

**TITLE:** PROTEASE AND AMYLASE ACTIVITIES FROM BACTERIAL ISOLATES FROM THE FOAM NEST OF THE FROG *Adenomera hylaedactyla*

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**ABSTRACT:**

Microbial biodiversity has been a major source of molecules with interest for biotechnological industries. For instance, amylases and proteases are hydrolases widely used in the food, cleaning and cosmetic industry. Their great request demands the search in underexplored environments as strategy to discover novel enzymes. Few studies have searched for enzymatic activities in anuran foam nests microbiota, making them valuable sources for the prospection of new biomolecules. Also, the findings so far have demonstrated that the foam nests are mainly composed by proteins and carbohydrates. Taking this into account, this study aims to access the microbial community associated with the foam nest of the frog *Adenomera hylaedactyla* and evaluate its potential for the production of proteases and amylases. The nests were collected in São Gonçalo do Amarante - CE, Brazil, during the rainy season with proper regard to Brazilian legislation. The colonization of the foam nests was evaluated by cell viable counting. Samples were serially diluted and plated in ATGE medium for 48 h at 30 °C. Simultaneously, samples from the soil surrounding the foam nests were also analyzed. Morphotypes were isolated, stained by the Gram procedure and screened for proteases and amylases. Bacterial isolates were cultured in ATGE supplemented with starch (0.1% w/v) for amylase activity and ATGE supplemented with skimmed milk (1% w/v) and ATGE supplemented with gelatin (3% w/v) for proteases. Activities were detected by the appearance of clear zones around the colonies after addition of a solution of lugol ( $I_2/KI$ ), for amylase, and a solution of  $(NH_4)_2SO_4$ , for gelatinase. Samples of foam nests presented  $5.0 \times 10^5$  ufc/g comprising 30 morphotypes, while the adjacent soil presented  $1.6 \times 10^5$  ufc/g and 21 morphotypes. There was a predominance of Gram-positive bacteria, with only two Gram-negative isolates. Nine (30%) isolates from the foam nests were positive for amylases and 20 (67%) positive for proteases, a higher number compared to the surrounding soil, 4 (19 %) positive for amylase and 7 (33%) positive for protease. The achieved results reinforced the presence of a microbial community in frog foam nests that can be explored for hydrolases enzymes and further studies are needed to better understand their biotechnological potential.

**Keywords:** Amylases, proteases, frog foam nests, bacterial hydrolases

**Development Agency:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and SERRAPILHEIRA