

Purification and characterization of trypsin from *Lophiosilurus alexandri* pyloric cecum

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Lophiosilurus alexandri, a native fish from São Francisco River, commonly known as pacamã, is a carnivorous catfish belonging to the *Pseudopimelodidae* family. It is highly valued for its organoleptic properties, such as tasty meat and absence of spines in the inside of the muscles. **OBJECTIVES:** Purify and characterize the trypsin from *Lophiosilurus alexandri*. **MATERIAL AND METHODS:** Five pyloric ceca were collected and transferred to a beaker containing 8.0 mL of extraction buffer. Using scissors, the tissues were grinded in small portions to facilitate maceration through a tissue homogenizer. The crude extract was subjected to saline precipitation and liquid chromatography using DEAE-Sepharose column equilibrated with 50 mM Tris-HCl buffer (pH 8.0). After the washing process, the adsorbed proteins were eluted by applying a saline gradient with 50 mM Tris-HCl buffer (pH 8.0) + 0.5 M NaCl. **RESULTS AND DISCUSSION:** Trypsin from *L. alexandri* was purified using only two purification processes: ammonium sulfate precipitation and anion exchange liquid chromatography in DEAE-Sepharose column, with recuperation of 86.97% and 44.91%, respectively. Trypsin mass was estimated as 24 kDa through SDS-PAGE, which showed only one band in silver staining. The purified enzyme showed an optimum temperature and pH of 50°C and 9.0, respectively, analyzed using BApNA as a substrate. Stability was well maintained, with high levels of activity at a pH of up to 11.0, including high stability at a temperature of up to 50°C after 60 min of incubation. The inhibition test demonstrated strong inhibition by PMSF, a serine protease inhibitor. Kinetic constants k_m and k_{cat} for BApNA were 0.517 and 5.0, respectively. The purified enzyme was also as active as casein, as analyzed by zymography. **CONCLUSION:** we consider trypsin a promising enzyme for industrial processes, owing to its stability in a wide range of pH and temperature and activity even under immobilization.

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