ID: 487

# Evaluation of *Bacillus* Strains for Protease Production

**Fikriye Alev Akçay, Ayşe Avcı**

Food Engineering Department, Faculty of Engineering, Sakarya University, Sakarya, Türkiye

## Abstract

Proteases have diverse industrial applications, particularly in laundry detergents and the leather industry, as well as in the food, textile, and pharmaceutical sectors. While plant- and animal-derived proteases are traditionally used, their production is limited by environmental and agricultural constraints. Microorganisms, especially *Bacillus* species and lactic acid bacteria, have emerged as key sources due to their high yield and ease of biotechnological manipulation, accounting for nearly 60% of global commercial enzyme sales. This study aimed to evaluate protease production in *Bacillus* isolates obtained from food and soil samples. Eight isolates were screened on milk powder-containing agar, and six demonstrated significant protease activity. The isolates ZGT9, EBTA7, and ZBP10 showed the largest clear zones (20.0, 18.8, and 18.5 mm, respectively) and were selected for further investigation. Crude enzymes were obtained from 48-hour nutrient broth cultures, and protease activity was assessed using 1% casein solution at pH 8 and 60°C for 15 minutes. Protein concentrations were determined using Bradford assay, with BSA as the standard. Total protein content ranged from 112.6 to 182.6 µg BSA eq./mL, while protease activity varied between 360.8 and 2530.8 U/mL. Specific activities ranged from 3221 to 13,862 U/mg. The isolate with the highest activity (EBTA7) was further analyzed to determine its optimal operating conditions. Enzyme activity was evaluated at various pH (7–11) and temperature (50–80°C) values. The enzyme showed maximal activity at 60°C and pH 9, with high relative activity at 50°C (76%) but a sharp decline above 60°C (35% at 70°C, 29% at 80°C). These results indicate that the enzyme is moderately thermophilic and functions best under alkaline conditions. Biochemical characterization and 16S rDNA gene sequence analysis confirmed that the strain belongs to the *Bacillus* genus.

***Key Words:*** *Microbial proteases, Bacillus, protease activity*

**Acknowledgment:** This study was supported by the Scientific Research Projects Unit of Sakarya University (Grant no: 2021-7-25-25).