Unlocking the Thar Desert's Potential: Enhancing Extracellular Protease Production in *Bacillus sp. DS06*

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ABSTRACT

Introduction: Proteases have got lot of attention in the recent time as the most significant enzyme because they are widely used in a variety of industries, such as detergent, diagnostics, food, feed, pharmaceuticals, leather, textile, waste management, and silver recovery. Proteases belong to diversified group proteolytic enzymes which cleave peptide bond present in proteins. The enzyme alkaline protease is accounting for nearly 60-65% of global enzyme market. Microbial proteases represent one of the largest classes of industrial enzymes and are more useful than proteases produced from plants and animals as microbes are easily optimized and grown in lab conditions, can be cultured on large scale in less time. Microbes are highly stable and active in extreme conditions. Among the microbial producers, members of genus *Bacillus* are especially valuable because of their ease in growth on a variety of substrates, high yield and heterotrophic nature.

The Thar Desert is a large arid and semi-arid regions having extreme drought conditions that are habitat for the existence of living things. Thermophilic bacteria can survive in high temperatures such as desert soil, geothermal vents, and hot springs. So only extremophilic can thrive in such kind of environments. Thermophilic organisms are known to be excellent sources of thermostable proteases.

Objectives: The main objective of this research is to isolate and identify thermophile *Bacillus* strain from Thar Desert soil through their colony characteristics, gram staining and 16S rRNA sequencing and then to screen the culture for protease activity on Casein agar plates as well as to optimize the media for economical protease production.

Methodology: In this study, several thermophilic *Bacillus* strains were isolated at 50°C from Thar Desert soil, further screened for proteases activity using casein as a substrate. The strain showing maximum proteases activity was selected and identified through standard morphological and molecular methods such as 16S rRNA sequencing. The strain DS06 further studied, and the physical and chemical parameters were optimized to produce the protease enzyme. The optimization of different fermentation parameters like (incubation time, Carbon/Nitrogen sources, pH, temperature, and ionic effects) were optimized for the economical protease production. Assay for the activity of the protease was determined through Kunitz assay method.

Result: The result of studies shows that strain DS06 is thermophile and can grow temperature at 55°C. The DS06 strain can give maximum enzyme yield at higher temperature in the presence of Carbon source glycerol and incubation time 48 hours at 150 rpm.

Conclusion: The thermophilic strain DS06 produced thermophile proteases which is highly desirable for industrial purposes, especially in sectors such as detergent formulation, leather processing, and bioremediation, where operational conditions are harsh.

Keywords: Protease, Desert, Thermophile, Casein, Temperature.

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