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## **RELEASE OF CYTOSOLIC PROTEASES VIA EXTRACELLULAR VESICLES IN *Oenococcus oeni* RAM11 UNDER STRESS CONDITIONS**

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*Oenococcus oeni*, a lactic acid bacterium predominant in wines and ciders, exhibits extracellular proteolytic activity against proteins from various substrates such as grape and apple juice. The expression of this enzymatic activity is induced by the environmental harsh conditions like nutritional stress, low pH and presence of ethanol and sulfur dioxide. Although two extracellular proteases have been characterized in *O. oeni*, specific genes for these proteins have not yet been identified in its genome. It is suggested that the proteolytic activity could be due to the release of cytosolic enzymes through extracellular vesicles (EV). The *O. oeni* RAM11 strain was exposed to nutritional stress in 0.05 M citrate buffer for 2 h. After centrifugation, the cell-free supernatant was used as a source of proteolytic enzymes against apple juice proteins. Proteolytic activity was evaluated by the colorimetric method of Doi using apple juice as substrate. The supernatant was also partially purified by precipitation with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Then, SDS-PAGE was performed on the partially purified supernatant. Protein bands on the SDS-PAGE gel were excised and studied by mass spectrometry (MS). The obtained spectra were then analysed using the Proteome Discoverer software. Based on the MS results, the presence of EVs produced by this strain was evaluated by electron microscopy using a scanning electron microscope (Zeiss, SUPRA 55VP). Extracellular proteolytic activity was evidenced in the stress supernatant of *O. oeni* (0.36 U/mL). MS analysis of the purified supernatant revealed the presence of proteins, including a metalloprotease, a serine protease, heat shock proteins, stress adaptation-associated proteins, and malolactic enzyme. Microscopy analysis revealed the presence of EVs approximately 40 nm in size. The presence of cytosolic proteins in the stress supernatant of *O. oeni* RAM11 and the electron microscopy observations suggest that cytosolic enzymes with proteolytic activity could be transported to the extracellular medium via vesicles.

Palabras clave: extracellular vesicles - protease activity - *Oenococcus oeni* - apple juice - microscopy