yield of production, some conditions need to be optimized accordingly.

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## H12

### Cloning, expression and characterization of a lipase from thermophilic bacterium *Geobacillus kaustophilus* and its immobilization studies

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In this study, cloning, expression and characterization of the lipase (gene id: GK1986) from thermophilic bacterium *Geobacillus kaustophilus* as well as its immobilization studies have been under taken. The lipase gene (GK1986) was amplified by PCR using primers designed based on the known DNA sequence (KEGG database), and cloned into pET28a(+) expression vector and expressed in *E. coli* BL21 (DE3). The recombinant enzyme was immobilized on to Eupergit C modified with glycine and utilized in the production of medium-chain fatty acids and their esters. The activity of the lipase was maximal at a temperature range of 55–60°C and the pH range of 7.5–9.0. The enzyme showed the highest activity toward C10–C12 lipids. The immobilized lipase was more stable in various organic solvents and at higher temperatures. *G. kaustophilus* lipase was first time cloned and characterized in this study. It is a thermostable organic solvent tolerant lipase and has significant sequence homology with L1 lipases from other Gram-positive bacteria. Also, it has higher specificity towards medium-chain fatty acids.

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## H13

### Isolation and characterization of α-amylase from thermophilic *Geobacillus stearothermophilus*

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α-Amylase (E.C.3.2.1.1) breaks down the α-1,4 glucosidic bonding of linear amylose and branching amylopectin which are the major building blocks of starch. This enzyme is of great significance in present day biotechnology with applications ranging from food, textile to paper industries. In this investigation, α-amylase production and activity optimum was determined from thermophilic *Geobacillus stearotermophilus* under various chemical and physical conditions. *G. stearotermophilus* was tested for producing α-amylase using different carbon/nitrogen sources and $CaCl_2$. Optimum temperature and pH for enzyme production were determined. α-Amylase activity was determined according to Bernfeld method. In the present study, it was found that the best medium for α-amylase production of *G. stearotermophilus* was nutrient broth. When it was grown in the presence of carbon/nitrogen sources, a decrease in α-amylase production was observed. It was seen that the amylase production was completely repressed by sucrose and lactose. In presence of 10 mM $CaCl_2$ to the medium increased amylase production by 5%. Optimum temperature and pH for maximum enzyme activity were 70°C and 7.0, respectively. α-Amylase of *G. stearotermophilus* can be purified because of its features in next steps.

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## H14

### Purification and characterization of a thermostable α-amylase from *Geobacillus stearothermophilus*

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Amylases (α-1,4 glucan-4-glucanohydrolases, EC 3.2.1.1) are enzymes which hydrolyse starch molecules to give diverse products including dextrins and progressively smaller polymers composed of glucose units. In this study deals with the purification and characterization of thermostable α-amylase. The enzyme was purified using ammonium sulfate precipitation (40–80% saturation), DEAE-cellulose ion exchange and Sephadex G-100 gel filtration chromatography. α-Amylase activity was determined according to Bernfeld method. SDS-PAGE (polyacryilamide gel electrophoresis) of these samples was carried out. Optimum temperature and pH were determined. The effects of surfactans, inhibitors, and some heavy metals on α-amylase activity were investigated. The optimum pH and temperature for enzyme activity were pH 7.0 and 70°C, respectively. This enzyme was fully stable at 60°C and retained 93%. The enzyme was activated by $Ca^{2+}$ and $Mn^{2+}$, but strongly inhibited by $Cu^{2+}$, $Zn^{2+}$, $Fe^{2+}$ and $Hg^{2+}$. The enzyme was activated by Triton X-100. The molecular mass of purified enzyme determined by SDS-PAGE. Thermostable α-amylase from *Geobacillus stearothermophilus* can be use industrial application.

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## H15

### Alteration of the coenzyme specificity of formate dehydrogenase from *Candida methylica* by site saturation mutagenesis

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FDH catalyses the conversion of formate and NAD+ to $CO_2$ and NADH. Its usefulness lies in the fact that it is capable of regenerating NADH in enzymatic reductions of aldehydes/ketons to form chirally pure alcohols. However, its highly specific to NAD+ coenzyme and it would be also desirable to regenerate NADPH by using NADP+ coenzyme. Although many attempts using rational design have been applied for changing coenzyme specificity of FDH, all mutants bind NADP+ very weakly. Here, we have used site saturation mutagenesis, a semi-rational approach, to improve the KM of cmFDH for NADP+. Firstly, in the coenzyme binding domain amino acid residues which are responsible for the coenzyme specificity have been determined by using Insight II program on a homology model of cmFDH based on *Pseudomonas* sp.101 and *Candida boidinii*. The randomization of these selected residues should produce