Production and Immobilization of L-Asparaginase from Natural *Bacillus* spp. Isolates

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Abstract

L-Asparaginase (EC 3.5.1.1) is an enzyme of significant pharmaceutical and industrial relevance due to its application in acute lymphoblastic leukemia therapy and its ability to mitigate acrylamide formation in thermally processed foods. The study aimed to isolate L-Asparaginase–producing *Bacillus* spp. strains from natural sources and perform their molecular identification, optimization of production, and covalent immobilization on agarose supports using different linker strategies. Protein-rich environmental sources, including wheat, potato, and maize fields, farm wastewater, decayed potatoes, and plant compost, were sampled to isolate *Bacillus* species with potential L-Asparaginase activity. A heat-shock pretreatment (80 °C, 15 min) was employed to select spore-forming bacteria, followed by serial dilutions and spread plating on Nutrient Agar. From 93 morphologically distinct *Bacillus* isolates, 26 exhibited L-Asparaginase activity on a phenol red-based screening medium. Among these, five isolates demonstrated high enzymatic activity under submerged fermentation conditions. They were subjected to 16S rRNA gene sequencing for molecular identification, and their sequences were deposited in the NCBI GenBank database, ensuring reliable taxonomic classification. L-Asparaginase produced from *Bacillus* sp. ADA01S (GenBank: PX117331.1) was selected as the producer strain because it showed the highest activity. Optimization experiments revealed that *Bacillus* sp. ADA01S produced maximum L-Asparaginase activity at pH 7.0 and 40 °C. The enzyme was subsequently precipitated using cold acetone and partially purified. Activity assays, based on Nesslerization, confirmed its efficiency under optimized fermentation conditions. Subsequently, the enzyme was covalently immobilized onto agarose supports through three different linker (Genipin, Sulfon, Glyoxyl) strategies. The immobilized enzyme retained high activity, exhibited improved thermal stability, and maintained reusability across multiple cycles. These findings demonstrate that natural *Bacillus* spp. isolates represent a promising biotechnological source of L-Asparaginase. The optimized production conditions and immobilization strategies developed in this study highlight the potential for both medical and food industry applications, particularly in reducing acrylamide levels in heat-processed products.

Key Words: L-Asparaginase, *Bacillus*, enzyme production, immobilization, biotechnology