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# Characterization of Protease Produced by Bacterial Strains from Tannery Wastes

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**ABSTRACT:** The study aims the isolation, characterization and applications of potential protease producing bacterial strains from tannery industrial wastes that contain large amounts of proteinaceous substances. Different isolates from tannery wastes are screened for the ability to produce protease. Then the potential organisms are tested using various biochemical tests. The organisms showed the maximum growth and enzyme production at  $p^H$  9 and temperature at 40°C. The crude and partially purified protease was preliminarily characterized.

**KEYWORDS :** Tannery wastes, bacterial strains, protease

## I.INTRODUCTION

In recent years, tannery industries have been significantly contributing in the economic development of developing countries. The waste produced from these industries has also increased excessively. The pollution control board's screening of waste disposal from these industries and awareness among the consumers create a plenty of pressure on the leather industries to develop a new method of leather processing by Sundararanjan et al.[1]. A major bottleneck in the leather processing is to dethrone the hair from the skin without inflicting damage. Traditionally hair is removed by using highly concentrated saturated lime and sodium sulphide by Thanikaivelan et al.[2]. The boundless utility of toxic chemicals (i.e., sulphide) pollutes the environment and also intensely affects the health of leather industry workers. Hence, the leather industries tend to implement enzymatic dehairing process, which is more economical and environmentally secure by George et al.[3].

## II.METHODOLOGY

### Collection of sample

The protein rich soil samples were collected from combined effluent of tannery industries at Thomiyapuram, Dindigul district in India. The soil samples were collected in sterile plastic bags and transported to the laboratory maintaining temperature around 4°C as early as possible for microbiological analysis.

### Isolation and Screening of Protease Producing Bacteria

The soil and effluent samples were suspended in water by vigorous vortexing and serial dilutions were made up to  $10^{-6}$  in sterile distilled water. 0.1 ml of appropriate dilution were added to petriplate on skim milk agar plate containing peptone (0.1%), NaCl (0.5%), Agar (2%) and skimmed milk (10%) and incubated at 37°C for three days. Proteolytic bacteria were screened. A clear zone formation around the colonies due to skim milk hydrolysis indicated protease production by the microbes. These colonies were picked and purified by streaking on skim milk agar. The cultures were subsequently sub-cultured and used regularly. Agar slants were prepared and preserved at 7°C for short term storage for further experiments. The protease production by the bacterial isolates were analyzed using standard methods by Anandharaj et al.[4].



**FT-IR spectroscopy attenuated total reflectance (ATR)**

ATR is an IR sampling technique that provides excellent quality data in conjunction with the best possible reproducibility of any IR sampling technique. It has revolutionized IR solid and liquid sampling through: Faster sampling, Improving sample-to-sample reproducibility, minimizing user to user spectral variation Most importantly, the improved spectral acquisition and reproducibility associated with this technique leads to better quality database building for more precise material verification and identification. ATR is clearly an extremely robust and reliable technique for quantitative studies involving liquids.

**III. EXPERIMENTAL RESULTS AND DISCUSSION**

**Isolation and Identification of Bacterial isolates**

The 10 different bacterial strains (8 strains from soil 2 strains from liquid) were isolated on skim milk agar medium. The isolates were checked for quantitative test of protease in liquid medium. Clear that all the bacterial under the study secreted protease enzyme at varied levels. The ten strains (8 strains from soil 2 strains from liquid) which was shown the protease activity are termed as M1S1, M2S1, M3S1, M4S1, M5S1, M7S1, M8S1, M9S1, M1S2 and M2S1. (M stands for microorganisms and S1 and S2 stands for sample 1 and sample 2. Among them two of the isolates (M5S1 and M1S2) showed higher production of protease. Hence the maximum protease producing strains were identified as protease producers and it was taken for further experimental studies and biochemical test. Based on the morphological characteristic and the biochemical tests, the bacterial isolates M5S1 and M1S2 were identified as *Bacillus sp.* and *Pseudomonas sp.* respectively.

**Estimation of Enzyme production**

Pure culture of each isolate was inoculated to the production medium ( contain peptone 0.1%,(w/v),glucose 1.5%, (w/v), NaCl 0.05%,(w/v), MgSo<sub>4</sub> 0.01%,(w/v) and another medium glucose 5.0g,peptone 7.5g,MgSo<sub>4</sub>.H<sub>2</sub>O 5.0g,KH<sub>2</sub>Po<sub>4</sub> 5.0g,FeSo<sub>4</sub>.H<sub>2</sub>O 0.1g,p<sup>H</sup> 7 incubated at 30°C for 48 -72 hours on shaker. At the end of the fermentation period, the culture was centrifuged at 6000 rpm for 15 minutes to obtain the crude enzyme extract which was used as enzyme sources and the protease activity was assayed.

**Table 1: Identified functional groups of protein from FT-IR - ATR (M5S1)**

S.No.	Frequency range	Absorbtion band location(cm <sup>-1</sup> )	Functional groups
1	939cm <sup>-1</sup>	910 + 990	=C-H bend group of Alkenes
2	1082cm <sup>-1</sup>	1000 – 1350	C-F stretch group of Alkyl halides
3	1391cm <sup>-1</sup>	1365 + 1395 (two bands)	-CH (CH <sub>3</sub> ) <sub>2</sub> or -CH (CH <sub>3</sub> ) <sub>3</sub> bend group of Alkanes and Alkyls
4	1647cm <sup>-1</sup>	1640 – 1670	C=C stretch group of Alkenes
5	3286cm <sup>-1</sup>	3265 – 3335	≡C-H stretch group of Alkynes



Table 2: Identified functional groups of protein from FT-IR - ATR (MIS2)

S.NO	Frequency range	Absorbtion band location( $\text{cm}^{-1}$ )	Functional groups
1	$564\text{cm}^{-1}$	500 – 680	C–Br stretch group of Alkylhalides
2	$1187\text{cm}^{-1}$ and $1273\text{cm}^{-1}$	1000 – 1350	C–F stretch group of Alkylhalides
3	$1503\text{cm}^{-1}$	1450 – 1600	ring C=C stretch group of Aromatic compounds
4	$1613\text{cm}^{-1}$	1590 – 1655	N-H bend group of Amides
5	$1701\text{cm}^{-1}$	1680 – 1710	C=O stretch group of carboxylic acids
6	$3406\text{cm}^{-1}$	2500 – 3500	O-H stretch group of carboxylic acids

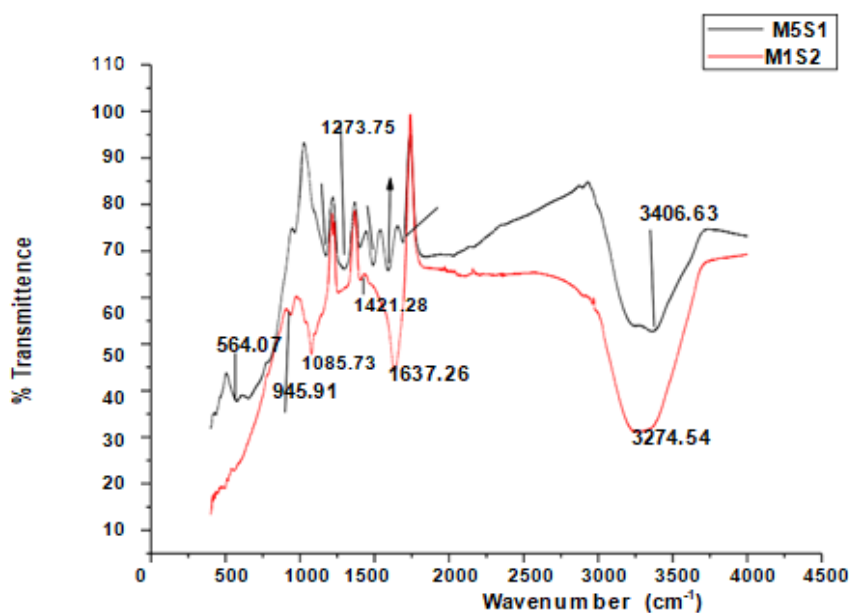


Figure 1: FT-IR Spectroscopy Attenuated Total Reflectance (ATR) of M5S1 andM1S2.

The IR absorption spectrum of the partially purified protease enzyme M5S1 and M1S2 was shown in Figure 1. The M5S1 absorption shows strong peak at  $3286\text{cm}^{-1}$  attributed to the  $\equiv\text{C-H}$  stretch vibration and peaks at  $1647\text{cm}^{-1}$  assigned to C=C stretch vibration, there by indicating the presence of Alkenes and Alkynes in the protein. The C-F stretch vibration frequencies observed at  $1082\text{cm}^{-1}$  confirmed the presence of Alkyl halides groups. The M1S2 absorption shows strong peak at  $3406\text{cm}^{-1}$  attributed to the O-H stretch vibration and peak at  $1701\text{cm}^{-1}$  assigned to C=O stretch, there by indicating the presence of carboxylic acids in the protein. The C–Br stretch vibrations frequencies observed at  $1187\text{cm}^{-1}$  and  $1273\text{cm}^{-1}$  confirmed the presence of Alkyl halides groups. The N-H bend vibrations frequencies observed at  $1613\text{cm}^{-1}$  confirmed the presence of Amides groups. Depending on the fingerprint characters of the peak position, shapes and intensities the other functional groups present in the protein are identified and listed in



the Table 1 and 2 and Figure 1.

Presence of C=O group and  $\equiv$ C-H stretching in the protein sample confirms the presence of amino acids and presence of amino group in their side chain. As there is no absorbance in between the region  $2220 - 2260 \text{ cm}^{-1}$ , indicates that there is no cyanide groups present in the protein. Similar functional groups were also observed in bromelain proteases from *Ananas comosus* with very slight variation in IR bands both in terms of intensity and frequency by Chandrasekaran and Saravanan [5].

#### IV.CONCLUSION

Different isolates from tannery wastes are screened for the ability to produce protease. Then the potential organisms are tested using various biochemical tests. The organisms showed the maximum growth and enzyme production at  $p^H$  9 and temperature at  $40^\circ\text{C}$ . The crude and partially purified protease was preliminarily characterized.

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