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Purification and Characterization of B-Galactosidase from Probiotic *Pediococcus acidilactici* and its use in Milk Lactose Hydrolysis and Galactooligosaccharide Synthesis

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β- galactosidase is a commercially important enzyme that was purified from probiotic *Pediococcus acidilactici*. The enzyme was extracted from cells using sonication and subsequently purified using ammonium sulphate fractionation and successive chromatographies on Sephadex G-100 and Q-Sepharose. The enzyme was purified up to electrophoretic homogeneity by 3.06 fold with specific activity of 0.883 U/mg and yield of 28.26%. Molecular mass of β-galactosidase estimated by SDS-PAGE and MALDI-TOF was 39.07 kDa. The enzyme is a heterodimer with subunit mass of 15.55 and 19.58 kDa. The purified enzyme was optimally active at pH 6.0 and stable with more than 97% activity in a pH range of 5.8-7.0. Purified β-galactosidase was optimally active at 50°C. Kinetic parameters K_m and V_{max} for purified enzyme were 400µM and 1.22×10^{-1} U respectively. Its inactivation by PMSF confirmed the presence of serine at the active site. The metal ions had different effects on enzyme. Ca^{2+}, Mg^{2+} and Mn^{2+} slightly activated the enzyme whereas NH_4^+, Co^{2+} and Fe^{3+} slightly decreased the enzyme activity. Determination of thermodynamic parameters revealed that the thermostability of β-galactosidase is less at higher temperature (60°C). Purified enzyme effectively hydrolysed milk lactose with lactose hydrolysing rate of 0.047 min^{-1} and $t_{1/2}$ of 14.74 min. This is better than other studied β-galactosidases. Both sonicated *Pediococcus acidilactici* cells and purified β-galactosidase synthesized galactooligosaccharides (GOSs) as studied by TLC at 30% and 50% of lactose concentration at 47.5°C. These findings indicate the use of β-galactosidase from probiotic bacteria for producing delactosed milk for lactose intolerant population and prebiotic synthesis. pH and temperature optima and its activation by Ca^{2+} shows that it is suitable for milk processing.

Biography:

Dr. Suman Singh is presently working as an Associate Professor, Dept of Biochemistry Kurukshetra University, Kurukshetra. She did her master's from National Dairy Research Institute, Karnal (India) and subsequently did Ph.D. in Biochemistry from Kurukshetra University, Kurukshetra. For the last 14 years, she is teaching at post graduate level and guiding research. She has also worked at the German Institute of Human Nutrition, Potsdam-Rehbrucke, Germany. She has completed two research projects and currently running a Young Scientist Award cum research project funded by Department of Science and Technology, New Delhi India. She has published more than 30 research papers in accredited scientific Journals. She is working on strain characterization *Pediococcus* species after exploring its *in-vitro* probiotic potential. Purified enzymes from the strain under study are being studied to be used in dairy, food and meat industries. Her focus is to find out action molecules and biomarkers of probiotics.