

# A Rapid Method for Detection of Proteolytic Activity Using Tannic Acid

<sup>1</sup>Jyoti Sharma, <sup>2</sup>Dr. Nupur Mathur, <sup>3</sup>Dr. Anuradha Singh, <sup>4</sup>Deepa Yadav,  
<sup>5</sup>Aditi Agarwal, <sup>6</sup>Prof. Pradeep Bhatnagar

Department of Zoology, University of Rajasthan, Jaipur, India

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**Abstract:** Routine proteolytic assays employ different developing reagents where the clarity of the hydrolyzed zone is not very sharp and distinctly visible. We here present an easy and improved assay to detect the presence of extracellular protease on milk agar plates. In the following method, 10% tannic acid was flooded post incubation on agar plates and observed for any zone of hydrolysis. The result of the present study clearly defines the method to be highly sensitive for detection of any extracellular protease produced by bacterial cells.

**Keywords:** Protease, Tannic acid, Zone of hydrolysis.

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## 1. INTRODUCTION

Proteolytic enzymes or proteases are a group of hydrolytic enzymes whose catalytic function is to breakdown proteins into polypeptides and free amino acids. They find significant applications in various industries like textile, leather, dairy, detergent and many more [1-2]. Proteases are found to dominate the global enzyme market representing one of the most significant groups of industrial enzymes accounting for approximately 60-65% of total global enzyme market and the sale is also supposed to increase to 1.5-2.5 billion dollars in the near future [3]. Being widespread in nature their sources include all forms of life i.e., plants, animals and microorganism. However microbial sources are highly preferred due to several advantages associated with them. Thus, the isolation of new microbial strains and their subsequent screening becomes mandatory due to several associated advantages.

Proteolytic screening with tannic acid represents an easy, rapid and highly efficient method for qualitative detection of extracellular protease on agar plates. Tannic acid (C<sub>76</sub>H<sub>52</sub>O<sub>46</sub>), a commercial form of tannin, is not a true acid but an acid like substance called a polyphenol which is naturally found in tea, coffee, oak, sumac bark and myrobalan. However the chemical should be handled with care as it causes potential health hazards such as damage to the eye, skin, respiratory tract, and gastrointestinal tract.

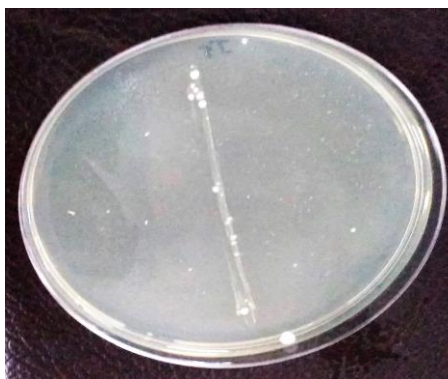
In the following research, effect of tannic acid on proteolytic screening was checked. Tannic acid was added at a concentration of 10% w/v to the agar plate and observed for the presence of any zone of hydrolysis along the line of growth.

## 2. MATERIALS AND METHODS

The microorganism employed in this study was *Bacillus* sp. isolated from soil and maintained on nutrient agar slants. The agar plates were prepared by mixing 15 gm of skim milk in 200 ml distilled water, 20 gm of agar-agar dissolved in 200 ml distilled water, and 600 ml of 0.2M phosphate buffer (pH 7.0). All the medium components were autoclaved separately to avoid milk coagulation and charring due to the presence of buffer salts. Post autoclaving the medium components were mixed aseptically and poured when hot. The test was performed by streaking the microorganism onto the plate followed by incubation at 37°C for 48 hrs. Thereafter 10% tannic acid was flooded on the milk agar plates and examined for the presence of zone of hydrolysis.



**Fig 1: Agar plate showing clear zone of hydrolysis after addition of 10% tannic acid**



**Fig 2: Agar plate showing no significant color change after addition of tannic acid**

### 3. RESULTS AND DISCUSSION

In the present study a very distinct zone was observed after the addition of 10% tannic acid on the agar plates. The intensity of zone produced is directly related to amount of protease produced by the organism. A dark green colored zone against a light background was evident in the plates showing positive reaction (Fig. 1) while no significant color change was witnessed in negative result (Fig. 2).

Tannins are polyphenolic compounds forming insoluble complexes with proteins. Being a very powerful protein precipitating agent they provide a sharp color contrast to the plate creating a very distinct zone of hydrolysis [4].

### 4. CONCLUSION

In conclusion it can be said that tannic acid provides a sharp clear contrast to the plate accounting for a much distinct zone of hydrolysis compared to precipitation with other developing agents. The method therefore serves to be easy, rapid and effective for screening of protease producing microorganisms.

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