



ISOLATION AND CHARACTERIZATION OF PROTEASE AND PROTEASE INHIBITERS OF BACTERIA ASSOCIATED WITH WASTE WATER, AIR AND SOIL.

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INTRODUCTION:

A protease is any enzyme that conducts proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein. Proteases are one of the most abundant classes of enzymes and are involved in a wide range of biological processes, including cell-cycle progression, cell signalling, proliferation and death, protein trafficking and immune response. Proteases are also involved in many human diseases, ranging from degenerative and inflammatory diseases to infectious diseases. Proteases of commercial importance are produced from microbial, animal and plant sources. They constitute a very large and complex group of enzymes with different properties of substrate specificity, active site and catalytic mechanism, pH and temperature activity and stability profiles. Industrial proteases have application in a range of process taking advantage of the unique physical and catalytic properties of individual proteolytic enzyme types [1]. This vast diversity of proteases, in contrast to the specificity of their action has attracted worldwide attention in attempts to exploit their physiological and biotechnological applications [2]. Proteases, the enzymes cutting long sequences of amino acids into protein fragments, are essential for all life forms. Proteases are necessary for the synthesis of 2 proteins, controlling protein composition, size, shape, turnover and ultimate destruction. Their actions are exquisitely selective; each protease splits very specific sequences of amino acids. Therefore, there are many different types of proteases; e.g. humans have over 5,000 different protease genes, accounting for 2% of total human genes [3]. Proteases show potential commercial applications. They are important enzymes in the medical, environmental, food, and chemical industries, etc. [4][5][6]. Proteases bind their substrates through hydrogen bond interactions with the substrate peptide backbone and by hydrophobic and electrostatic contacts between the substrate side chains and well-defined pockets within the active site. There are seven distinct classes of proteases (aspartate, cysteine, glutamate, metallo proteases, serine, threonine and the newly identified asparagine peptide lyases²), grouped according to the amino acid or ion that catalyses peptide bond cleavage,

and the mechanism of substrate cleavage determines which type of chemical entity can be used to inhibit each protease family³. For cysteine, serine and threonine proteases, an electrophilic group can covalently modify the catalytic residue in a reversible or irreversible manner. In the case of metalloproteases, functional groups that coordinate the catalytic metal can achieve potent inhibition. For all protease families, potent transition state analogues can be designed that are based on structural and enzymatic studies. The activity of proteases is inhibited by protease inhibitors. One example of protease inhibitors is the serpin superfamily, which includes alpha 1-antitrypsin, C1- inhibitor, antithrombin, alpha 1-antichymotrypsin, plasminogen activator inhibitor-1, and neuroserpin. Natural protease inhibitors include the family of lipocalin proteins, which play a role in cell regulation and differentiation. Lipophilic ligands, attached to lipocalin proteins, have been found to possess tumour protease inhibiting properties.

MATERIAL AND METHOD:

Isolation of Bacteria:

Isolation of Bacteria, we are taken at. Department of Biochemistry, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar. Isolation is done with help of different natural sources like waste water, Air and soil, which were collected from the garden, industrial sewage, different market places, from different localities of Chhatrapati Sambhajinagar, at 2024.

Collection of the sample from different sources:

Waste water: Isolation of bacteria from waste water was performed by spread plate method as [7]. Sample was spread aseptically to Nutrient agar plates in uniformly. The plates were incubated at 37°C for 24 hr. The bacterial isolates were further sub cultured to obtain pure culture on Nutrient agar were maintained at 4°C.

Air: Isolation of bacteria from air was performed as Nutrient agar plates [7] were exposed to the air for 5-10 min. and after the exposure the plates were incubated at 37°C for 24 hr. The bacterial isolates were further sub cultured to obtain pure culture on Nutrient agar were maintained at 4°C.

Soil: Bacteria was performed by serial dilution and Streak plate method as [7]. Soil sample taken was serially diluted in sterilized distilled water to get a concentration range from 10^{-1} to 10^{-6} . A volume of 0.1 ml of each dilution was transferred aseptically to Nutrient agar plates. The sample was Streak in Four Quadrant uniformly. The plates were incubated at 37°C for 24 hr. The bacterial isolates were further sub cultured to obtain pure culture on Nutrient agar were maintained at 4°C.

Composition of media used in isolation of Bacteria:

Nutrient Agar: Sodium chloride (NaCl) 10.0g, Peptone 10.0g, Yeast Extract 1.0g, Agar-Agar 15.0g, P^H was maintained 7.5, Distilled water 1000ml used for isolation of Bacteria from different samples like the Waste water, Air and soil.

Identification of Bacteria:

After the growth of Bacteria on Petri Plates, the Morphological observations of colonies were external features, Colony color, Shape of colony, Surface of Colony, Colony Pattern, growth rate, Opacity of colony, Margin of Colony, Elevation of colony & microscopic characteristics of shape, size and spore colour of Bacteria. Macroscopic & microscopic features of Bacteria were helpful in accurate identification of Bacteria. The identification of bacteria was done by using various research papers, monographs & other literature such as, Practical Atlas for Bacterial Identification [8], Biochemical Test for Identification of Medical Bacteria [9].

Table No. 01: Isolation of Bacteria from Different natural sources

| Sr. No | Name of Bacterial species | Waste water | Air | Soil |
|--------|---------------------------|-------------|-----|------|
| 1 | <i>Bacillus sp.</i> | ++ | + | + |
| 2 | <i>Pseudomonas sp.</i> | + | - | ++ |
| 3 | <i>E. coli</i> | ++ | ++ | +++ |
| 4 | <i>Salmonella sp.</i> | + | - | + |
| 5 | <i>Vibrio sp.</i> | + | + | ++ |

Activity of Protease:

The Obtained Bacterial colony were taken on a glass slide mixed well in the saline water and this mixed culture solution is spotted on the gelatine X-ray film and incubated for 30 minutes at room temperature. After 30 min. the gelatine film were washed gently under tap water with the help of small paint brush. On the gelatine film the clear-cut zone of inhibition is observed. It shows the protease activity in the bacteria.

Preparation of pure culture:

The colonies obtained from above culture were mixed with Nutrient Broth and that were further used as the pure standard culture or bacterial sample. The activity of an enzyme was found in the extra cellular moiety. The culture was centrifuged at 6000 rpm for 10-15 min. we get the supernatant and suspended cell debris further this are tested for the activity of protease from this test we confirmed that the enzyme is extra cellular.

Preparation of extract for Protease Inhibition Activity:

Extractant that supported maximal extraction of the protein protease inhibitor from the Fruit, Nut and Leaf of different plant materials was selected after standardization of the extraction protocol with different solutions: viz NaCl 15% (w/v), NaOH 0.2% (w/v), HCl 0.05 M, phosphate buffer 0.1 M (pH 7.0) [10] and

deionized water. Extraction was carried out by homogenizing 25 g of sample in 100 ml of the extracting solution in an electrical blender. The prepared homogenate was incubated at room temperature (RT, 28 ± 2 °C) on a rotary shaker for 30 min at 150 rpm. Later the slurry was filtered through cheesecloth and the filtrate was centrifuged at 10,000 rpm for 15 min at 4 °C for the removal of any cell debris that remained in the preparation [11]. The clear supernatant obtained was used as the crude extract for the assay of protease inhibitor activity.

Table No.02: Activity of Protease Inhibitor with Fruit, Nuts and Leaf Extract different plant

+: Positive Protease activity; -: Negative Protease activity

| Sr. no. | Plant Sample Code | Local Plant Name | Botanical Name of Plant | Protease Inhibition Activity | | | | |
|---------|-------------------|------------------|----------------------------|------------------------------|-------------------------|----------------|-----------------------|-------------------|
| | | | | <i>Bacillus spp.</i> | <i>Pseudomonas spp.</i> | <i>E. coli</i> | <i>Salmonella sp.</i> | <i>Vibrio sp.</i> |
| 1 | A1 | Neem | <i>Azadirachta indica</i> | - | - | - | - | - |
| 2 | A2 | Chiku | <i>Manilkara zapota</i> | - | - | - | - | - |
| 3. | A3 | Mango | <i>Mangifera indica</i> | + | - | + | - | - |
| 4. | A4 | Tantani | <i>Tridax procumbens</i> | + | - | + | - | - |
| 5. | A5 | Jamun | <i>Syzygium cumini</i> | + | - | + | - | - |
| 6. | A6 | Badam | <i>Terminalia catappa</i> | + | - | + | - | - |
| 7. | A7 | Tulsi | <i>Ocimum sanctum</i> | - | - | - | - | - |
| 8. | A8 | Sadafuli | <i>Catharanthus roseus</i> | - | - | - | - | - |
| 9. | A9 | Vad | <i>Ficus benghalensis</i> | + | - | + | - | - |
| 10. | A10 | Pimpal | <i>Ficus religiosa</i> | - | - | - | - | - |

Result and Discussion:

During the investigation of Bacterial isolation from different Natural sources like waste water, Air and soil. It is clear from table no. 01. Among total of 20 isolates the 05 bacterial species was identified, it was clear from the table no. 01. The highest occurrence of bacterial species from the source of soil from these source 09 isolates. Next to that 07 isolates were isolated from different waste water source. The lowest occurrence observed on the Air is only 04 isolates. Among all these sources are 05 different bacterial species are isolates e.g. *Bacillus sp.*, *Pseudomonas sp.*, *E. coli sp.*, *Salmonella sp.*, and *Vibrio sp.* The morphological studies of all these bacterial species were carried out and the result is mentioned in Figure no. 01 were noted. All these bacterial species are producing the protease enzyme, it is confirmed to protease activity on gelatine X-ray film (Figure no. 02). All bacterial species are showing the clear zone Inhibition of protease. Among these 05 bacterial species are activity of protease inhibitor with different plant Fruit, Nut and Leaf extract such as

Azadirachha indica, *Manilkara zapota*, *Mangifera indica*, *Tridax procumbens*, *Syzygium cumini*, *Terminalia catappa*, *Ocimum sanctum*, *Catharanthus roseus*, *Ficus benghalensis*, *Ficus religiosa*, it is all from the table no. 02. Among these 10 plants extract the protease inhibition was observed in *Mangifera indica*, *Tridax procumbens*, *Syzygium cumini*, *Terminalia catappa* and *Ficus benghalensis* extracts against *Bacillus* and *E. coli* (Figure no. 03).

Conclusion:

During this investigation more occurrence was seen in the soil as compare to waste water and air. Total 05 bacterial species were isolated from different natural sources; these Bacterial species are capable to produce protease enzyme, it is clear from gelatine X-ray film protease activity. We could extract, purify and characterize the enzymatic activity of protease Inhibition from the Fruit, Nut and leaf extract of different plant. *Manilkara zapota*, *Mangifera indica*, *Tridax procumbens*, *Syzygium cumini*, *Terminalia catappa* and *Ficus benghalensis* these plant extract shows the positive activity against *Bacillus* and *E. coli*. This study indicates natural plant sources can be used for control of Bacterial growth. Bacterial proteases are among the important hydrolytic enzyme and used extensively since the advent of enzymology. It has great importance due to its wide applications in detergent industries, bioremediation, food industries, and leather processing.

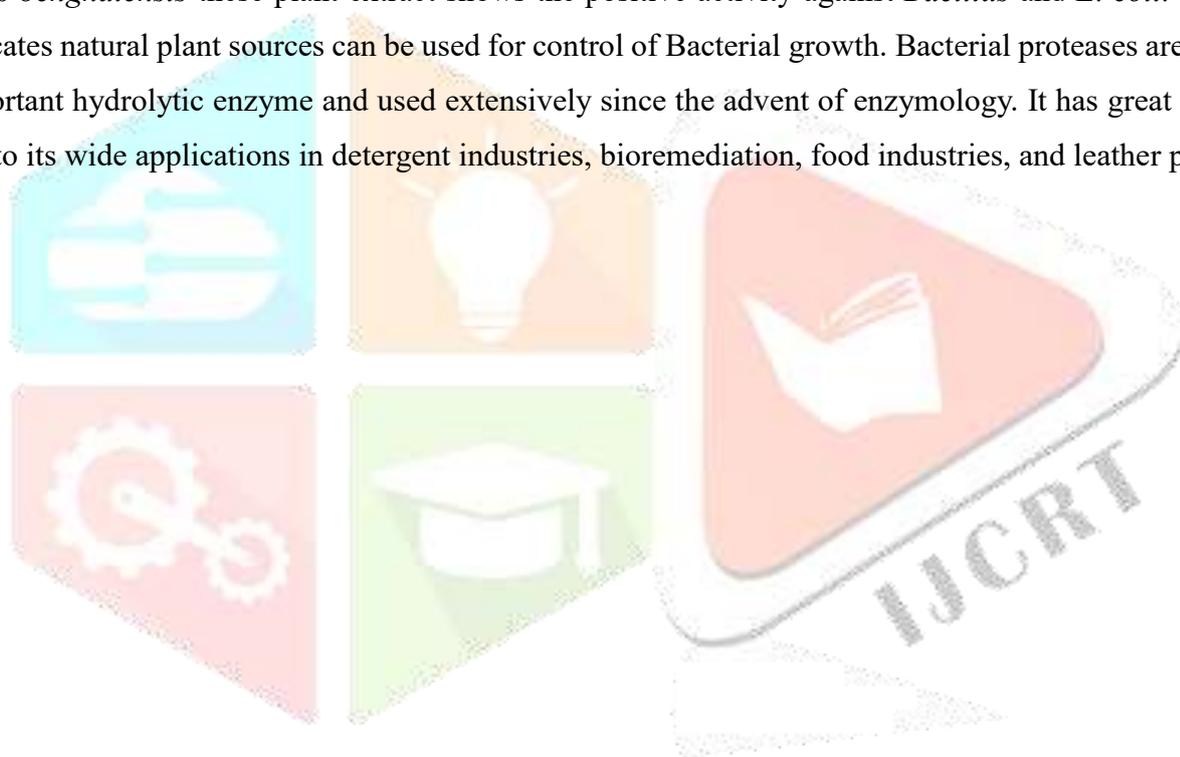
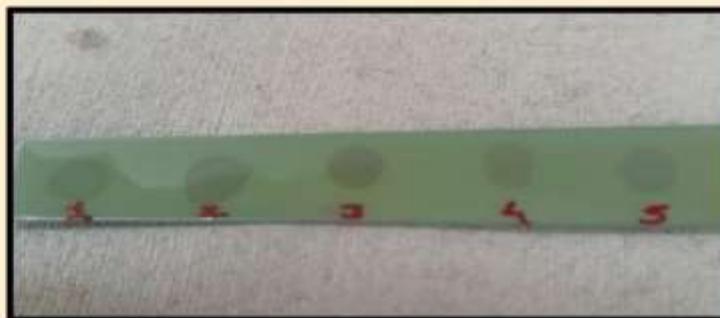
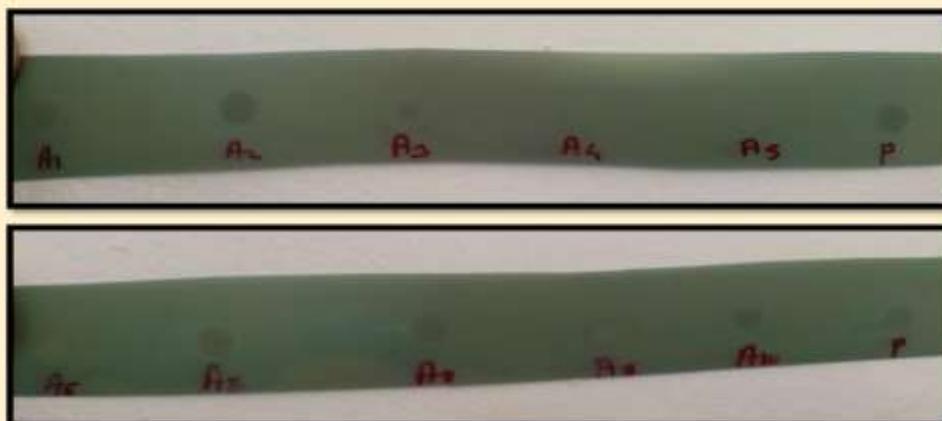


FIGURE NO. 01: PURE CULTURE PROTEASE PRODUCING BACTERIA**FIGURE NO. 02: CLEAR ZONE SHOWS THE PROTEASE ACTIVITY ON GELATINE X-RAY FILM****FIGURE NO 03: EXTRACT + ENZYME ON GELATINE X-RAY FILM SHOWS ZONE OF CLEARANCE AND ZONE OF INHIBITION**

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