Purification of a Protease from the Visceral Mass of *Mytella charruana* and its Use to Obtain Antimicrobial Peptides

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Introduction: Proteases are enzymes that selectively catalyze the hydrolysis of peptide bonds, which results in the degradation of proteins. These enzymes are largely used in industry and present many applications in biotechnology. Storage proteins are produced during the development and their main function is to storage nutrients. On its native structure, these proteins do not present any antimicrobial activity, but when degraded, they can release some bioactive peptides. **Objectives:** To purify a protease from the visceral mass of *M. charruana* and evaluate its potential to generate antimicrobial peptides from casein hydrolysis. **Material and Methods:** Visceral mass of *M. charruana* was homogenized with 0.1 M Tris-HCl pH 8.0 and, after centrifugation, the extract was evaluated for total proteolytic activity assay using azocasein as substrate (Azeez et al., Phytochemistry 68:1352–1357, 2007). One unit of activity was defined as the amount of enzyme that gave an increase of 0.01 in absorbance at 366 nm. The extract was submitted to gel filtration chromatography using Sephadex G-75 and posteriorly to an ion exchange chromatography using DEAE-Sephadex. The DEAE adsorbed peak was submitted to SDS-PAGE and then used to hydrolyze casein. The hydrolysate was evaluated for antimicrobial activity against *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae* and *Micrococcus luteus*. **Results:** The extract presented a proteolytic activity of 339 U/mg, which increased to 1,420 U/mg after the ion exchange chromatography (purification fold: 4.2). DEAE peak presented a single band in the SDS-PAGE between 30 and 45kDa and was able to hydrolyze casein. The hydrolysate inhibited the growth of all bacteria tested, but was not bactericide. **Conclusion:** The enzyme was purified by gel filtration and ion exchange chromatography and was able to generate antibacterial peptides when used to hydrolyze casein.

Key-words: Protease; casein; antibacterial activity

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