

TITLE: PRODUCTION OF ACID AND ALKALINE PROTEASES BY THE CYANOBACTERIA *Synechococcus* sp. GFB01 FROM AMAZON REGION.

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ABSTRACT: Proteases are hydrolytic enzymes responsible for the cleavage of peptide bonds. These proteins account for almost 60% of the commercialized enzymes worldwide due to their innumerable industrial applications. Then, its high demand requires the prospection of new proteases that exhibit features of commercial interest, such as substrate specificity and optimum temperature and pH. A cyanobacteria isolated from Amazon region showed great potential to produce proteolytic enzymes. In previous works it was shown that *Synechococcus* sp. GFB01 was able to synthesize proteases, which activity was affected positively by the ions K^+ and Mn^{2+} , and negatively by the inhibitors 1,10-Phenanthroline, EDTA and PMSF, indicating metallo- and serino-proteases production. Subsequently, this work aimed to analyze the influence of pH in the proteolytic activity produced by *Synechococcus* sp. GFB01. This microorganism was cultured in BG11 medium at 25°C under illumination. The biomass was collected through centrifugation at 4.000g for 30min, and the cells were lysed by sonication. The extract obtained was poured into a chromatography column containing Diaion-HP20 hydrophobic resin, which was washed with water. For the protease assay, Azocasein was dissolved in different buffers (50mM) at the pH range from 5 to 9 to a final concentration of 2%. The aqueous fraction obtained was incubated with the substrate for 60min at 35°C. The reaction was stopped using TCA 10%, and incubated for 15min at 0°C, then submitted to centrifugation at 2.800g for 5min. The supernatant was collected and mixed with NaOH 2M and the solution analyzed by a spectrophotometer at 440nm. The results indicated the presence of two proteolytic activities: one acid and one alkaline. The former has its peak activity at the pH 5.5 (Absorvance=0,106±0,015) and the latter has at the pH 7.5 (Absorvance=0,140±0,009). In comparison, the remaining pH tested maintained their activities below 0,090. Then, these results are consistent with previous work indicating the production of more than one protease. Therefore, this work was able to show that the cyanobacteria *Synechococcus* sp. GFB01 produces more than one protease. However, it is still necessary further works to better characterize these acid and alkaline enzymes, determining which is the serino- and metallo-proteases.

Keywords: proteases, cyanobacteria, amazon.

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