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Characterization of Protease Enzyme Produced from Locally Isolated *Bacillus sp.*

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ABSTRACT

This study aimed to characterization of protease enzyme purified from locally isolated *Bacillus sp.*. A total of 50 soil samples were taken for this purpose. Only 35 (or 70%) of the sample were found to have given rise to bacterial growth. As the first phase in the cultivation of bacteria, the samples were grown on mannitol egg yolk polymyxin agar and nutrition agar. More than 20 bacterial isolates were discovered, according to the findings. The results of the cultural, microscopic, and biochemical examinations used to identify these isolates revealed that 15 of them were Bacillus spp. Additionally, the use of the Vitek 2 technique ensured that each of the 15 isolates belonged to this species. Examining these isolates' capacity to create proteases revealed that all 15 of the Bacillus sp. isolates were capable of doing so, albeit to varying degrees. The isolate with the number S9 was the best at producing proteases, with a specific activity value of 40.19 U/mg proteins. On a casein hydrolyzed medium plate with a diameter of 3.0 mm and 2.2 mm, respectively, they demonstrated a clean zone. After 42 hours and 72 hours of incubation at 37°C, respectively, the strain 9 produces 1.01 units of proteolytic enzyme per milliliter. Proteolytic activity decreases with longer incubation times. It has potential for industrial use because the isolated Bacillus sp. was generating protease.

***Keywords*-** Bacillus, Casein hydrolyze and Protease.

I. INTRODUCTION

The family Bacillaceae, which includes many saprophytes and several parasites, is home to the genus Bacillus, which is composed of rod-shaped, gram-positive, endospore-producing, typically aerobic bacteria (as B. anthracis of anthrax). Although their primary habitat is dirt, Bacillus species are found all over the environment. The typical habitats for these organisms include decomposing organic debris, dust, vegetables, water, and some species are naturally occurring flora. In the hospital context, outbreaks and fake epidemics have been linked to contaminated dialysis equipment, hospital linen, and disinfection (ethyl alcohol). [1]

The genus' numerous species have a wide range of physiological traits that enable them to coexist in all types of natural habitats. Per cell, just one endospore forms. The spores are immune to radiation, desiccation, heat, cold, and disinfectants. [2] The majority of Bacillus types are not harmful to humans; however May, which is a soil bacterium, accidentally infects people. B. anthracis, which causes anthrax in people and domestic animals, is a rare exception. In the food sectors, Bacillus species are well known as problematic organisms that cause food to spoil. [3]

Numerous Bacillus species can produce large quantities of enzymes that are employed in many different sectors, such as the synthesis of alpha amylase for the hydrolysis of starch and protease subtilisin for detergents. [4]

One of the inside enzymes that degrades proteins is called protease. Enzymes that are internal to the cell are those that are not released into the medium until the cell has broken down. Regarding the external enzymes, they are naturally expelled without the cell's breakdown. [5] Proteases occur in all organisms, from prokaryotes to eukaryotes to viruses.

Protease animations have a significant role and significance in what they can enter in several industries (food stuffs such as cheese and meat softening) as well

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as in natural treatments such as skin burn treatment. It was found that microorganism protease enzymes play a significant and significant function in these organisms' resistance to aberrant circumstances and the diseases that cause them. It was found that microorganism protease enzymes play a significant and significant function in these organisms' resistance to aberrant circumstances and the diseases that cause them. [6]

II. MATERIALS AND METHODS

2.1 Isolation of Bacillus sp.

A total of 50 samples were obtained from various sources (soil, food, and human) were screened by biochemical test and Vitek2 system to isolate *Bacillus sp.*[7]

2.2 Analyzing the ability of isolated Bacillus species to produce proteases

A) A semi-qualitative screening

Each Bacillus sp. isolate was streaked on nutritional agar medium and let to grow for 24 hours at 37°C. A single colony was then put on a plate of skim milk agar media after incubation. The plate was incubated for 24 hours at 37°C. Based on observations of a distinct halo zone surrounding the colony, the isolate's capacity to generate protease was noted.

B) quantitative protease screening

Protease production was achieved by measuring the specific and enzyme activity.[8]

2.3 Protease Charateriztaion

1- Optimizing carbon source.
2- Optimizing concentration of carbon source.
3- Optimizing nitrogen source.
4- Optimizing concentration of nitrogen source.
5- Optimizing phosphate source.
6- Optimizing concentration of phosphate source
7- Optimizing PH.
8- Optimizing incubation temperature.

III. RESULTS

3.1 Isolation of Bacillus sp.

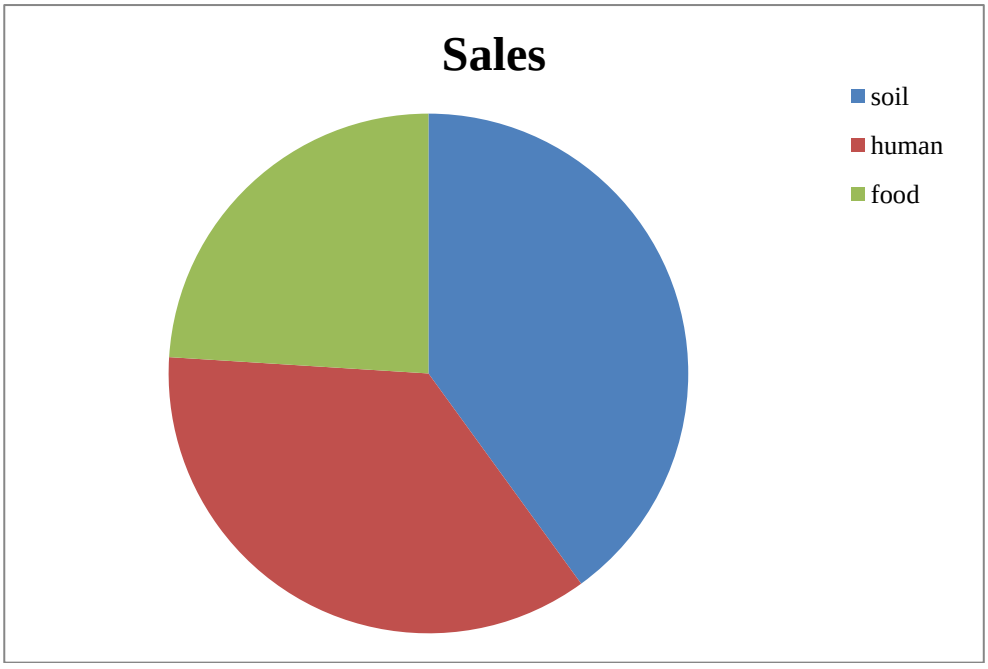

Figure (3-1): According to the sources, the probable Bacillus isolates are dispersed in a pie shape.

Table (3-1): Biochemical evaluation of isolated *Bacllius sp.*

Test	Result
Catalase test	**Positive**
Coagulase test	**Positive**
Indole test	**Negative**
Motility	**Positive**
Methyl Red test	**Negative**
Nitrate test	**Positive**
Oxidase test	**Positive**
Spore forming	**Positive**
Voges-Proskauer test	**Negative**

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3.2 Bacillus species' capacity to make proteases

A) A semi-qualitative screening

The study's findings demonstrated that all Bacillus sp. isolates hydrolyzed the skim milk agar medium by creating varying degrees of hydrolysis halos. The halos' diameters ranged from 8 to 18 mm. The largest protease producing isolate, S9 (from a soil sample), has a hydrolysis halo diameter of 18 mm. On the other hand, the S11 isolate (from a human sample) was the smallest and produced a zone with a diameter of only 8mm.[9]

B) A quantitative screening

The findings of testing Bacillus sp. capacity to produce proteases while growing in Luria-Bertani broth showed that the protease-specific activity of the culture filtrates ranged between 30.24 and 37.11 U/mg protein. S9 isolation, which had the highest specific activity among these isolates, was the most effective, while S11 isolate had the lowest specific activity. These findings led to the choice of isolate S9 for the study characterisation of the protease enzyme produced by Bacillus sp.isolates. The ability of bacteria to produce proteases was related to the diversity of the genes involved in producing proteases. [10]

3.3 Optimal conditions of protease production

Table (3-2): Effect of carbon source on protease production by *Bacllus sp.* S9 isolate.

Carbon source	Specific activity (U/mg protein)
Amylose	2890
Glucose	1856
Galactose	1956
Sucrose	1632

Table (3-3): Effect of amylose concentration on protease production by *Bacillus sp.* S9 isolate.

Amylose concentration	Specific activity (U/mg protein)
0.5	25.3
1.0	23.5
1.5	19.8
2.0	17.3

Table (3-2): Effect of nitrogen source on protease production by *Bacllus sp.* S9 isolate.

Nitrogen source	**Specific activity (U/mg protein)**
Peptone	**1630**
Sodium nitrate	**971**
Tryptone	**2064**
Yeast extract	**2134**

Table (3-3): Effect of yeast extract concentration on protease production by *Bacillus sp.* S9 isolate.

Amylose concentration	**Specific activity (U/mg protein)**
0.5	31.2
1.0	44.6
1.5	50.4
2.0	37.3

Table (3-2): Effect of nitrogen source on protease production by *Bacllus sp.* S9 isolate.

Phosphate source	**Specific activity (U/mg protein)**
K2HPO4	**94.2**
KH2PO4	**103.6**
Na2HPO4	**96.3**
NaH2PO4	**100.2**

Table (3-3): Effect of KH2PO4 concentration on protease production by *Bacillus sp.* S9 isolate.

KH2PO4 concentration	**Specific activity (U/mg protein)**
0.005	47.3
0.05	52.5
0.1	60.4
0.15	44.2

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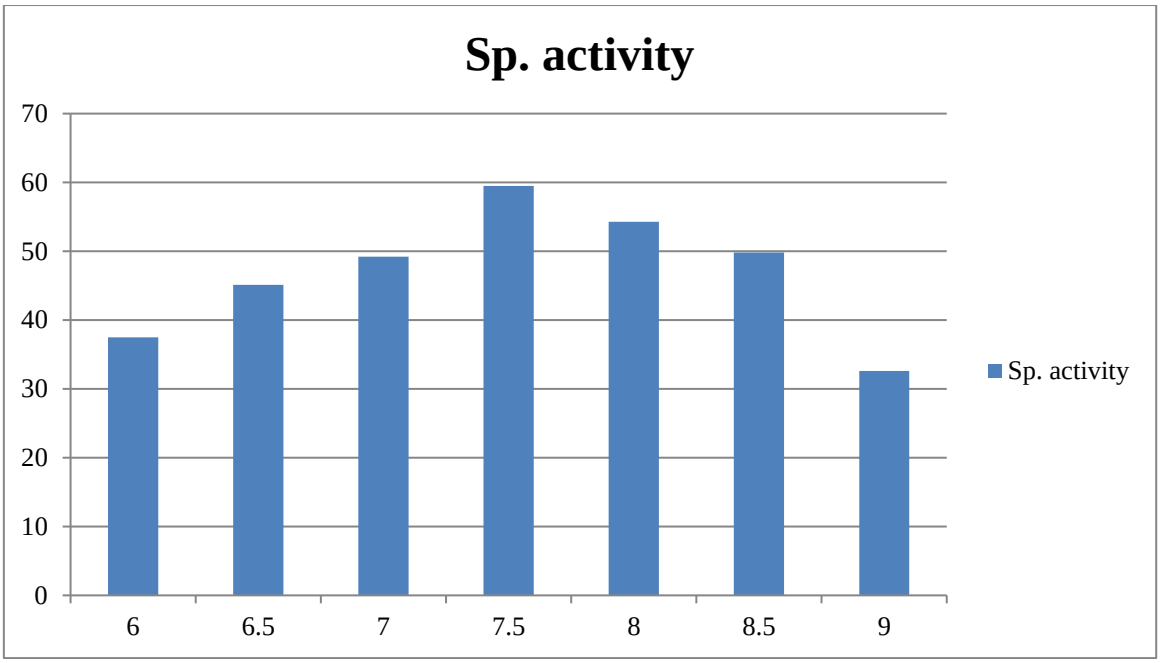

Figure (3-2): Effect of medium pH on protease generated by S9 isolate after 24 hours at 37°C and 150 rpm in a shaker incubator.

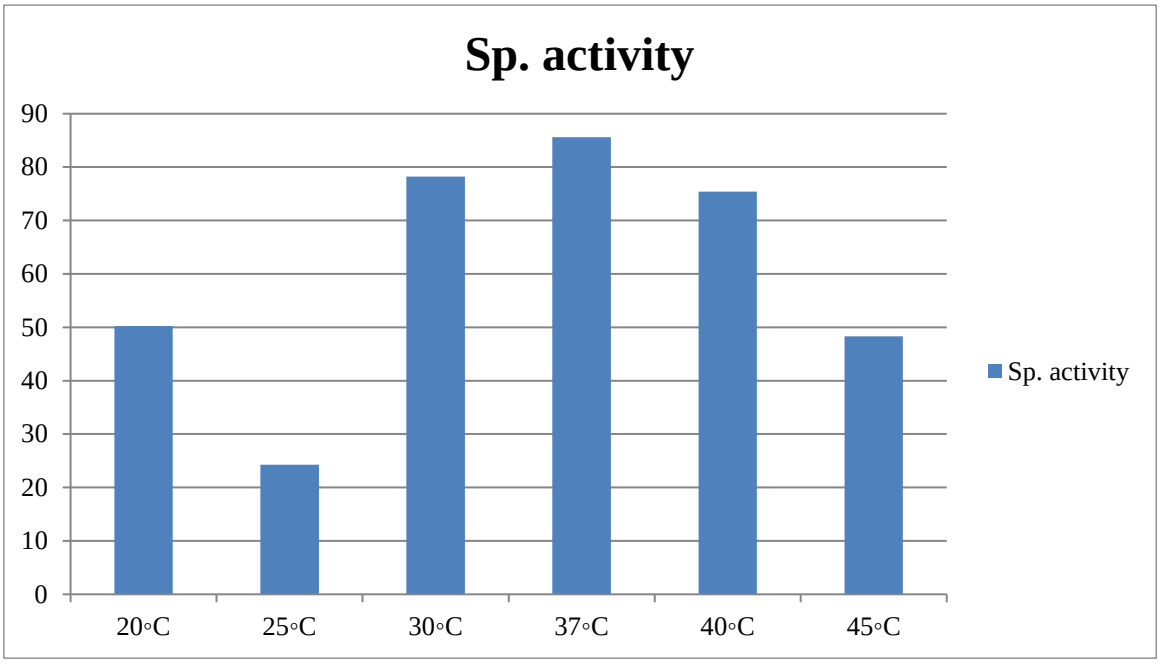

Figure (3-3): Optimal temperatures for the protease released by the S9 isolate after 24 hours at 37°C and 150 rpm in a shaker incubator.

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