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Identification of Protease Producing Bacteria from Different Samples

V.P.Mohan Babu, R.Kanakadurga, R.Hema Kalai Rani

Dept. of Biotechnology, Adhiyamaan College of Engineering, Tamilnadu, India

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Abstract - *This study is about the isolation and identification of protease producing bacteria Pseudomonas fluorescens from three different samples soil, water and milk by performing antibiotic sensitivity assay and the bacterial activity zone was measured using the antibiotic sensitivity scale for serially diluted microbes(bacteria). The number of colonies formed for soil, milk and tap water were noted. Similarly the inhibition zone near the streptomycin added well was measured.*

Key Words: Protease, *Pseudomonas fluorescens,* soil, water, milk, antibiotic sensitivity.

1.INTRODUCTION

Protease is a digestive enzyme responsible for proteolysis by hydrolysis of peptide bonds in proteins. Now-a-days due to its vast applications, it is essential to produce protease by microbial techniques. Serial dilution is a technique that is used for counting the number of bacteria that is present in the given sample based on geometric progression of decreasing concentration. The process of counting the number of bacteria present in the sample after serial dilution is called colony counting and it is measured by CFU/ml (Colony Forming Unit). The inhibition zone produced by bacteria is measured by antimicrobial activity by agar well diffusion method. The formation of clear zone by hydrolysis was observed by enzymatic activity.

2. Materials And Methods

2.1 Sample Collection

The Soil sample was collected from the road side. Tap water was collected from the Jayagen Biologics Lab, Chennai. Milk sample was collected from a shop at Chennai.

2.2. Serial Dilution

1 g soil was taken in a test tube with 10 ml of distilled water and concentration was noted as 10^{-1}. Then the test tube was shaken well. 1 ml of solution from the above test tube was pipetted out to a new test tube. Then distilled water was added to make up the solution to 10 ml and concentration was noted as 10^{-2}. Similarly concentrations were made up to 10^{-7}. Similarly, all the three samples were serially diluted up to a concentration of 10^{-7}.

2.3. Colony Counting Method

Nutrient agar were prepared and autoclaved at 121℃. 1 drop of clotrimazole (antifungal agent) was added to the medium in lukewarm condition. Then the medium was poured into six petriplates under aseptic conditions. After solidification of agar, concentrations of 10^{-6} and 10^{-7} of the samples were taken from all the three samples and poured onto the petriplates. The petriplates were incubated for 24 hrs at a temperature of 37℃. After incubation, bacterial colonies were counted and were expressed in CFU/ml.

Table -1: Number of colonies present in the samples

Colonies of bacteria			
	Soil	Tap water	Milk
10^{-6} concentration	54 X 10^6 CFU/ml	22 X 10^6 CFU/ml	69 X 10^6 CFU/ml
10^{-7} concentration	6 X 10^7 CFU/ml	34 X 10^7 CFU/ml	65 X 10^7 CFU/ml

2.4. Antimicrobial Activity

The three agar plates were spread with equal volumes of the three samples of concentration 10^{-7}. 4 wells were created in each petriplate using cork borer (or) tip aseptically. The wells were named as E, S, A, B. In the wells A and B, 50 µl and 100 µl of bacterial culture were added. Well E was used as blank. In the well S, 20 µl of Streptomycin was added which acted as the Standard. After incubation at 37℃ for 24 hours, the zones of inhibitions were measured.

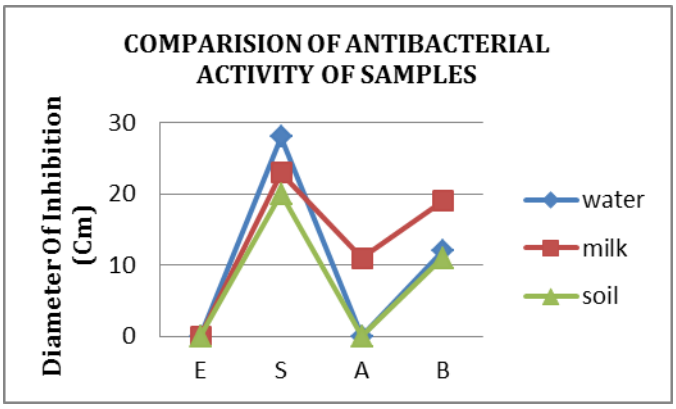

Chart -1: comparison of antibacterial activity.

2.5. Enzyme Assay

A loopful of soil sample (concentration of 10^{-7}) was inoculated into the broth and incubated for 24 hrs. After incubation, the culture was centrifuged at 10,000 rpm for 15 minutes at room temperature. The pellet was discarded and the supernatant was collected in another tube. 50 ml of nutrient agar was prepared and sterilized with two petriplates. After sterilization, the agar was poured into the plates under aseptic conditions and allowed to solidify. After solidification, two wells were created in the two agar plates using cork borer. The wells were named as A and B. 50 µl and 100 µl of supernatant was added into the wells and the petriplates were incubated at 37 ℃ for 24 hrs. Then, saturated solution of ammonium sulfate was poured into the plates. The results were recorded.

3. RESULTS AND DISCUSSION

3.1. Colony Counting Method

The results described indicate that the sample milk contained more number of bacterial colonies compared to the other samples (soil as well as the tap water). This is due to the presence of *Lactobacillus*.

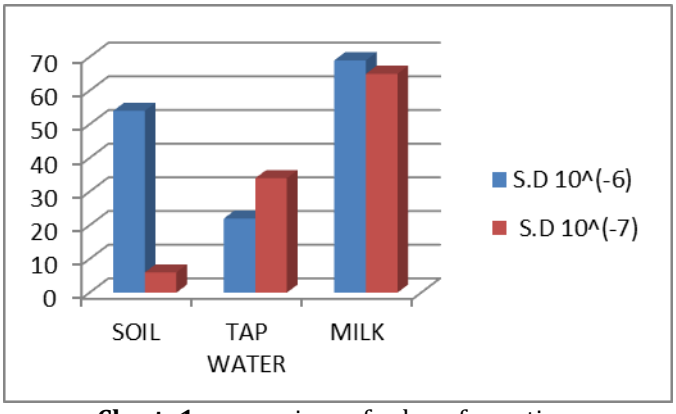

Chart -1: comparison of colony formation.

3.2. Antibacterial Activity

In this antimicrobial activity test, the results showed that the standard solution produced zone of inhibition around the wells than the other wells. As the blank contained no solutions as well as cultures, no zones of inhibitions were found. The wells A and B showed zones of inhibitions.

3.3. Enzyme Assay

Appearance of clear zone around the bacterial supernatant transferred wells in both the petriplates indicates that the bacteria produces protease enzyme.

4. CONCLUSION

In this work, the appearance of clear zone of inhibition on treatment with saturated ammonium sulfate solution indicates the presence of protease producing bacteria. As the bacteria fluoresces under UV light, it was confirmed as *Pseudomonas fluorescens.*

Our further studies include the isolation of several bacterial colonies that produce protease enzyme and improve the microbial production of protease enzyme in industries.

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