amylases, Proteases, Lipases, anuran

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