TITLE: Purification and biochemical characterization of a polyurethanase secreted by *Serratia liquefaciens* L135 isolated from raw milk

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ABSTRACT:
Serratia liquefaciens L135 isolated from raw milk it is able to secrete a heat-stable lipase which was identified as polyurethanase by liquid chromatography coupled to mass spectrometry (LC-MS/MS). Lipases that exhibit high thermal stability, activity in alkaline pH values and in the presence of surfactants, have high potential for industrial application and often represent a sustainable solution for many industries, reducing the impacts on the environment by replacing chemical catalysts. Therefore, the aim of this work was to purify and characterize biochemically, the polyurethanase of *S. liquefaciens* L135, aiming at future biotechnological applications. *S. liquefaciens* L135 was cultivated in Brain Heart Infusion broth (BHI) for 24 h at 30 °C then, supernatant obtained from this culture was concentrated in Amicon ultrafiltration device and separated by HPLC size exclusion chromatography. The lipolytic activity of fractions was detected at 410 nm using *p*-nitrophenyl palmitate as substrate. The purified enzyme was characterized. Purification resulted in a specific activity of 2,793 U/mg and a purification factor of approximately 3.1 fold with 21.5% recovery. The polyurethanase showed maximum activity at pH 8.0 and at 30 °C. Its activity was stimulated in the presence of Ca^{2+} and Ba^{2+} ions and inhibited by the Zn^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} ions at the final concentration of 10 mM. In the presence of dithiothreitol (DTT), β-mercaptoethanol and sodium dodecyl sulfate (SDS), the lipolytic activity was increased. However, in the presence of Tween-80, Triton X-100 and ethylenediaminetetraacetic acid (EDTA), lipolytic activity was inhibited. Thus, in this work it was possible to verify that heat-stable polyurethanase with lipase activity from *S. liquefaciens* L135 presented characteristics that allow directing this enzyme for possible biotechnological applications.

Keywords: polyurethanase, heat-stable, biotechnological application.

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