St. Xavier's College
(Autonomous), Mumbai

Department of Life Science and Biochemistry

2019-2020

SLSC501PR

Internal Assessment Project Report

Isolation and identification of unknown protease producing bacteria from soil

Name of the student: Mercy Stephen

UID Number: 172027 **Date of Submission:** 9th September,2019

Isolation and Identification of unknown protease producing bacteria from meat sample

Introduction

Proteases are present in at least 1-5% of the genome of infectious organisms .Protease are vital in the imitation and spread of infectious diseases .Protease hydrolyze peptide bonds present in the polypeptide chain of amino acids .Proteases because of their property of specificity and selectivity in protein modification used in various industries such as the textile ,silk, baking, pharmaceuticals etc .Protease can be isolated and purified using fermentation process in a relatively short time .Some of the protease producing bacteria in chicken respiratory tract are *Streptomyces griseus,Staphylococcus aureus, Aerococcus irridans*.

As the poultry meat consumption is steadily increasing worldwide by 14.2 kg per year, it is necessary for slaughter houses to maintain a clean environment and see that the equipments used for cutting and storing meat are free of contaminations. Meat provides an optimal habitat for microorganisms to grow in it.

This study evaluated minced chicken meat for their protease bacteria by using techniques like hexagonal streak plate on Skim Milk Agar plate, Nutrient Agar plate, Gram Staining Biochemical tests, Motility tests and gel electrophoresis. [1]

Materials and Methods

Sample Collection

The minced chicken meat sample was bought from a poultry shop and was kept for one day to rot in a loosely bound container.1 g of the minced meat was taken and diluted in a 9 ml saline aseptically.

Nutrient Agar Plate screening

The sample was ten fold serially diluted till 10^-3 dilution. The viable count was observed of 10^-1 and 10^-2 dilutions on a nutrient agar plate of containing 0.5% peptone, 0.3% beef extract, vitamins, carbohydrate, nitrogen and salt using spread plate method.

To observe the colony morphology of the isolated protease producing colony, the colonies were streaked on nutrient agar plate using hexagonal plate method. The protease producing colony was restreaked to ensure that pure culture colony was isolated.

Gram Staining

Gram staining of the original sample was done .And the colonies were observed under 100X microscope. After isolating a pure protease producing colony gram staining was again performed to ensure that the isolated colony was a pure culture.

Capsule staining

Capsule staining was done on the isolated protease producing colony to observe for capsule present in the isolated colony.

Biochemical Characterization

For biochemical characterization of the protease producing colony, tests like Methyl Red ,Indole Production, Voges Proskauer, Citrate Utilization,Urea,Nitrate,Sugar,7 % NaCl,H2O2 for Catalase tests were performed as per standard protocols.

Motility test

Motility tests was also performed on the Identified protease producing colony

Gel Electrophoresis

Genomic DNA of the culture having protease was used for analysis of 16S rRNA using PCR through Gel electrophoresis the steps taken are as follows-

In an overnight inoculated Super Optimal Broth media 1.5 ml of the culture was taken.

↓

Spun at 14,000 rpm/5 min

↓

Supernatant discarded .Tris EDTA Buffer(400 µl) is added to the pellet. + 10% SDS (100 µl) at 60°C for 10 min

↓

Equal amount of $CuCl_3$ was added

↓

Centrifuged for 20 minutes (4°C , 14,000rpm)

↓

Aqueous phase was separated in a fresh sterile tube.

↓

Chilled ethanol was added (double the amount removed)

↓

µl the mixture mixed with µl ethidium dibromide was loaded on to the Agarose gel for gel electrophoresis

↓

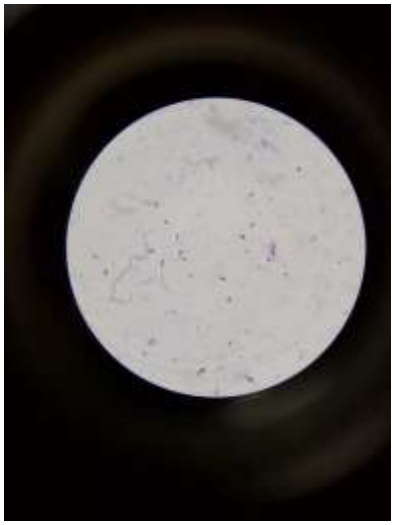
The bands were observed on the computer used UV rays

Protocol

Ten fold serial dilution was done till 10^{-3} dilutions , 10^{-1} and 10^{-2} were plated out in duplicates on Nutrient Agar plates. The viable count of the colonies could not be determined as it was a mix culture.

↓

Gram staining of the original suspension was done .Mixed colonies were observed on the gram stain.



↓

Original suspension was streaked on SMA plate using T shaped plating technique. Growths of various isolated colonies having clearings around them were seen. This showed that the casein was broken down and this proved the presence of protease.

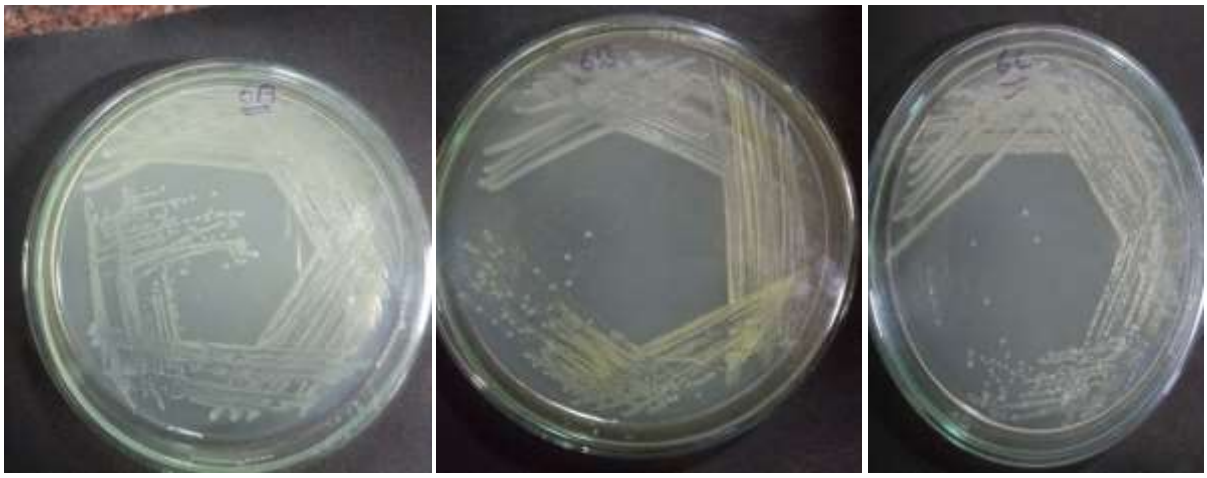
10^{-1} NA duplicate plates and SMA plates

10^{-3} NA duplicate plates

10^{-2} NA duplicate plates and SMA plates

There colonies were picked from this SMA plate and named 6A, 6B and 6C and were streaked on NA plate using hexagonal streak plate technique.

↓



Isolated colonies from each of the NA plate were streaked on SMA plates to check the protease producing activity of the isolates.

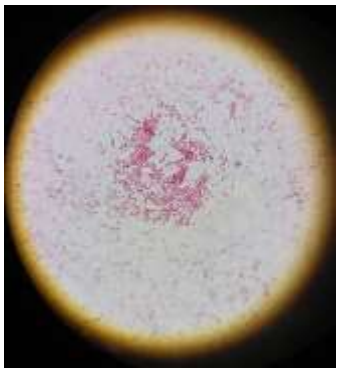
6A and 6C showed the same colony characteristic and they did not show any clearing on the SMA plate,hence were rejected.

6B showed prominent clearing on the SMA plate indicating the presence of protease producers.But due to the mixed nature of the culture on NA plates ,they were restreaked on the SMA plate .Only one type of colonies were observed on the plate.

6B isolate restreaked on SMA plate

6B protease producing isolate
Restreaked on NA

Gram staining and capsule staining of 6B was done.



Shape	Color	Size	Gram Stain
Short rods	**Pink**	**0.4 μm**	**Negative**

↓

Genomic DNA was extracted using the the protocol in fig 1 .Gel electrophoresis was run for the genome of the isolated protease producing bacteria, using PCR for analysis of 16 S rRNA. 6B showed that shredding of the DNA had occurred during extraction but faint color bands were observed

↓

Biochemical tests and motility tests were performed on the isolated protease producing colonies .The tubes were kept for duration of 24 hrs at 37°C

S No	Tests	Results
1	Motility Test	Mobile
2	Capsule	Capsule not present
	Biochemical Tests	
I	Indole test	Negative
2	Methyl Red	Positive
3	Voges Proskauer	Negative
4	Citrate Utilization	Negative
5	Urea	Positive
6	Nitrate	Positive
	Sugar tests	
1	Maltose	Negative
2	Galactose	Positive
3	Lactose	Negative
4	Xylose	Positive
5	Mannitol	Negative
6	7% NaCl	Positive
7	H2O2 for catalase	Positive

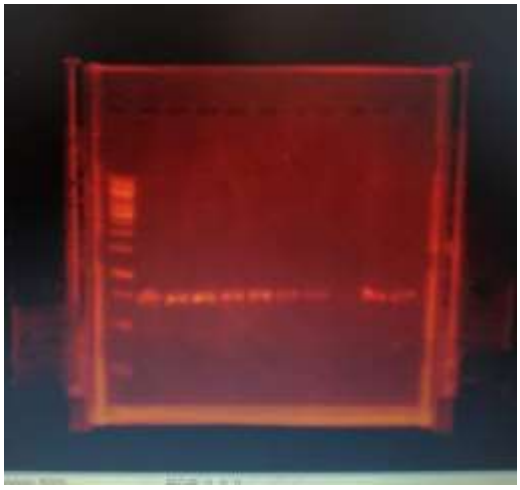


Fig 1 showing DNA bands

Galactose test here color changes from Red to faint Pink

Maltose does not change colour

S**ucrose** test Colour changes from Red to white

Mannitol Motility test showed motile 6 B organisms.

Degradation of Xylose helps the pH to drop which changes the color of phenol red to brown.

Lactose changes from red to yellow

Urea biochemical test positive.

Methyl Red test positive

Citrate Utilization test negative

The positive results show us that the isolated protease producing organism is *Proteus mirabilis.*

Discussion

The procedure taken to isolate the bacteria could have been more easy if more tests like qualitative and quantitative tests for proteins could have also been measured ,to ensure that the isolated is Protein producing or not. Its fermentation time was not recorded and the protease producing organisms optimal pH, temperature was not observed.

References

1. Gayathri Thanigaivel, Anu Swedha Anandhan. Isolation and Characterization of Microorganisms from Raw Meat Obtained from Different Market Places in and Around Chennai. J Pharm Chem Biol Sci 2015; 3(2):295-301
2. ISOLATION AND IDENTIFICATION OF PROTEASE PRODUCING BACTERIA FROM SOIL Vishnu Priya V G (1), Preethi S (1), Karthikeyan S (1) and Ramesh Babu N G
3. Raut, S., Sen, S. K., Kabir, N. A., Satpathy, S., & Raut, S. (2012). Isolation and characterization of protease producing bacteria from upper respiratory tract of wild chicken. *Bioinformation*, *8*(7), 326–330. doi:10.6026/97320630008326
4. Faria Fatima, Neelam Pathak, and Smita Rastogi Verma, "An Improved Method for Soil DNA Extraction to Study the Microbial Assortment within Rhizospheric Region," Molecular Biology International, vol. 2014, Article ID 518960, 6 pages, 2014. https://doi.org/10.1155/2014/518960.
5. Rouger, A., Tresse, O., & Zagorec, M. (2017). Bacterial Contaminants of Poultry Meat: Sources, Species, and Dynamics. *Microorganisms*, *5*(3), 50. doi:10.3390/microorganisms5030050