ENZYMIC STUDIES ON β-GALACTOSIDASE FROM *LACTOBACILLUS BIFIDUS* AND *GALDIERIA SULPHURARIA*

Ph.D. DISSERTATION

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Title : Enzymic Studies on β-Galactosidase from *Lactobacillus bifidus* and *Galdieria sulphuraria*

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Abstract : The enzyme β-galactosidase (EC 3.2.1.23) was observed in an alga: *Galdieria sulphuraria* (strain 107.79) and in bacteria: *Lactobacillus bifidus* (NCTC-S1). The red alga *Galdieria sulphuraria* and the bacterium *Lactobacillus bifidus* are the organisms known to be able to grow on lactose as the sole carbon source. These microbial sources should exhibit high activity of β-galactosidase. This research employed experiments in the cultivation and identification of *L. bifidus* and *G. sulphuraria.* After that β-galactosidase from *G. sulphuraria* and *L. bifidus* was purified by column chromatography and then characterized. The properties of this enzyme were studied by UV-visible spectrophotometry. In comparison with *L. bifidus* β-galactosidase, *G. sulphuraria* β-galactosidase was not significantly different (V_{max} values were 7.72×10^{-5} and 7.13×10^{-5} $Mmin^{-1}ml^{-1}$, respectively), but the substrate affinity was slightly less (K_m values were 9.49×10^{-2} and 13.09×10^{-2} M, respectively). After SDS-PAGE, the molecular weight of subunit for *L. bifidus* β-galactosidase was 55 kDa and that of *G. sulphuraria* β-galactosidase was 52 kDa. The β-galactosidase from *L. bifidus* and *G. sulphuraria* had optimum temperature values of 40°C at optimum pH of 6.0 and 50°C at broad pH optimum around 6.5. The β-galactosidase from *L. bifidus* was unstable, while *G. sulphuraria* β-galactosidase was stable up to 50°C for several hours. The time required for one catalytic cycle of β-galactosidase

from *L. bifidus* and *G. sulphuraria* was about 2.69×10^{-4} min and 2.36×10^{-4} min. Due to the activation energy value of 2.19 $kcalmol^{-1}$ of *G. sulphuraria* β-galactosidase, its catalyzed reaction is slower than that of *L. bifidus* β-galactosidase with the activation energy value of 1.55 kcal mol^{-1}. The enzyme unit of β-galactosidase from *L. bifidus* and *G. sulphuraria* was 120 EU and 312 EU, respectively. The reaction order for this enzyme from the two sources followed the first-order kinetics. In this work, optimization factors for immobilization of enzyme and conversion of lactose in milk powder into glucose were also determined. At 4°C, β-galactosidase from the two sources was completely immobilized onto EUPERGIT®C acrylic polymers after 2 days. There had been no changes on the operational stability of the immobilized enzyme within one month study. Immobilized *L. bifidus* β-galactosidase and *G. sulphuraria* β-galactosidase digest lactose from milk powders with release of approximately 95% of glucose.