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Proteolytic Activity of a *Lactobacillus* Species Isolated from Rumen

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Abstract: A protease-producing bacterium was isolated from rumen of goat and identified as a *Lactobacillus* species. Optimum protease producing temperature, medium pH and incubation period of this isolate were 37°C, 8.0 and 48 h, respectively. The crude enzyme of *Lactobacillus casei* showed maximum activity at 50°C temperature and pH 8.0.

Key words: Proteolytic activity, *Lactobacillus casei*

INTRODUCTION

Proteases are group of enzymes, which hydrolyze peptide bonds in proteins and peptides. Microbial proteases have a number of commercial applications[1-3]. A major commercial use is the addition of microbial proteases to domestic detergents for the digestions of proteinaceous stains of fabrics. It has been reported that the production of extra cellular protease by different microorganisms can be strongly influenced by culture conditions[4-8]. So, it becomes necessary to understand the nature of proteases and their catalytic potentiality under different conditions. Thermostable and stability at wide range of pH are desirable properties of any enzyme for industrial application.

The present study, reported the isolation and characterization of a bacterium capable of producing a protease active at alkaline pH.

MATERIALS AND METHODS

Microorganism: The bacterial isolate *Lactobacillus casei* (SYC) were collected from rumen of goat.

Isolation: During isolation of proteolytic microbes enrichment media technique was followed and the isolates were purified by repeated pour and streak plate method.

Screening for proteolytic ability: The bacterial isolate SYC was screened for proteolytic activity by egg albumin, skimmed milk casein and gelatin hydrolysis method. Three screening broth such as peptone-yeast extract-dextrose broth (contained peptone 2%, yeast extract 1%, dextrose 2%)[9], tryptone- yeast extract-dextrose broth (contained tryptone 1%, dextrose 0.1%, yeast extract 0. 5%)[10] and gelatin-yeast extract-glucose broth (contained gelatin 1%, glucose 1%, yeast extract 0.2%, K_2HPO_4 0.3%, KH_2PO_4 0.1%, $MgSO_4.7H_20$ trace)[11] were used for the final selection of the isolate.

Identification of protease producing bacteria: Morphological, cultural and physiological characteristics of the bacterial isolate SYC were studied and these were compared with standard description of Bergey's Manual of Determinative Bacteriology[12].

Protease activity: Enzyme assay was done by the modified method of Hayashi *et al.*[13], as followed by Meyers and Ahearn[14]. Three milliliter of culture filtrates, 3 mL phosphate buffer and 3 mL 1% casein was taken in a 25 mL test tube. Then the test tube was placed in a water bath at 35°C for 1 h. After reaction, 5 mL 20% TCA was added with the solution for stopping the reaction, after 1 h, the solution was filtered by Whatman no. 540 (Ashless). From the filtrate solution 1 mL enzyme substrate mixture was taken into a test tube and 2 mL 20% Na_2C0_3 was added to it. To this mixture 1 mL of Folin Ciocalteu Reagent was added and immediately the contents of the tube were mixed well. After 30 min 6 mL distilled water was added to it and the absorbance of the solution was measured at 650 nm in a spectrophotometer and calculated the amounts of amino acids released from a standard curve plotted from known concentration of tyrosine. The enzyme activity was expressed in Unit. One unit of enzyme was defined as the amount of enzyme that releases 1 μg of tyrosine/mL of crude extract/h.

Culture conditions

Effect of incubation time: To investigate the optimum incubation time for maximum activity of protease, the present study was carried out at different incubation period such as 24, 48, 72 and 96 h.

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Effect of medium pH: The effect of medium pH on the growth and production of protease by selected bacterial isolate was studied at different medium pH i.e. 5.0, 6.0, 7.0, 8.0 and 9.0.

Effect of temperature: The culture medium was incubated at different temperature i.e. 10, 27, 37 and 45°C for optimum enzyme production. The effect of temperature on biomass characteristics, biomass yields and protease production was recorded.

Characterization of protease

Effect of temperature and pH on protease activity: The effect of pH and temperature on activity of protease was investigated by incubating the reaction mixture at pH value ranging from 5.0 to 9.0 using citrate phosphate buffer with different temperature such as 35 to 55°C.

RESULTS AND DISCUSSION

Using enrichment technique twenty-three microbial strains were isolated. Among these one bacterium, SYC showed strong proteolytic ability by hydrolyzing different natural protein such as casein, egg albumin and gelatin and was chosen for further studies.

Production: Although three different broth medium were used during final selection but the isolate SYC exhibited better protease production (activity) in peptone-yeast extract-dextrose broth medium and this medium was finally selected for further studies.

Identification: The morphological, cultural and physiological characteristics of this isolate are summarized in Table 1. The bacterial isolate was identified as belonging to the genus *Lactobacillus* and closely similar to *Lactobacillus casei* Hasen and Lessel.

Effect of incubation period on protease production: Table 2 demonstrates the effect of incubation period on the production of protease by the isolate *L. casei*. It showed maximum protease production at 48 h of incubation period. The highest biomass yield was recorded after 72 h of incubation. The yield of biomass gradually increased with the incubation time. The pH of the culture filtrates was found to range from 6.15 to 6.61.

Effect of medium pH on protease production: The bacterial isolate *L. casei* showed maximum enzyme production and biomass yield at medium pH 8.0. The biomass characteristics was variable with different medium pH. The pH of the culture filtrates was varied from 5.95 to 8.97 (Table 3). Protease production at alkaline pH was also reported by Fermor and Wood[15], Shin *et al.*[16].

Effect of temperature on protease production: The effect of temperature on production of protease was also studied. The biomass characteristics, biomass yield and protease production at different incubation temperature by *L. casei* were profile in Table 4. The highest protease production was recorded at incubation temperature 37°C

Table 1: Morphological, cultural and physiological characteristics of the isolate SYC

Vegetative cells	Short rod
Spore staining	Non spore former
Gram staining	Gram positive
Acid fast staining	Non-acid fast
Motility test	Positive
Agar colonies	Form-circular-margin-entire, elevation- convex, glistening surface and yellow in color
Agar slant	Filiform growth
Nutrient broth	Turbid growth
Glucose broth	Turbid growth
Asparagin broth	Negative
Catalase test	Negative
Oxygen relationship	Aerobic
Indole test	Negative
Nitrate reduction test	Negative
Growth in synthetic medium	Negative
Inorganic salt medium	Negative
Growth in citrate medium	Scanty turbid growth
Liquefaction of gelatin	Positive
H_2S production	Negative
Proteolysis test	Positive
Casein hydrolysis	Hydrolyzed
Voges- Proskaur test	Negative
Starch agar	Hydrolyzed
Growth at different temperature	10, 27, 30, 37, 40 and 50°C - ++ ++ +++ +++ -
Growth at different NaCl concentration (%)	0 1 2 3 4 5 6 7 +++ ++ - - - - - -
Gelatin hydrolysis	Positive
Urease test	Negative
Oxidase test	Positive
Fermentation of different carbohydrates	Alkali:Galactose, Raffinose, Mannitol Sucrose, Rhamnose, Arabinose, Cellubiose, Starch, Inulin, Xylose. No fermentation: Glucose

Table 2: Effects of incubation periods on the production of protease by the isolate *L. casei*

Incubation periods	pH of the culture filtrates	Biomass charact-eristics	Biomass yield (absorb-ance at 600 nm)	Protease activity (U mL^{-1})
24	6.61	Turbid growth	0.827	321
48	6.58	Turbid growth	1.141	369*
72	6.23	Turbid growth with some sedimentation	1.480	221
96	6.15	Turbid growth with some sedimentation	1.381	150

Note: Initial color of the medium Golden Yellow; pH 7.0; temperature 37°C

Table 3: Effects of medium pH on the production of protease by the isolate *L. casei*

Medium pH	pH of the culture filtrates	Biomass charact-eristics	Biomass yield (absorb-ance at 600 nm)	Protease activity (U mL^{-1})
5.0	5.95	Turbid growth	0.463	473
6.0	7.14	Turbid growth	1.109	516
7.0	8.09	Turbid growth	1.409	557
8.0	8.86	Turbid with some sedimentation	1.509	884*
8.5	8.88	Turbid with some sedimentation	1.411	808
9.0	8.97	Turbid with some sedimentation	1.001	684

Note: Initial of the medium pH 7.0; temperature 37° C

Table 4: Effects of temperature on the production of protease by the isolate *L. casei*

Temp. (°C)	pH of the culture filtrates	Biomass charact-eristics	Biomass yield (absorb ance at 600 nm)	Protease activity (U mL^{-1})
10	6.89	Turbid growth	0.373	504
27	7.12	Turbid growth	0.960	561
37	7.48	Turbid growth	0.944	630*
45	7.56	Turbid with some sedimentation	0.374	621

Note: Initial medium pH 7.0; temperature 37° C

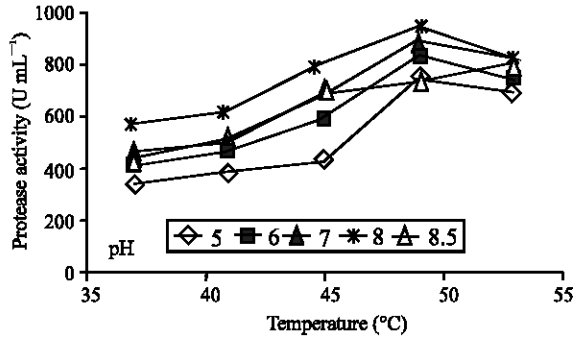

Fig. 1: Effect of temperature and pH on activity of protease

but the highest yield of biomass was observed at 27°C. The pH of the culture filtrates was found to range from 6.89 to 7.56. The isolate prefer 37°C for maximum production of protease, which are in concurrence with the report of Al-saleh and Zahran[17], Shumi *et al.*[4].

Characterization of protease

Effect of temperature and pH on activity of protease: The crude enzyme of *L. casei* showed maximum activity at pH 8.0 and temperature 50°C (Fig. 1). Similar results also reported by Rajakumar[18], Thangam and Rajkumar[19], Saad and Hoda[20]. From the above results it can be concluded that the proteolytic enzymes of *L. casei* is thermostable and active at wide range of pH, which are significant of any enzyme for industrial applications.

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