3. "Bacteria a best source for Protease (Peptidase)"

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Abstract

The bacterial soil sample was collected from Costal area of Dhanu beach. Which were subjected to estimate the protease producing bacteria from soil. Bacteria secrets protease enzyme extracellular. Protease Enzyme was active at optimum pH. The cultivation of bacteria on SAM (Skimmed Agar milk) plate medium a zone of inhibition observed after incubating the plate for 2-3 day. Study the colony characterization indicates the isolated bacteria were *bacillus spp*. Further protease assays were done by biochemical testing of bacteria test sample. The assay involved starch hydrolysis, MR-VP, catalase , sugar fermentation etc.

Key- soil, serial dilution, *bacillus spp*, Protease, Enzyme assays.

1. Introduction

Enzyme is the biocatalyst .Enzymes are substrate specific and stereo-specific in nature which works on optimum pH. Protease a group of enzyme hydrolyses the peptide covalent bond present in primary, secondary or tertiary structure of protein. Some protease cleave the polypeptide chain at terminal called exo-peptidase and cleave at internal is called endo-peptidase. Protease enzyme produced in plant, animal, Archae and micro-organism. The *bacillus spp*. is widely used for production of protease enzyme and has wildly application in many industries like detergent, pharmaceutical, leather, food and biotechnology companies etc. Their are different groups of protease which work on different pH i.e. a) Acidic protease Work at pH 2-5, b) Neutral protease work at pH 7-7.5, and c) Basic protease work at pH above 8.The *bacillus spp* was easy to cultivate , screened and have widely used in industries for the production of protease enzyme. In this article shows that, the isolation and characterization of bacteria colonies that produced protease and performed the biochemical's assays for protease enzyme produced from isolated bacterial strain.

2. Objectives

a) Collection and isolation of soil bacteria.
b) Characterization of bacterial colonies.
b) Perform different biochemical assay for protease enzyme production.

3. Method and materials

Sample collection

The soil sample was collected from costal areas of dhanu breach, Mumbai. Take 0.5 gm of soil sample and start with serial dilution method. A final diluted sample (test tube) was used for bacterial isolation. Take 1ml of bacterial suspension from final dilute and spread on the general Nutrient Media plate containing following compositions ,concentration gm/100 ml [Glucose -1, Pepton-0.3 , yeast extract -0.5,KH2PO4 - 0.1 , $MgSO_4.7H_2O$ -0.02, Na_2CO_3-1 , Agar- 2 , PH- around 8] and incubate the plate at37ºC for 2 days.

Growth Media for Selection of enzyme producing strain

Pick up the bacterial colonies grown on nutrient plate medium and streak on the SAM plate medium (streak plate method). The Skimmed Agar milk plate Medium, contain following composition with concentration gm/100ml [Yeast Extract-0.3, Skim Powder-1, casein enzyme hydrolyate-1, dextrose-0.5, Agar-1, D/w-100ml, pH-7.2]. After 24 hrs of incubation the bacteria shows the zone of inhibition.

Morphological analysis and Identification of bacterial strain

The bacterial colonies characterization were studied and the identification of the bacterial strain i.e. *bacillus spp.* To understand the protease producing capacity for given isolate, perform different assay such as, Indole test, Oxidase test, Voges Proskauer test, Methyl red test, starch hydrolysis, sugar fermentation etc.

4. Result and discussion

Bactria grown properly on general nutrient medium (Fig-A). The selected colonies on general nutrient medium were grown further on SAM medium plate (Skimmed Agar milk). After Incubation of plate for 24 hrs gives zone of inhibition, indicates the protease enzyme was released from bacterial strain (Fig B). Further Study the bacterial Colonies the isolated bacterial strain was *bacillus spp.* (Fig-B). The bacteria shows positive result of different assay such as Methyl red, Oxidase test, Starch Hydrolysis, Catalase test (table. No. 1) indicates the bacteria released protease enzyme .The bacteria also shows positive result of MR-VP TEST (Fig. C). The bacterial strain was able to hydrolyzed starch that indicate release of protease, the plate shows zone of inhibition around the colonies (Fig .D). The sugar fermentation assay give the positive result (Table.No.2.) an indicate hydrolysis of sugar molecules.

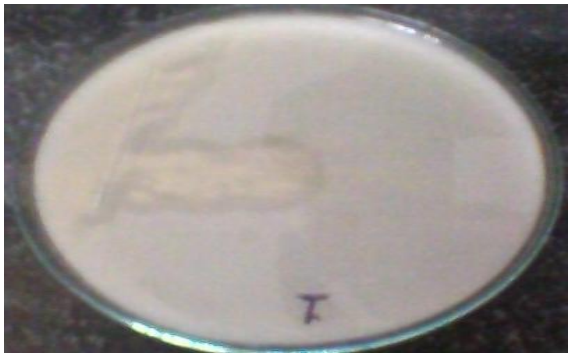

Fig .A. General Nutrient Media plate

Fig .B. Skimmed Agar milk plate Medium

Characteristics	**Colony observation / test**
Size	5 mm
Shape	Circular
Color	White
Margin	irregular
Opacity	Opaque
Consistency	Smooth
Elevation	Convex
Gram character	Gram positive rods
Indole production	Negative
Vogues-proskaur test	Negative
Methyl red	Positive
Citrate utilization	Negative
Oxidase test	positive
Starch Hydrolysis	Positive
Catalase test	Positive
Indole production	Negative
Vogues-proskaur test	Negative
Methyl red	Positive

Table .1. Morphological analysis, Identification of bacteria & different test

Test **Control**

Fig .C. MR-VP TEST

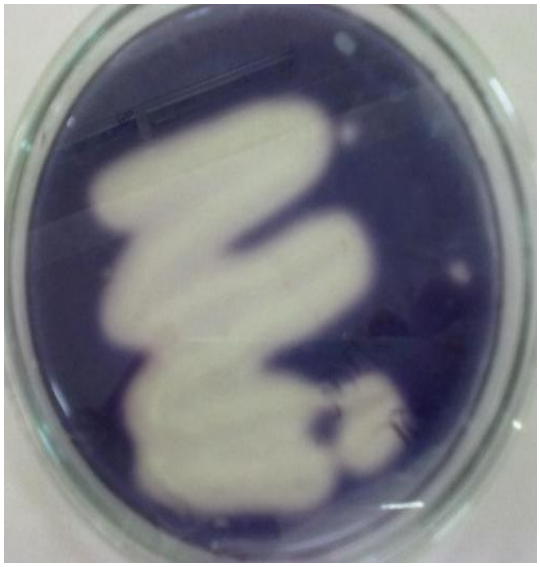

Fig .D. Starch hydrolysis plate

Sugar	Fermented
Glucose fermentation	-
Sucrose fermentation	-
Lactose fermentation	+
Maltose fermentation	-

Table. No .2. SUGAR FERMENTATION

5. Conclusion

The Enzyme protease synthesized and secreted in the presence of protein resources like casein, peptone and yeast extract and able to hydrolyzed it. The Skim agar milk is used to demonstrate the hydrolysis activity of this enzyme and observed a zone of inhibition of bacteria *bacillus spp*. The medium is composed of nutrient agar supplemented with milk that contain the protein substrate casein which were hydrolyses by the bacteria at pH in between 7.2-7.4. The zone around the colonies confirmed that protease actively synthesized and secrets extracellular, observed on skim milk agar plate medium. The assays shows conformation about bacterial strain can produce protease enzyme.

6. Acknowledgement

This research was supported by Dr. Shriniwas .V. Joshi [Principal of GES, Jawhar college dist-Palghar]. Who provided insight and expertise that greatly assisted the research, although they may or may not agree with all of the interpretations, conclusions of this paper or article.

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