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Isolation and Characterization of Alkaline Serine Protease Producing Microorganisms from Soil

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ABSTRACT

Protease is most important group of enzymes. Protease enzyme breaks protein into smaller peptides or amino acids. They are essential for many biological processes; they help in digestion and catabolism of protein. Protease producing bacteria & molds were isolated from soil sample.Soil sample was enriched in milk broth & spread on plates of milk agar & incubated at 30^0 C for 48 h.The isolates obtained were *Bacillus spp* , *Pseudomonas spp* , *Micrococcus spp* , and *Aspergillus niger* .They were characterized on the basis of morphological, cultural & biochemical properties. The screening for promising isolates on same media & another basis of selection ratio (size of zone of clearance in mm divided by area of growth of organism in mm) the best promising isolates were *Bacillus spp Pseudomonas spp* , *Micrococcus spp* , and *Aspergillus niger* . Further optimization study is in progress. Protease producing microorganisms produced the clear zone around the colony. Protease are used in various industries such as food and dairy and extensively in the pharmaceutical industries for preparation of medicines

Keywords: *Protease, soil, enzyme, Bacillus spp, Pseudomonas spp, Aspergillus spp, Micrococcus spp*.

1. INTRODUCTION

Proteases are detected in all living species, including eukaryotes such as mammals, plants, fungi, protists, and the prokaryotic domains bacteria and archaea, where they serve critical activities in their metabolism (Rao *et al*., 1998). Alkaline proteases and other commercial enzymes are best obtained from bacteria and fungi because they are easy to cultivate, maintain, genetically transform, and are not impacted by seasonal fluctuations. In addition, they can be produced on a large scale using cheap culture media involving agro-industrial wastes and produced outside the cells as extracellular metabolites facilitating the downstream separation processes (Kotb *et al*., 2023). Furthermore, they can be generated in huge quantities utilizing inexpensive growth media with agricultural and industrial wastes, and they can be generated extracellularly as metabolites, which will help with the subsequent separation operations (Huang *et al*., 2019). Proteases are mostly used for flavour improvement, cheese manufacturing, meat tenderization, therapeutic medication research, biologics production, protein-based stain removal, and other applications in the food, beverage, pharmaceutical, and detergent sectors (Asha and Palaniswamy, 2018). The present study was aimed to isolate and characterize protease producing microorganism from soil. The soil sample were collected and subjected for enrichment for the isolation of microorganism having protease enzyme producing activity.

2. MATERIALS AND METHOD

1) Collection of soil & root sample: Soil samples were collected from nearby A/p wing Tal – Karad Dist - Satara. Soil sample were collected in clean sterile bag

2) Enrichment and isolation of protease degradation microorganisms:

A) Enrichment:

To enrich Protease producing microorganism from soil samples, 1 gm of each soil samples were inoculated into 100 ml of Nutrient broth and incubate at 300 c on shaker for 4 to 6 days.

B) Isolation:

1) Isolation of organism was carried out by spread plate method and streak plate method.

2) For bacterial isolation 1 mL enriched sample was serially diluted and from 10-5 dilution, 0.1 mL sample was plated into Nutrient agar.

3) Plates were incubated under aerobic condition at 30 ^{0}C for 24 h.

c) Screening of isolate for Protease degradation microorganisms:

1.Bacterial isolates grown on Nutrient agar medium were further screened on milk agar medium.

2.Once the incubation is completed, the isolates showing clear zone on milk agar were picked up & separately transferred on nutrient agar slant. These isolates were positive for Protease producing microorganism.

3.Slant was incubated to get sufficient growth. The purified culture used for further studies i.e., cultural and morphological characteristics.

3) Identification and characterization of the isolates-

The colony characterization of well isolated colonies was recorded as their size, shape, margin, color, elevation, consistency. The suspension of well isolated colony was prepared and was used to study its Gram nature by Gram staining and motility by hanging drop technique. The biochemical tests performed were starch hydrolysis, Voges-Proskauer (VP), Oxidase test, and sugar fermentation test. Isolates obtained in pure cultures were characterized by morphology, colony characteristics, and various biochemical tests recommended in the Bergey's Manual of Determinative Bacteriology (1997).

4) Preparation of biomass

The Nutrient broth is used for production of microbial biomass. Four isolates were inoculated into separate broth and kept at 300c on rotary shaker for 6 to 7 days. After 7 days broth was centrifuged at 3000 rpm for 10 min. The pellets were discarded and supernatant was collected for production of protease.

3. RESULTS AND DISCUSSION

1) Result of Sample collection

Sr.no	Sample no.	Date of sample collection	Sample Location	Amount of sample
1.	1	06 March 2024	Soil Sample	5gm

Table no. 1 Result of Sample collection

2) Enrichment result

After the inoculation of 1 gm soil samples were added in 100ml of Nutrient broth and incubation at 37^0c for 4-6 days, increase in turbidity of broth was observed.

3) Isolation result

Microorganisms were isolated on nutrient agar. Ones the isolation was completed then isolated microorganisms was identified by the help of screening.4

4) Screening Result

Four microorganisms where show clear zone of Protease producing around the colony on milk agar.

ISOLATES	TENTATIVE IDENTIFICATION
SR-I	*Micrococcus spp*
SR-II	*Aspergillus spp*
SR-III	*Bacillus spp*
SR-IV	*Pseudomonas spp*

Table no. 2 Tentative identification of isolate:

4)Morphological Characterization results

Colony character	**SR-I**	**SR -II**	**SR -III**	**SR-IV**
Size	1 mm	1 mm	1 mm	1.5 mm
Shape	Circular	Irregular	Circular	Irregular
Color	Creamy White	Black	White	Pale yellow
Margine	Regular	Irregular	Rough	Regular
Elevation	Flat	Concave	Convex	Convex
Opacity	Opaque	Opaque	Opaque	Opaque
consistency	Moist	Dry	Mucoid	Smooth Mucoid
Morphological Characters				
Gram nature	Gram Positive	Gram Negative	Gram positive	Gram Negative
Morphology	Cocci	Filamentous	Short rod	Rod
Motility	Motile	Non-Motile	Motile	Motile

Table no. 3 Colony character and Morphological character

5) Results of biochemical test of the isolates.

Test	**SJ-I**	**SJ-II**	**SJ-III**	**SJ-IV**
Indole	-	+	-	-
MR	+	+	-	+
VP	-	-	-	-
Oxidase	-	-	-	-
Catalase	-	+	-	-
Urease	-	+	-	-
Dextrose	-	(+)	+	+
Sucrose	-	(+)	-	+
Manitol	-	(+)	+	+
Lactose	-	(+)	-	-
Amylase	(-)	(+)	(+)	(-)

Table no.4 Biochemical result

6) Result of protease production

All four isolates were considered good protease producers, exhibiting clearance zones higher than 1.5 mm on milk agar plates incubated for 20–30 h at 37 °C (Singh et al., 2010).

4. DISCUSSION

The present study was aimed to isolate and characterize protease producing microorganism from soil. The soil sample were collected from local village. All the sample were subjected for enrichment for the isolation of microorganism having protease enzyme producing activity. Total four isolate were obtained from enrichment. All isolate subjected to primary screening of protease producing Microorganism the obtained isolated designated as SJ1, SJ2, SJ3, SJ4. These isolate further selected characterization form the colonies character and biochemical characterization. All four isolates were considered good protease producers, exhibiting clearance zones higher than 1.5 mm on milk agar plates incubated for 20–30 h at 37 °C

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