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Research Article

Isolation and characterization of cold-active protease producer from ice factory samples

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Abstract

Two psychrophilic organisms were isolated from the soil sample of ice factory, Phule market, Nanded, Maharashtra. Of these one efficient Cold-active protease producer, casein hydrolysing Gram positive, round shaped motile bacterium was selected and designated as IF1. The isolate was identified by morphological and biochemical characters as *Peptostreptococcus magnus* on the basis of Bergey's manual of systematic bacteriology.It have optimum growth at pH 7 and 4°C temperature. Further extraction, partial purification and characterization of alkaline protease were carried out. After dialysis, catalytic activity of IF1 cold-active protease was determined as 279 IU/ml.

Keywords: Ice industry, *Peptostreptococcus magnus*, Protease, Psychrophiles.

INTRODUCTION

Proteases refer to a group of enzymes whose function is to catalyse hydrolysis of proteins. Proteolytic enzymes play an important role in digestion as they breakdown the peptide bonds in the protein foods to liberate the amino acids to fulfil the need of body. Beside this, protease has also been used in various forms of therapy and clinical studies like oncology, inflammatory conditions, blood rheology control, and immune regulation[1].

Detergent fortified with cold active protease can be used in cold wash treatment to improve washing quality of detergent. In present investigation we therefore worked on isolation, characterization of efficient protease producer using ice factory sample[2, 3].

MATERIALS AND METHODS

A. Sample collection

Soil samples were collected from an ice factory of Nanded district, Maharashtra. The samples were collected in sterilized polythene bags and transported to the laboratory [4-7].

B. Isolation of psychrophiles

1 gm of composite soil sample was transferred to a 250 mL flask containing 100 mL sterile distilled water and shaken in cooling shaking incubator for 5 °C at 120 rpm for 20 min. Serial dilutions were made and 0.1 mL of aliquots were spread on nutrients agar plates. The plates were incubated for 5 days at 5 °C [8-14].

C. Screening of isolate for cold-active proteolytic activity

Selected isolates were spot inoculated on skimmed milk agar plates and incubated at 5 °C temperature for 5 days. After incubation, plates were observed for the formation of zone of clearance around the colonies[10-14].

D. Identification of efficient cold-active protease producer

Gram staining of selected isolate was performed. Catalase activity, indole production, citrate

utilization, lipase, Pectinase, amylase and Urease production tests were performed. Methyl red, Vogues-Proskauer and carbohydrate fermentation tests were performed by standard procedures. Appropriate positive and negative controls were used[14-20].

E. Cold-active protease production

0.5 mL culture of selected isolate was inoculated in 250 mL of tryptone yeast extract glucose broth containing tryptone 5 g/L, yeast extract 2.5 g/L and glucose 1 g/L (pH 7.2). Protease production was carried out at 5 °C temperature and 100 rpm agitation speed for 48 h in shaking incubator[14-23].

F. Partial purification

The cold active protease was partially purified from fermented broth by centrifuging at 8,944 ×g and 5 °C for 20 minutes. The supernatant was collected and filtered through Whatman filter paper 41µm. To the supernatants ammonium sulphate was added slowly with continuous stirring to the final concentration of 80 % saturation and this solution was allowed to stand for 24 h at 4 °C and further centrifuged at 8,944 ×g for 20 min. The precipitate was resuspended in 50 mM Tris HCl buffer having pH 8.0 and further precipitated with acetone by adding slowly to the final concentration 80 % saturation and left for 1 h at 4°C. The pellet was collected by centrifugation at 8,944 ×g for 20 min at 5°C and resuspended in 20 mM Tris HCl buffer (pH 8.0) and dialyzed against 500 ml of same buffer containing 1 mM $MgCl_2$ over night at 5 °C with stirring conditions. The dialysate was centrifuged at 5,724 ×g for 20 min at 5 °C and supernatant was collected[14-23].

G. Determination of protein content and proteolytic activity assay

The proteolytic activity was determined by using casein as substrate. The substrate contained 3.75 ml of 1.0 % casein in 100 mM Tris-HCl buffer and 1 mM $MgCl_2$ at pH 8.0. The 0.5 ml of protease sample was added to the substrate and incubated for half an hour at 5 °C. After incubation, the reaction was quenched with 0.5 mL of 10 % trichloro acetic acid. The quenched reaction mixture was centrifuged at 10,000 rpm for 10 min to pellet precipitated protein and absorbance for the supernatant was determined at 280 nm. One unit of proteolytic was defined as the amount of enzyme that produced an increase of absorbance at 280 nm of 0.1 under the conditions of the assay[14-24].

RESULTS AND DISCUSSION

Isolation, screening and identification

Twelve colonies were appeared on nutrient agar plates. Of these, two morphologically distinct colonies were selected and designated as IF1 and IF2. The extracellular protease producer was screened by streaking bacterial strains on skimmed milk agar and incubated for 48 hrs 4°C.

The isolate IF1 has showed largest size of zone of clearance than IF2. Therefore IF1 isolate was selected for further studies.

IF1 isolate was Gram positive, motile and round shaped bacterium and positive for Catalase, Methyl red, citrate utilization and casein hydrolysis and negative for Indole production, VP, Urease, Cellulase and Pectinase production (Table 2).

It does not utilize any of the fructose, glucose, maltose and sucrose as a carbon source.

Morphological and biochemical characters of IF1 isolate have been given in Table1 and Table 2 respectively.

On the basis of biochemical characterization the isolates IF1 was identified as *Peptostreptococcus magnus* by comparing with standard strain of Bergeys Manual of systematic bacteriology.

Protease production and assay

The *Peptostreptococcus magnus* secretes large extracellular enzyme protease in the surrounding medium.

The 80% ammonium sulphate saturation leads the precipitation of the protease at 4°C and fractional precipitation with acetone. The excess salt removed from protease by means of a dialysis. The proteolytic activity was determined by using casein as substrate in Tris HCl buffer. Catalytic activity of IF1 protease was determined 279 IU/ml.

CONCLUSION

Efficient protease producer was isolated from soil sample of Ice factory and identified as *Peptostreptococcus magnus* based on morphological, microscopic, biochemical and physiological characters. *Peptostreptococcus magnus* can be used for production of protease and further in enzyme biotechnology field.

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Table 1
Colony Characters of IF1

Characters	Isolate IF1
Size	1 mm
Shape	Circular
Color	Off white
Margin	Entire
Surface	Smooth
Elevation	Raised
Consistency	Sticky
Opacity	Translucent

Table 2
Biochemical Characteristics of IF1

Test	Result	Test	Result	Test	Result
Catalase	+	Amylase	+	Protease	+
Indole production	-	Urease	-	Fructose	-
Methyl red	+	Lipase	+	Glucose	-
VP	-	Cellulase	-	Maltose	-
Citrate utilization	+	Pectinase	-	Sucrose	-

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