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Short Communication

Optimization of thermostable alkaline protease production from species of *Bacillus* using rice bran

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A protease producing microorganism was isolated from soil collected from a detergent industry and identified as *Bacillus* species. Isolate K-30 produced thermostable alkaline protease utilizing rice bran. The optimum conditions for protease activity was 55°C at pH 9 with 4% inoculum in the medium containing 1% rice bran after 96 h of incubation. Beef extract, tryptone and yeast extract were good nitrogen sources while lactose, starch, and sucrose were suitable for enzyme production. The extracellular production of the enzyme, its thermostable nature and compatibility with most commercial detergents are features which suggest its application in the detergent industry.

Key words: Alkaline protease, rice bran, thermostability, detergent.

INTRODUCTION

Proteases are essential constituents of all forms of life on earth including prokaryotes, fungi, plants and animals. Proteases are highly exploited enzymes in food, leather, detergent, pharmaceutical, diagnostics, waste management, and silver recovery. The protease enzyme constitutes two thirds of total enzymes used in various industries and this dominance in the industrial market is expected to increase by the year 2005 (Gupta et al., 2002). Of all proteases, alkaline proteases produced by *Bacillus* species are of great importance in detergent industry due to their high thermostability and pH stability. For production of enzyme for industrial use, isolation and characterization of new promising strains using cheap carbon and nitrogen source is a continuous process (Parekh et al., 2002). Rice bran, a by product of the milling of rice is a good source of proteins at present underutilized as a food material. The potential of producing rice bran at the global level is 27.3 million ton. The present study was undertaken to examine the effectiveness of rice bran as potent alternative protein source in production medium and optimization of enzyme production conditions using rice bran.

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MATERIALS AND METHODS

Screening of microorganism

For isolation of protease producing organism, soil samples were collected from the vicinity of Nellore detergent industry in Andhra Pradesh. Briefly, 1 g of the sample was suspended in 100 ml sterile distilled water, agitated for 45 min on a shaker at 50°C and 0.2 ml was spread on casein agar plates (nutrient agar with 1% casein) and incubated at 30°C for 7 days. Enriched sample was plated over nutrient agar containing 0.4% gelatin (Harrigan et al., 1966). After incubation for 24 h, plates were flooded with 1% tannic acid. Colonies showing clear zone were picked and purified. A total of 75 isolates were screened for protease production by using casein digestion method. Two isolates K-21 and K-30 which showed maximum activity were selected and maintained on nutrient agar at 4°C. The cultures were examined for various morphological and biochemical characteristics as per Bergey's Manual of determinative Bacteriology (Holt et al., 1994).

Protease assay

The proteolytic activity was assayed by casein digestion method (Manachini et al., 1989) at 55°C, pH 8.0 in 50 mM Tris-HCl buffer. One unit of protease activity is defined as the amount of which liberates 1 □g of tyrosine min^{-1} under experimental conditions.

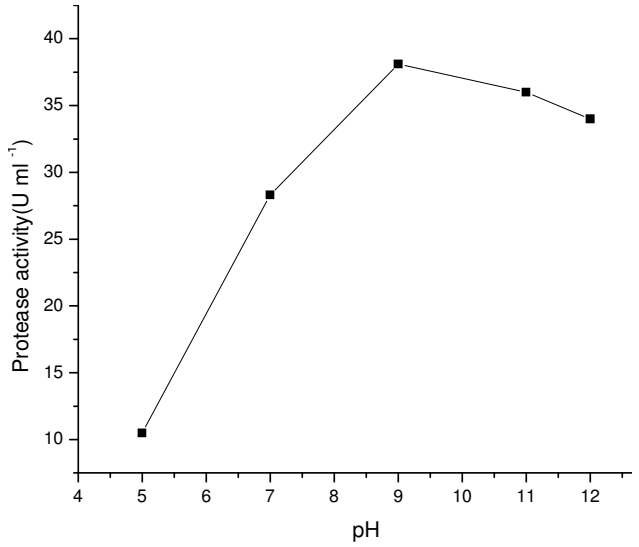

Figure 1. Production of protease by *Bacillus* sp. K-30 at different pH using rice bran.

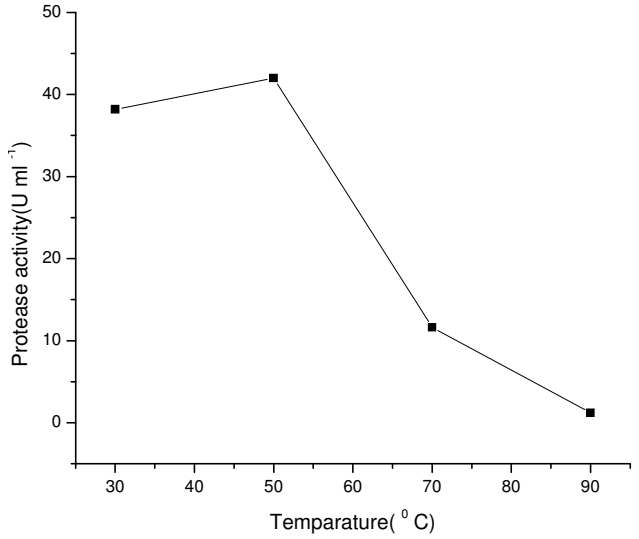

Figure 2. Production of protease by *Bacillus* sp. K-30 at different temperature using rice bran.

Optimization of culture conditions for enzyme production

The cultural conditions (pH, temperature, incubation period and different sources of C and N) were optimized for maximum enzyme production using yeast extract casein medium (Tsujibop et al., 1990) containing (g/L) glucose, 10; casein, 5; yeast extract, 5; KH_2PO_4, 2; and Na_2Co_3, 10. The medium was incubated at 30°C for 48-72 h on shaker. At the end of fermentation period, the culture medium was centrifuged at 10,000 rpm for 15 min to obtain the crude extract, which was used as enzyme source. Protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard. Protease production was studied at different pH (5-12), temperature (30-80°C), inoculum size (1%-10%), substrate concentrations (rice bran 0.125 to 2%) The time course of enzyme production was followed up to 148th hour.

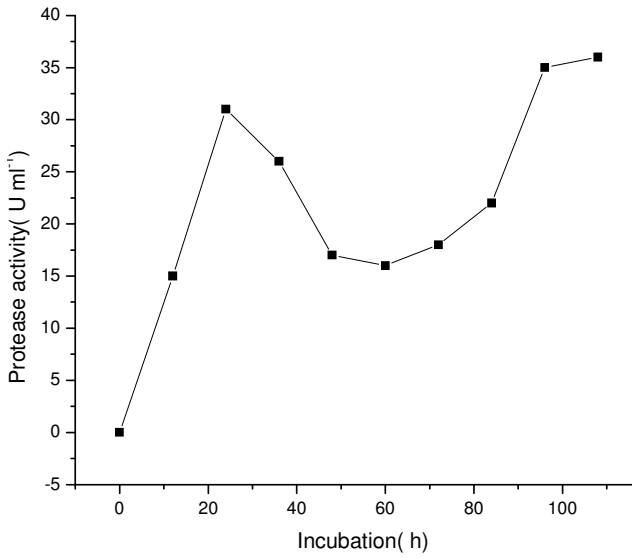

Figure 3. Protease Production pattern of *Bacillus* sp. K-30 at optimum pH and temperature.

RESULTS AND DISCUSSION

Using morphological and biochemical characteristics (Holt et al., 1994) these isolates were identified as *Bacillus*. The optimum pH and temperature for production of protease by K-30 using rice bran was 9 and 50°C, respectively (Figures 1 and 2). The pH of the culture strongly affects many enzymatic processes and transport of compounds across the cell membrane. Majority of the thermophilic bacilli are found to grow at pH and temperature range of 5.8-8.0 and 50-65°C, respectively (Zeikus, 1979). Alkaline protease production is found to be maximum at pH 9-13 (Borris, 1987). The mechanism of temperature control in enzyme production is not well understood (Chaloupka, 1985). A link exists between enzyme synthesis and energy metabolism in *Bacillus*, which is controlled by temperature and oxygen uptake (Frankena et al., 1986).

By growing these cultures at optimum pH and temperature, the activity of protease was estimated at different intervals of growth. The maximum protease production was recorded after 96 h of incubation at 50°C (Figure 3). *Bacillus* usually produces extra cellular protease during late exponential phase (Ward, 1985).

The use of cheap sources of carbon and nitrogen like wheat bran, rice bran, casein, soy meals are important as these can significantly reduce the cost of production of protease. Therefore, the effects of various carbohydrates

Table 1. Effect of different carbon and nitrogen sources on protease production by *Bacillus* sp. K 30.

Carbon source (1%, w/v)	Protease activity (U ml^{-1})	Nitrogen source (1%, w/v)	Protease activity (U ml^{-1})
Wheat bran	81.1	Beef extract	75
Rice bran	42.1	Yeast extract	65
Casein	40	Tryptone	62
Maltose	35	Potassium nitrate	28
Skin milk	35.3	Glycine	23
Lactose	32	Peptone	65
Starch	62	Gelatin	55
Sucrose	60	Soybean meal	45

and organic nitrogen sources were evaluated at optimum pH, temperature and incubation time with respect to enzyme yield. Wheat bran supported the maximum production of protease enzyme however rice bran also had comparable results along with casein and soy meal. The best nitrogen source for protease production was beef extract, while yeast extract and tryptone were comparable (Table 1). Addition of inorganic nitrogen sources in the production medium resulted in low enzyme yield as also reported by Fujiwara et al. (1987). Among the ten carbon sources studied, starch, sucrose, and lactose proved appreciably good for the protease production. Lactose, starch, soy meal and sucrose are considered good for industrial protease production (Sonnleitner, 1983).

The appreciable high enzyme activity and stability at high temperatures and pH, suggest that K-30 can be a potential producer of alkaline protease by using cheap substances like wheat bran, rice bran, and soy meal. *Bacillus* species have been successfully used in degradation of pertinacious waste into useful biomass by many investigators (Atalo et al., 1993; Yeng et al., 2000). In this study, we further demonstrate that *Bacillus* sp. are useful in deproteinisation of rice bran and can be used as production medium for thermostable alkaline protease which can find application in detergent industry.

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