TITLE: Identification of a polyurethanase with lipase activity secreted by *Serratia liquefaciens* L135 isolated from raw milk

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ABSTRACT:
Serratia liquefaciens, a psychrotrophic bacterium present in raw milk, is able to produce heat-stable enzymes, such as proteases and lipases, which remain active after heat treatments used in the dairy industry. Lipases hydrolyze triacylglycerols that release fatty acids responsible for undesirable flavors in milk and dairy products. The objective of this work was to identify the lipase produced by *S. liquefaciens* L135 to better understand its structure and secretion system. *S. liquefaciens* L135 was cultured in BHI for 24 h at 30 °C, the supernatant obtained from this culture was concentrated in Amicon® ultrafiltration device and separated by HPLC size exclusion chromatography. The lipolytic activity of fractions were detected using the substrate *p*-nitrophenyl palmitate and by zymography using the fluorescent substrate methylumbelliferyl-butyrate. The band with lipolytic activity was excised from the zymogram gel, subjected to trypsinolysis and then, identified by liquid chromatography coupled to mass spectrometry (LC-MS/MS). Two single peptides were recovered by LC-MS/MS, with 5% coverage and 100% identity with polyurethanase of *S. liquefaciens* deposited in UniProtKB. The polyurethanase identified had a theoretical molecular weight of 64,8 kDa, confirming the value estimated by zymography. This enzyme of 615 amino acid residues presents a theoretical isoelectric point of 4.35. The polyurethanase from *S. liquefaciens* L135 has the highly conserved sequence of serine hydrolase (GXSXG) at position 205-209, which is characteristic of lipases. According with the SignalP 4.1 program, the polyurethanase does not require a signal peptide for its transmembrane translocation. One of the strategies used by Gram-negative bacteria to secrete proteins is through the secretory system of type I by the sec-independent pathway and, these proteins have glycine-rich repeats such as GGXGXDXXX that complex with calcium ions. The sequence of polyurethanase identified showed three glycine-rich repeats and, through the molecular modeling, it was possible to verify that one of these sequences can complex with calcium. Therefore, in this work, it was possible to identify that the lipase secreted by *S. liquefaciens* L135 is a polyurethanase and is secreted by the type I secretion system. This information will contribute to elucidate mechanisms of inactivation of the secretion of this enzyme, since the action of heat-stable lipolytic enzymes are undesirable in milk and dairy products.

Keywords: deterioration; heat-stable; secretion; serine hydrolase.

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