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## Effect of symbiotic relationship of *Lactobacillus bulgaricus 77* and *Streptococcus thermophilus 95/2* on beta-galactosidase and lactic acid production

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Enzyme production which is a growing field of biotechnology has an annual world sale close to billion dollars. Therefore isolation of new strains producing novel enzymes is important for industrial enzyme production.

*S. thermophilus* and *Lactobacillus bulgaricus* are particularly promising microorganisms for the production of beta-galactosidase and lactic acid which have commercial importance in food and pharmaceutical industries (Cortes et al., 2005; Somkouti et al., 1996). Beta-galactosidase enzyme is known to eliminate problems related to whey disposal, lactose crystallization and lactose intolerance individuals (Kim and Rajagopal, 2000). With this perspective, traditional yogurt samples collected from Toros mountain region of Turkey were used for isolation of *S. thermophilus* and *L. bulgaricus* strains. In this study, the goal was to investigate the symbiotic relationship between *S. thermophilus 95/2* (St95/2) and *L. bulgaricus 77* (Lb77) in lactic acid, biomass and beta-galactosidase production (not considered so far in the literature) by using response surface methodology. Therefore the effect of the ratio (Lb77:St95) of two strains and media formulation was investigated. As a result, the ratio (Lb77:St95/2) of the strains and media formulation had significant effect on all responses ($p < 0.01$). The predicted enzyme activity (2.1348 U/ml), lactic acid (22.47 g/l) and biomass (52.15 g/l) at optimum conditions were very close to the actual values obtained experimentally (2.1265 U/ml, 22.18 g/l and 49.15 g/l, respectively). The optimum conditions determined were such as, to use these two cultures together in a ratio of 3:2.6 (Lb77:St95/2) in a medium containing whey (5%), corn steep liquor (4%), potassium phosphate (2%) and peptone (2%) at 43 °C for 8 h. Symbiotic relationship of these cultures provided 60% more beta-galactosidase activity and 72% more lactic acid compared to the results obtained by using Lb 77 only. Similarly, this relationship provided 10% more beta-galactosidase activity and 74% more lactic acid when St95/2 was used alone. As a result this study brought a new a perspective in producing the products of interest using the two strains in a symbiotic relation as opposed to conventional single organism use.

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doi:10.1016/j.jbiotec.2007.07.405

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## Kinetic and thermodynamic properties of crude and three-phase partitioned polygalacturonase from *Aspergillus sojae*

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Commercial preparations of pectinolytic enzymes derived from fungi are well known to have high biotechnological value in the industry. Since potential applications of pectinases in various fields of food, paper, textile and waste water treatment are increasing, it is important to understand the properties of these enzymes for efficient and effective usage. Furthermore, kinetic studies can provide some information on reaction mechanism of enzyme whereas estimation of thermodynamic parameters like $\Delta G^*$, $\Delta H^*$, $\Delta S^*$ helps understand the probable mechanism of denaturation; hence both are important features in any enzymatic process (Xiong et al., 2005). Therefore, the aim of this work was to investigate the kinetic and thermodynamic properties of the crude and three-phase partitioned extracellular polygalacturonase (PG) produced by *Aspergillus sojae* ATCC 20235, which has not been considered as pectinase producer so far. The deactivation process was modelled as first-order kinetics. The crude and purified PG had a deactivation energy ($E_d$) of 151.81 and 285.97 kJ mol$^{-1}$, respectively. The half-lives ($t_{1/2}$) of the crude and purified PG at 85 °C were estimated as 61.3 and 14.1 min, respectively. In addition, the thermodynamic study clearly demonstrated that the crude PG was more stable than the purified PG. Furthermore, comparison with the kinetic parameters results showed that the $K_m$ value of crude PG has a higher affinity for the polygalacturonic acid and the purified PG possesses a lower catalytic activity. This was attributed to the presence of certain impurities in the crude preparation. Moreover, the purified PG was completely inhibited in the presence of $Mn^{2+}$ and SDS and induced significantly by EDTA, glycerol and beta-mercaptoethanol. The results of kinetics and thermodynamics for this enzyme were very comparable to the studies on commercial pectinases (Pectinex 3XL, Rapidase C80, and Pectinase CCM) in the literature (Ortega et al., 2004). As a result, this enzyme can be considered as a potential candidate to be used in various industrial applications.

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doi:10.1016/j.jbiotec.2007.07.406