Title of thesis: Purification of β-Galactosidase Enzyme by Probiotic *Limosilactobacillus fermentum* LF08

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Thesis Abstract

This thesis aims to evaluate the purification protocol for β-galactosidase from the probiotic strain *Limosilactobacillus fermentum* LF08. Several critical steps were taken during the purification process, including enzyme fermentation, determining the optimal ammonium sulfate saturation, and cell lysis. A glucose and galactose ratio of 1:3, 1% (v/v) inoculation, and 16 hours of fermentation at 37°C was applied during fermentation to achieve the highest enzyme activity. Optimal ammonium sulphate saturation was found to be 75%, leading to the highest β-galactosidase activity, consistent with previous findings. Lysozyme incubation at 45°C for 4 hours resulted in the highest β-galactosidase activity compared to other temperatures. The purification process included precipitation, dialysis, and FPLC chromatography. The enzyme had a specific activity of 11,572 U/mg after cell disruption and 23,075 U/mg after precipitation. However, after dialysis, the particular activity dropped to 3,315 U/mg, indicating a possible loss of essential cofactors or coenzymes. The overall yield remained stable at around 41%, indicating that the purification protocol is efficient.

The *Limosilactobacillus fermentum* LF08 purification protocol was found to be effective when compared to other studies on β-galactosidase purification from various probiotic strains. These findings have important implications for industrial applications and future research.