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Isolation and Screening of Protease Producing Bacteria from Soil

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ABSTRACT
Proteases execute a large variety of functions and have important biotechnological applications. Proteases represent one of the three largest groups of industrial enzymes and find application in detergents, leather industry, food industry, pharmaceutical industry and bioremediation processes. In the present study soil sample is collected from college primesis and used for screening of protease producing bacteria. On the basis of colony size, texture, and microscopic characteristics, the isolates were identified as Bacillus sps .The two Isolated Bacillus sps were screened for protease producing ability on casein agar and gelatine agar plates. The zone formation around the bacterial colony indicated the protease positive strain which may be due to hydrolysis of casein and gelatine. Hence the strains were identified as a protease producer and it was taken for further experimental studies and biochemical test. The extraction of protease enzyme is important due to its wide industrial applications.

Keywords: *Proteases, Bacillus sps, Enzyme, Proteolytic Ubiquitous*

1. INTRODUCTION

Proteases (EC 3.2.21.24) are the single class of enzyme which occupies a pivotal position with respect to their applications in both physiological and commercial fields. These are degradative enzymes which catalyze the total hydrolysis of proteins by the cleavage of peptide bonds[1]. Proteases are classified according to their structure or the properties of the active site. There are several kinds of proteases such as serine-, metallo-, carboxyl-, acidic-, neutral-, and alkaline proteases [2]. Proteolytic enzymes are ubiquitous in occurrence, found in all living organisms, and are essential for cell growth and differentiation. There is renewed interest in the study of proteolytic enzymes, mainly due to the recognition that these enzymes not only play an important role in the cellular metabolic processes but have also gained considerable attention in the industrial community [3]. Proteases can be produced from wide diverse sources such as plants, animals and micro-organisms. The majority of commercial alkaline proteases are produced by bacteria, especially *Bacillus* sp[4].

Several *Bacillus* species involved in protease production are e.g. *B. cereus*, *B. sterothermophilus*, *B. mojavensis*, *B. megaterium* and *B. Subtilis* [5]

However, until today, the largest share of the enzyme market has been held by detergent alkaline proteases active and stable in the alkaline pH range. They constitute 59% of the global market of industrial enzymes, which is expected to exceed $ 2.9 billion by 2012. Proteases are robust enzymes with considerable industrial potential in detergents, leather processing, silver recovery, medical purpose, food processing and also in waste water treatment [6].

2. MATERIALS & METHODS

2.1 Collection of sample: Soil sample was collected from college premises in a sterial boiling tube.

2.2 Isolation of Pure culture.

1 gram soil sample is dispensed in 9ml of sterile distilled water. This is mixed vigorously and 0.5ml from this is taken and added to another tube with 4 ml sterile distilled water to get a dilution of 10-1. This serial dilution is repeated to get dilutions of 10-2, 10-3, 10-4, 10-5, 10-6, 10-7, 10-8 and 10-9. For the isolation of organisms, 0.1ml of each dilution was plated onto a nutrient rich medium by spread plate method for the propagation of microbial growth. The plates were incubated at 37 0C for 48 hours. Colonies with different characteristic features were obtained and a two colonies were picked, maintained as pure cultures on nutrient agar slants and stored at 4°C[7].

2.3 Characterization of Protease producing organism

Protease producing organism are identified and characterized by morphological characters and biochemical tests.

2.3.1Morphological characterization:

To study the morphological features the pure culture was spread on nutrient agar media plates and incubated for 24 hour at 37°C and Colonies were observed. Gram staining is done to study the gram nature.

2.3.2Biochemical characterization:

Different Biochemical tests were carried out for the isolated pure culture, which includes indole, methyl red, voges-proskauer, citrate, urease and oxidase .[8]

2.4 Screening for protease production:

screening for protease production is done by inoculating the cultures on starch agar, casein agar and gelatine agar. Organisms were also streaked on lipase agar plates to check its multiple enzyme activity.[9]

3. RESULTS & DISCUSSION

RESULTS

The isolated two bacteria are identified as *Bacillus sps* by observing their morphological and biochemical tests.

Table 1 Morphological *Features Bacillus sps1*

Colony Features	**Result**
Shape	Irregular
Size	Medium-Large
Texture	Mucoid
Colour	Cream
Elevation	Flat
Density	Opaque
Margins	Irregular
Gram's Reaction	
Gram nature	Positive
Shape	Rods
Size	Short
Arrangement	Chains/Pairs

Figure 1 Gram staining *Bacillus sps1*

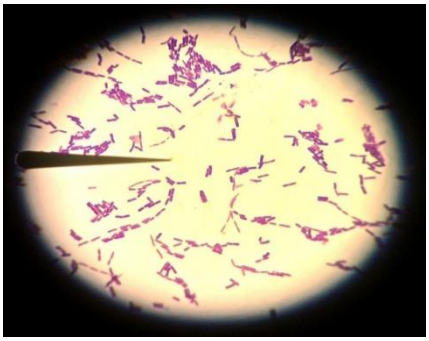


Table 2 Morphological Features *Bacillus sps2*

Colony Features	**Result**
Shape	Irregular
Size	Medium-Large
Texture	Mucoid
Colour	Cream
Elevation	Flat
Density	Opaque
Margins	Irregular
Gram's Reaction	
Gram nature	Positive
Shape	Rods

Size	Long
Arrangement	Chains/Pairs

Figure 2 Gram staining *Bacillus sps2*

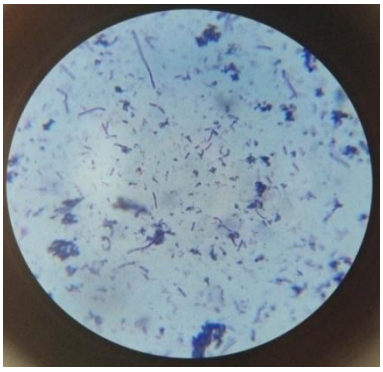


Figure 3 Starch agar plate

Figure 4 Lipase agar plate of *Bacillus sps1*

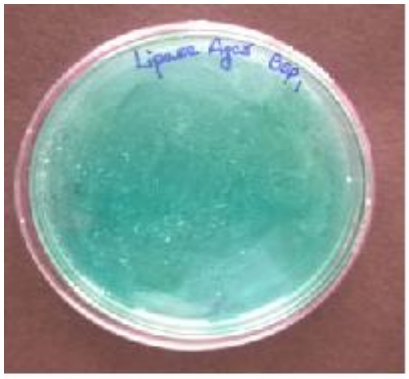


Figure 5 Lipase agar plate of *Bacillus sps2*

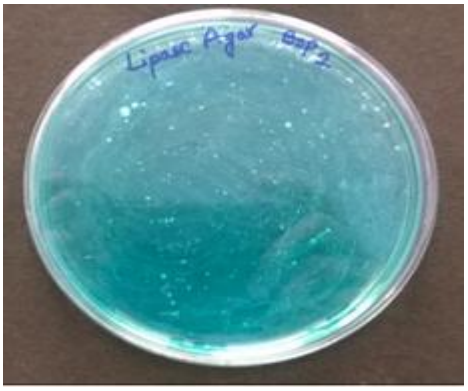


Figure 6 Caesin agar plate of *Bacillus sps1*

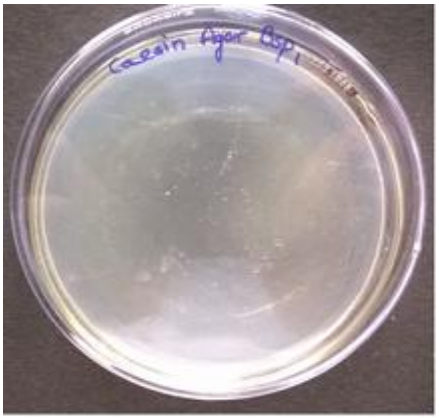


Figure 7 caesin agar plate of *Bacillus sps2*

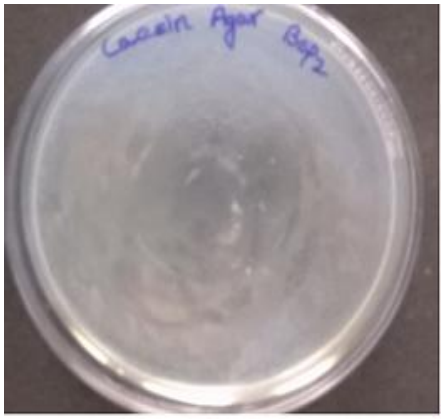


Figure 8 Gelatin agar plate of *Bacillus sps1*

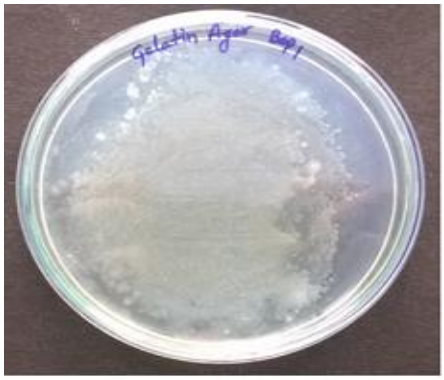
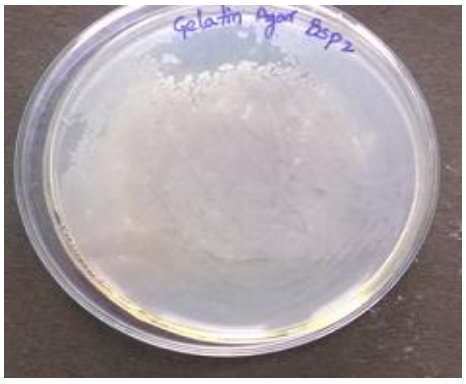


Figure 9 Gelatin agar plate of *Bacillus sps2*



4. CONCLUSION

Proteases are the enzymes having wide range of applications in industry. The two isolated bacteria are identified as *Bacillus sps* .The two species of bacteria streaked on starch, casein, lipase and gelatine agar plates showed zone of hydrolysis indicating the production of protease enzyme.. Catalase and oxidase tests are also positive. The organisms proved to show multiple enzyme activity. Hence the isolated bacteria proved to be potential producers of protease enzyme.

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Author Profile

I **Kummari Swathi finished** Msc from Andhra University, Awarded Gold medal and Best outgoing postgraduate award. Working as a junior faculty at st. francis college with 3+ years of teaching experience. Presented 2 papers at national conferences and won best poster award. Participated in various seminars, symposiums and workshops. Working on 3 different projects, one research paper accepted for international journal.