Isolation, Characterization and Optimization of Alkalophilic and Thermotolerant Lipase from Bacillus subtilis Strain

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Abstract : The thermotolerant, solvent stable and alkalophilic lipase producing bacterial strain was isolated from the water sample of the foothills of Trikuta Mountain in Kakryal (Reasi district) in Jammu and Kashmir, India. The lipase-producing microorganisms were screened using tributyrin agar plates. The selected microbe was optimized for maximum lipase production by subjecting to various carbon and nitrogen sources, incubation period and inoculum size. The selected strain was identified as Bacillus subtilis strain kakrayal_1 (BSK_1) using 16S rRNA sequence analysis. Effect of pH, temperature, metal ions, detergents and organic solvents were studied on lipase activity. Lipase was found to be stable over a pH range of 6.0 to 9.0 and exhibited maximum activity at pH 8. Lipolytic activity was highest at 37°C and the enzyme activity remained at 60°C for 24hrs, hence, established as thermo-tolerant. Production of lipase was significantly induced by vegetable oil and the best nitrogen source was found to be peptone. The isolated Bacillus lipase was stimulated by pre-treatment with Mn2+, Ca2+, K+, Zn2+, and Fe2+. Lipase was stable in detergents such as triton X 100, tween 20 and Tween 80. The 100% ethyl acetate enhanced lipase activity whereas, lipase activity were found to be stable in Hexane. The optimization resulted in 4 fold increase in lipase production. Bacillus lipases are 'generally recognized as safe' (GRAS) and are industrially interesting. The inducible alkaline, thermo-tolerant lipase exhibited the ability to be stable in detergents and organic solvents. This could be further researched as a potential biocatalyst for industrial applications such as biotransformation, detergent formulation, bioremediation and organic synthesis.

Keywords: bacillus, lipase, thermotolerant, alkalophilic

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