Isolation and Screening of Protease Producing Organisms from Soil Sample

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Abstract: **Protease are among the most widely used enzyme in industries like, detergent, food, leather and pharmaceuticals. Protease performs proteolysis by breaking peptide fraction and amino acid. In the present study bacteria were isolated from soil of various regions of Vapi. A total of 14 bacterial colonies were isolated from soil sample. All 14 isolated colonies shows zone of clearance on casein agar plate which indicates their ability to produce protease enzyme. The isolated colony of bacteria showing protease activity was quantified by enzyme assay. Bacterial isolate P-1 shows maximum enzyme activity under submerged fermentation.**

Keywords**: Protease, enzyme activity, soil, Submerged fermentation**

I. INTRODUCATION

Protease is an enzyme that breaks protein into smaller peptide fraction and amino acid by a process known as a proteolysis(Singhal P et al**.,** 2012)..Protease are differing in properties such as substrate specificity, active site, catalytic mechanism, pH and temperature optima (Sumantha et al.,2oo5) . Protease can be found in animal, plant, bacteria, archae and viruses (Rani K et al., 2012). Proteases are widespread in nature, microbes serve as a source of these enzymes because of their rapid growth and limited space required for their cultivation and the ease with which they can be genetically manipulated to generate new enzymes .Proteases represent three largest groups of industrial enzymes and find application in detergents, leather industry, food industry, pharmaceutical industry, biotechnology and bioremediation processes(Gupta, R et al.,2002). Probably the largest application of proteases is in laundry detergents where they help removing protein based stains from clothing (Banerjee et al., 1999). In textile industry, proteases may also be used to remove the stiff and dull gum layer of sericine from the raw silk fiber to achieve improved luster and softness. Proteases are also useful and important components in biopharmaceutical products such as contact-lens enzyme cleaners (Anwar, A et al .,2000). In this study we aimed to isolate newer source of protease producing bacteria from the local soil sample.

II. MATERIAL AND METHOD

Materials

Nutrient agar, Casein, all carbon and nitrogen sources, Follin phenol reagent and others chemicals used were of analytical grade. All these reagents and media were prepared in distilled water.

Methods

Bacterial strains isolation and Screening

Various Bacterial strains were isolated from soil samples collected from the various region of Udwada, Gujarat-396185, India. The soil samples were suspended in sterile distilled water and appropriate dilutions of soil solution were spreaded on the casein agar plate contained (gm/liter):10 casein; 10 peptone ; 5 glucose ; 5 KH2PO4; 20 NaCl ; 5 MgSO4.7H2O; 0.2 FeSO4.7H2O; 30 agar ,pH 8.0. The plates were incubated at 30°C for 24 hrs. After incubation period the casein agar plates were observed for colonies showing zone of clearance and are purified by streaking on casein agar plate. The bacterial isolates showing zone of clearance were stored and maintained in nutrient agar slants at 4˚C.

Enzyme production

Alkaline protease was produced in submerged fermentation, which was carried out in 250 ml Erlenmeyer flasks containing 100 ml of liquid medium for enzyme production.

Inoculum preparation

An isolated colony, from the preserved culture plate was transferred into 50 ml Erlenmeyer flask Containing nutrient broth. The flask was incubated at30°C for 24 hrs at 150 rpm. The freshly grown 24 hrs old culture with 1.0 O.D. at 600 nm is used as inoculum to inoculate in production medium.

Inoculation of production medium

The sterilized production medium containing (/liter):10g casein; 10g peptone ; 5g glucose ; 5g KH2PO4;20g NaCl ; 5g MgSO4.7H2O; 0.2g FeSO4.7H2O, Ph 9.0 was inoculated with 1% of the inoculums of bacterial strain and incubated at 30ºC for 48 hrs under static condition. The samples were Collected at regular intervals and centrifuged at 5000rpm for 20 min. The cell-free supernatant was used as the source of crude alkaline protease enzyme and the pellet was used to measure the cell biomass.

Protease assay

The enzyme was assayed in the Reaction mixture containing 2.0 ml of 0.5% casein solution in 0.1M Carbonate – Bicarbonate buffer pH 9.5 and 1ml enzyme solution in a total

volume of 3.0ml. Reaction mixture was incubated for 5 min at 30°C. The reaction was terminated by adding 3ml of 10% ice-cold trichloroacetic acid (TCA). The tubes were incubated for 1 hr at room temperature. Precipitate was centrifuged at 5000rpm for 15 min and after then supernatant was collected. 5ml of 0.4 M Sodium carbonate and 0.5 ml of Follin phenol reagent were added to 1ml of supernatant, vortexed immediately, then incubated for 30 min at room temperature and OD was taken at 660 nm. Concentration of tyrosine in the supernatant was read from a standard curve for tyrosine already prepared. One unit enzyme activity was taken as the amount of enzyme producing 1µg of tyrosine under standard assay conditions and expressed as units ml-1 enzyme.

Effect of different medium composition on protease production

In present study, bacterial strain with high protease activities were optimized with different production medium. The fermentation was carried out in 250ml Erlenmeyer flask with 100ml of the respective media, which is then inoculated with 1% of the inoculum of bacterial stain and incubated at 30°C under static condition for 48 hrs.

Effect of Time course on protease production

Effect of time course was performed with 1% of the inoculums of bacterial strain. The cultures were incubated for different time period (0, 6, 12, 18, 24, 30, 36 and 48 hrs) at 30°C with shaking at 150rpm. At the end of incubation period, the samples were

III. RESULT AND DISCUSSION

Isolation and screening of protease producing microorganisms

In present study, a total of 14 morphologically different bacterial strains were isolated from different soil samples and were identified as protease producers, which shows zone of clearance around the colonies on casein agar plates. In quantitative screening under submerged fermentation conditions, bacterial strains p1showed highest production (**11.1** U/ml/min) compared to other strains (Table 1).

Thus, P1strain was selected for further study as it exhibited highest protease activity. Fig.1Shows zone of clearance around the colony of the bacterial strain P1 on milk agar plates. The bacterial isolate P1 was found to be Gram-negative, rod shaped bacteria, motile, non-capsulated, and non-sporulated bacteria .

Effect of different medium composition on protease production

In order to determine the optimum medium composition for maximum protease production, various medium composition was inoculated with 1% of inoculums of the selected bacterial strain and was incubated at 30°C for 48 hrs under static condition . The results obtained shows thet a high protease activity was obtained in media 6 which contained (g/liter): 5g casein; 10g glucose; 5g yeast extract; 2g KH_2PO_4; O.5 g NA_2CO_3; pH 8.0 compare to other medium composition s show in Figure 3.Thus, above medium composition was selected for further process parameter.

Effect of Time course on alkaline protease production

According to the results observed at different time intervals, it was noted that a high protease activity (13.12 U/ml/min) obtained at 48 hrs of incubation time. And also high biomass content (1.89) was recorded at 48 hrs of the incubation period as shown in Fig.4 P1 has ability to produce maximum protease in the period of 48 hrs. This finding is comparable with the findings of Vasantha, and Abhilash et al., (2012) who reported high proteolytic activity in *pseudomonas species* at an incubation time of 48 hrs.

IV. SUMMARY AND CONCLUSION

The main objective of our study was to characterize protease producing bacteria and to check their ability for enzyme production. In this study, 14 bacterial strains shows zone of clearance around colony on casein agar plate. Bacterial isolate P1shows large zone of clearance. And P1 were characterized as Gram-negative, rod shaped bacteria, motile, non-capsulated and non- sporulated bacteria. All the above results regarding the optimization of condition in fermentation process as well as activity of the proteolytic enzymes indicate the potential use of the P1 bacterial strain as biotechnological tools for various industrial activities.

Tables and Figures;

Table 1: Morphological and cultural characteristics of different bacterial strainS

Sr.No	sample	Bacterial strain	Colony characteristics	Gram's staining	Motility	Capsule staining	Endospore staining
1	Kitchen waste	**P1**	**Small, round, entire, convex, translucent**	**Gram negative, thin rods**		**Non-capsulated**	**Non-Sporulated**
2	Chicken field soil sample	**P2**	**Large,round,irregular, mucoid,opaque**	**Gram positive thine and thick rod**	**Non- motile**	**Non-capsulated**	**Sporulated**
3	Cattle field, soil sample	**P3**	**Medium,round,entire, Smooth,translucent**	**Gram positive thick rod**	**Motile**	**Capsulated**	**Non-sporulated**

4	Farm, soil sample	**P4**	**Medium,round,entire, Smooth,moist,opaque**	**Gram positive short thick rod**	**Motile**	**Non-capsulated**	**Sporulated**
5	Dairy waste, soil sample	**P5**	**Large,round,irregular, Smooth,opaque**	**Gram positive thine and thick rod**	**Motile**	**Non-capsulated**	**Sporulated**
6	Laundary waste, soil sample	**P6**	**Large,round,irregular, Smooth,opaque**	**Gram positive slender rod with round end**	**Non- motile**	**Capsulated**	**Non-sporulated**
7	Farm, soil sample	**P7**	**Medium,circular,entire, Smooth,moist,opaque**	**Gram positive short thin rod**	**Non- motile**	**Non-capsulated**	**Sporulated**
8	Cattle field , soil sample	**P8**	**Small,round,irregular, Smooth,opaque**	**Gram positive thick rod**	**Non- motile**	**Non-capsulated**	**Sporulated**
9	Laundry wast, soil sample	**P9**	**Medium,round,irregular, Smooth,translucent**	**Gram positive short rod**	**Motile**	**Capsulated**	**Non-sporulated**
10	Dairy waste, soil sample	**P10**	**Medium,round,smooth, Butyrous,opaque**	**Gram positive thin and tnick rod**	**Motile**	**Non-capsulated**	**Sporulated**
11	Chicken field, soil sample	**P11**	**Large,round,entire Smooth,mucoid,opaque**	**Gram negative thin rod**	**Non- motile**	**Capsulated**	**Non-sporulated**
12	Farm, soil sample	**P12**	**Large,circular,entire, Butyrous,opaque**	**Gram positive short rod**	**Motile**	**Non-capsulated**	**Sporulated**
13	Laundary waste, soil sample	**P13**	**Small,round,irregular, Mucoid,opaque**	**Gram positive thin and tnick rod**	**Motile**	**Non-capsulated**	**Sporulated**
14	Kitchen waste, soil sample	**P14**	**Large,irregular,smooth, Moist,opaqe**	**Gram positive thick rod**	**Motile**	**Capsulated**	**Non-sporulated**

Table 2: Screening and protease enzyme activity of different bacterial strains

Sr.no	**Bacterial strains**	**Zone of clearance (mm)**	**Protease activity(U/ml/min)**		
			24hrs	**48hrs**	**72hrs**
1	**P1**	**16**	**9**	**11.1**	**10.3**
2	**P 2**	**13**	**0.8**	**2.4**	**1.9**
3	**P 3**	**5**	**6.6**	**8.4**	**7.6**
4	**P 4**	**7**	**9**	**8.1**	**7**
5	**P 5**	**1**	**7.8**	**2.4**	**5.7**
6	**P 6**	**4**	**5**	**6**	**7.9**
7	**P 7**	**2**	**2.9**	**0**	**0**
8	**P 8**	**6**	**2**	**6**	**4**
9	**P 9**	**3**	**6**	**7.2**	**5.5**
10	**P10**	**5**	**5**	**3.6**	**4**
11	**P 11**	**15**	**8.6**	**10.4**	**9.5**
12	**P 12**	**13**	**4.2**	**3.2**	**2**
13	**P 13**	**9**	**9.6**	**8.4**	**7.2**
14	**P 14**	**11**	**8.3**	**7.2**	**7**

Figure 1: zone of clearance on milk agar plate

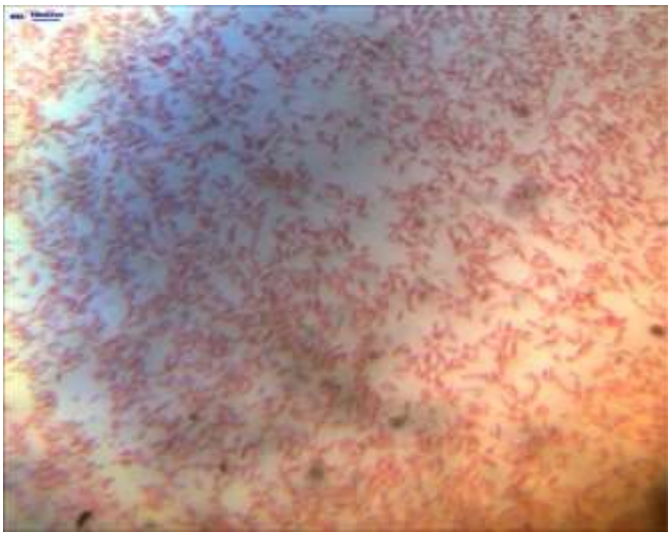

Figure 2: Gram negative rod shaped organisms stains

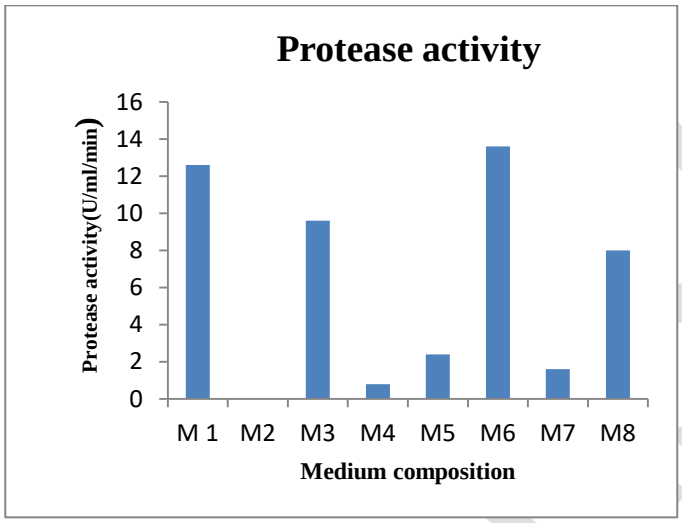

Figure 3 Effect of different medium composition on protease production

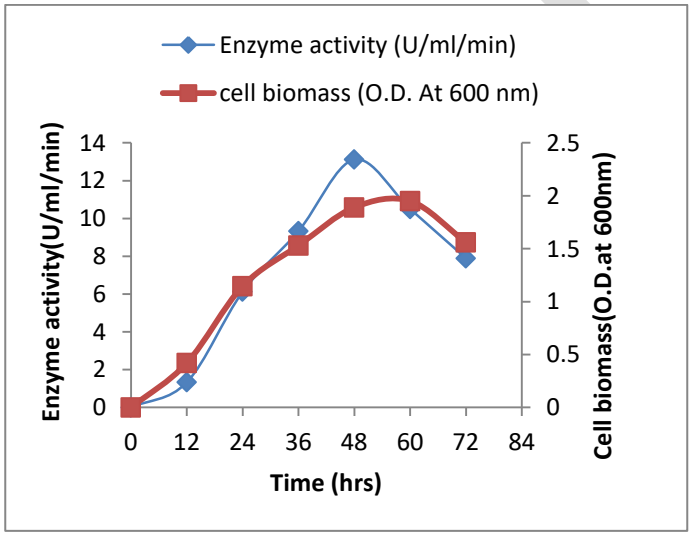

Figure.4.Effect of time period on protease production

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