Screening & Optimization of Protease Enzyme from Marine Derived bacteria

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Abstract- **The study investigated the optimum conditions of temperature, pH, metal ions, nitrogen source and carbon source on bacterial protease production. Protease producing bacterial species were isolated from marine environment located near Valsad. The optimum condition observed for protease production was pH 9.0 in the laboratory medium after 24 hrs of incubation with isolate Vsd4. The study gave the evidence that this bacterial isolate could be potentially applied in enzyme production processes.**

Key words: Valsad, Protease enzyme, optimization of protease enzyme.

I. INTRODUCTION

The world's ocean's coastline is of 312,000 km and it has been used since long period of time for variety of purposes. Marine microbes are found to a potential source for commercially available enzymes. Industries require enzymes which are able to perform at range of physico-chemical conditions and therefore selection of suitable enzymes having optimal performance at desired conditions is of prime importance. Microbial protease represents about 60% of all the industrial enzyme's sales in the world due to their applications in several industrial sectors (Gupte R., 2002). The Proteolytic enzymes with higher activity, stability, extreme pH & temperature are main driving force in search of novel bacteria. Among all the proteases, bacterial protease are most significant because of the abundance and cheap economically. Alkaline protease has considerable industrial appliance due to wide applications in food industries, silver recovery, detergent, waste water treatment, etc. This organism can be exploited because of its ability to produce important compound which can be used for industrial application. The focus of this study is to determine such activity and to identify its role for production of industrially valuable compound. The present study is aimed at isolation and determining the optimum condition for protease activity.

II. MATERIALS AND METHODS

A. Sample collection and enrichment techniques

Soil, sediment and water samples were collected from Tithal beach located at Valsad , in South Gujarat coastal area . Soil and sediment samples were collected in separate polythene bags and water samples were collected in sterile container and transported to the laboratory as soon as possible. The samples were processed within four hours of collection by enriching in marine broth prepared in sea water and allowed to remain on a mechanical shaker for 2- 3h at 150 rpm.

B. Isolation of Marine Bactria

A serial dilution method has been followed after enrichment method for isolation of marine bacteria. The samples were plated on marine agar plates and the plates were incubated at 37°C for 2- 3 days. After incubation the isolates were stored on nutrient agar slants at 4°C for further uses. Twenty one isolates were purified for protease production.

C. Screening for Extra cellular enzymes

All the isolates were screened for the extracellular enzyme protease on Skim milk Agar plate and then incubated for 48 hrs for hydrolysis of protein. After incubation for 2 days, a clear hydrolytic zone formed isolates were selected for further studies & maintained on marine agar slants. The isolates were screened for protease production. Then, potential isolates was/were analyzed for its production at lab scale.

D. Production of Protease medium

One of the bacterial isolates named as DN2/ was selected and used in this study. For protease enzyme production media containing glucose 0.5% (W/%), peptone 1 g, $FeSO_4$. 7H2O 0.1 g, KH_2PO_4 0.5 g, $MgSO_4$ 0.5 g, and NaCl 3 g at pH 7.0 (N.S.Nisha, 2014) was used. Inoculum was developed in marine broth for 24h. 1% inoculums was added to 50 ml of the production medium and then the flaks were incubated at 37°C for 48h. The samples were withdrawn after fermentation and centrifuged for 15 min at 5000 rpm. Then, the supernatant has been used as crude enzyme for protease assay.

E. Protease Assay

Ten ml of the culture medium was taken for centrifuge from which take 3 ml supernatant, 3 ml phosphate buffer, 3 ml 1% casein as substrate .this mixture was incubated for 30 min at 37°C. Then, 2 ml 20% TCA was added. Again the mixture was further incubated for 30 min at room temperature. By adding this precipitation formed so after incubation period the mixture was centrifuged to get supernatant.

Then, take 1 ml supernatant, 2 ml 20% sodium carbonate , mix it well & then add 1 ml Folin ciocalteu reagent (1:10). Mix well & incubate it at room temp for 30 min. To this add 6 ml DW to stop the reaction & absorbance was read at 660 nm using UV viz spectrophotometer

against a reagent blank using a tyrosine standard (Lowry et al., 1951).

F. Optimization of protease enzyme activity

Effect of pH on protease production

Adjust the production medium for optimizing the pH for protease production to different pH value. For which the production medium was separately prepared at pH 6,7,8,9, and 10. The production medium was inoculated with potential isolate and incubated at 37°C.

Effect of temperature on protease medium

The production medium was adjusted at pH 9 and was inoculated with selected bacterial strain. The production medium was incubated at different temperatures from 37 and 50°C for 48h.

Effect of carbon sources on protease production

Activity of the alkaline protease was determined by carrying out at different temperatures viz., 37°C & 50°C. The production medium was inoculated with bacterial strain and then incubated at different temperature.

Effect of nitrogen sources on protease production

Different nitrogen sources like casein, yeast extract, peptone, urea, ammonium chloride were used to determine their effect on protease production.

Effect of metal ions on protease production

The metal ions can influence the production of protease and it was determined by supplying with different metal ions such as KCL, MnSO4, CaCl2, and CuSO4.

III. RESULTS AND DISCUSSION

Twenty one bacterial strains were isolated form marine soil and water samples. All the isolates were screened for protease production by agar plate method. Out of twenty one 10 isolates produced protease enzymes, but one isolate gave maximum zone of hydrolysis on skimmed milk agar plate.

Isolation and Screening of Bacterial strain

In this study, a protease enzyme was produced by a bacterial strain Vsd4 isolated from coastal area of Valsad, Gujarat, India. This strain was as Gram Positive, aerobes, positive for catalase & positive for oxidase.

Morphological characteristics of the Potential Isolate

Tests	**Results**
Pigments	Yellow
Gram reaction	Positive
Cell shape	Cocci
Motility	Non – motile

Biochemical and phenotypic characterization

Tests	**Results**
Gram stain	**+**
Motility	**-**
Catalase	**+**
Oxidase	**+**
Indole production	**-**
Methyl Red	**-**
V – P Test	**-**
Citrate test	**-**
H2S Production	**-**
Urea Hydrolysis	**-**
Phenylalanine deaminase	**-**
Gelatin Hydrolysis	**+**
Sugar Fermentation	
D – Glucose	**+**
D – Fructose	**-**
D – Lactose	**-**
Maltose	**-**
Mannitol	**-**
Xylose	**+**
Nitrate Reduction	**-**
Lipase production	**+**

Effect of pH on protease activity

In the present study, the effect of pH on protease production by isolate revealed that the optimal pH was 9 which have 7.52 U/ml. The protease production was reduced at higher pH like 10. (Figure 1). This gives the attribution that as pH increases much higher then inactivation of enzyme would be occurred. **(**Tsujibo et al., 1990; Mukesh Kumar et al., 2012).

Effect of temperature on protease activity

The effect of temperature on protease production showed that the maximum protease activity was found at 37°C (7.42 U/ml) and minimum activity has been observed at 70°C (2.1 U/ml). At higher temperature the protein and nucleic acids would be denatured. So at higher temperature the inactivation of protein would be carried out. (Figure 1)

Effect of Carbon sources on protease production

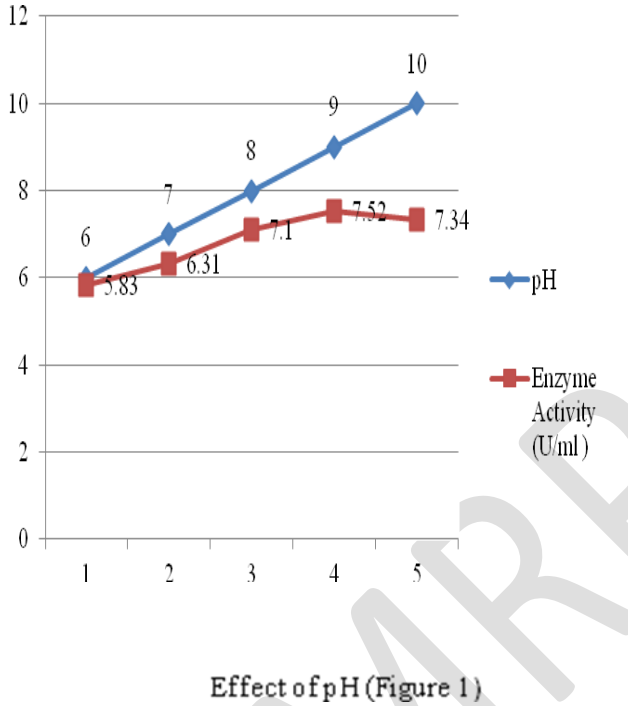
There were five different carbon sources have been used for determination of protease production. After harvesting, the maximum protease activity was observed in glucose (7.89 U/ml) than the other carbon source like fructose, lactose, sucrose, maltose (Figure 3).

Effect of nitrogen sources on protease activity

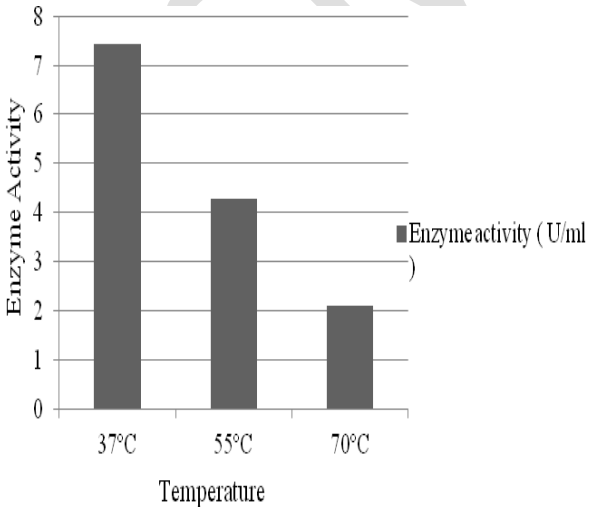
The nitrogen source is an important source for the growth of organism which favors higher enzyme or metabolites production (N.S. Nisha et al., 2014). In this present study organic nitrogen source has higher protease production than the inorganic nitrogen compounds. (Figure 4)

Effect of metal ions on the protease activity

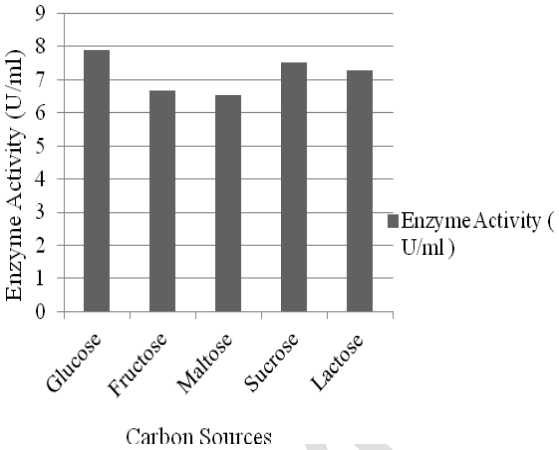
The effect of various metal ions on the protease production was observed. The protease enzyme activity was enhanced by supplementing Calcium carbonate and Potassium chloride. The enzyme activity was enhanced by adding K^{+}, Ca^{+2} salts to the medium for better protease production. By adding Manganese sulphate would be decreased the protease production. (Figure 5)



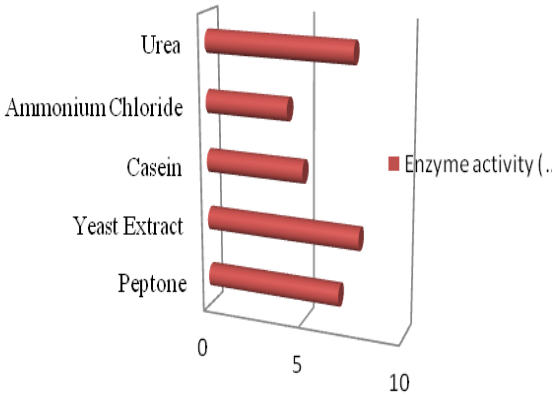
Effect of pH (Figure 1)



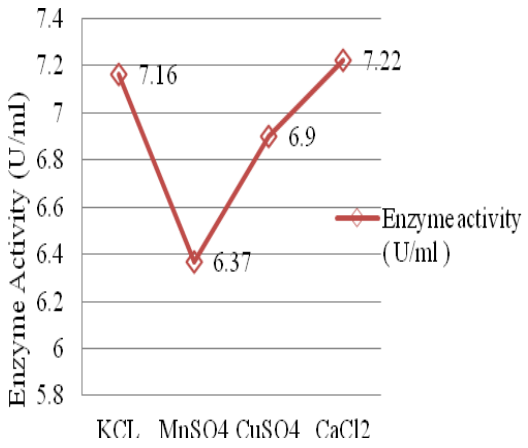
Enzyme Activity vs. Temperature (Figure 2)



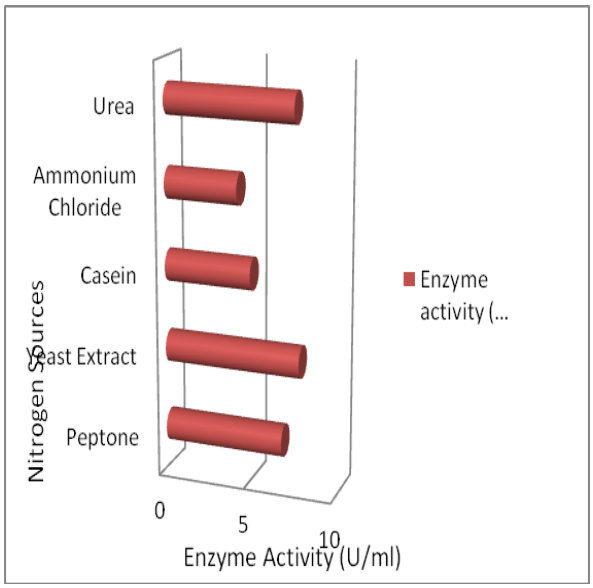
Enzyme Activity vs. Carbon Sources (Figure 3)



Nitrogen Sources Vs Enzyme Activity (Figure 4)



Metal Ions Vs Enzyme Activity (Figure 5)



Enzyme Activity vs. Nitrogen Sources (Figure 3)

IV. CONCLUSION

The marine source gives a wider scope of isolating potential microorganisms which can withstand wider change in physiocochemical conditions and producing economically favorable enzyme production. The use of glucose as a carbon source and organic nitrogen as nitrogen source increased the enzyme production. The addition of metal ions like K and Ca salts increased the production of enzyme. The isolate Vsd4 can be further exploited for the protease enzyme production and scaled up for its industrial application.

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