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PROTEOLYTIC ACTIVITY OF A THERMOPHILIC STRAIN OF *NOCARDIA* SP. -TP8 ISOLATED FROM SOIL

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MICROBIAL enzymes, particularly the proteases, have wide-ranging applications in food, chemical, leather, medical, photographic and pharmaceutical industries. During the last few decades attention has been focused on the industrial production of enzymes from thermophilic micro-organisms because they are far superior to the enzymes from mesophiles by virtue of their higher thermostability. Losses are less during purification and due to the higher growth temperature of the organisms, industrial processes are beset with less fermentation contamination and, finally, no cooling costs[1] are involved.

Extracellular proteases are more abundant than intracellular proteases. The former are easy to detect and isolate and consequently they serve as important tools in industry and research[2]. There is extensive literature on production and properties of proteases from various micro-organisms such as *Bacillus* sp.[3-5], *Pseudomonas maltophilia*[6], *Cephalosporium* sp.[7], *Vibrio splendidus*[8], *Vibrio harveyi*[9], *Aspergillus* sp.[2,10], *Mucor* sp.[2,11] and *Streptomyces* sp.[11]. We have come across a hitherto unreported thermophilic strain of *Nocardia* sp., which has a remarkable ability to produce an appreciable amount of extracellular protease. This paper describes the isolation and proteolytic activity of the thermophilic *Nocardia* strain.

The strain was isolated during an investigation to obtain a thermophile that could be exploited for production of extracellular protease on a commercial scale. Soil samples were collected from different parts of West Bengal. Colonies were isolated using dilution plating method under incubation at 45°C and transferred to nutrient agar slants. For the isolation of proteolytic strains, plates with nutrient agar (Hi-Media, India) containing gelatin (0.4%) were inoculated at the centre with strains isolated as above. After two days of incubation the plates were flooded with 12.5% mercuric chloride solution. Micro-organisms forming clear zones around their colonies were identified as protease producers. The zone of widest diameter was produced by a strain that was subsequently identified to be a thermophilic strain of *Nocardia* sp. The organism was designated as *Nocardia* sp. -TP8. The identification of the strain to the genus level was supported by the Commonwealth Mycological Institute, Kew, England.

One ml of cell suspension (6×10^7 cells) was inoculated into 100 ml flasks containing 25 ml of the medium. The medium contained 2.5% mannitol as the carbon source, 0.5% peptone as the protein source and 0.1% dipotassium hydrogen phosphate. Growth was found to be rapid in a rotary shaker at 45–47°C at an initial pH of 7.5, and after an incubation period of 24 h the culture filtrate was centrifuged at 10,000 r.p.m. for 15 min and the supernatant was utilized for assaying the enzyme.

Protease activity in the supernatant was evaluated by a modification of Anson's method[12]. The reaction mixture consisted of 1 ml of diluted supernatant and 1 ml of a selective protease inhibitor preincubated for 20 min at 37°C in a water bath. After preincubation 1 ml of casein solution in 0.4 M Tris-HCl buffer, pH 7.7, was added and the mixture was incubated for 30 min at 37°C in a water bath. The reaction was terminated by the addition of 3 ml of 5% trichloroacetic acid. The mixture was allowed to stand for 1 h and was then filtered. To 1 ml of the filtrate, 5 ml of 0.44 M Na_2CO_3 and 1 ml of Folin reagent were added and absorbance at

660 nm was measured after 30 min. One unit of protease activity was defined as the amount of enzyme that liberated 1 μg of tyrosine per minute under the conditions specified. Total proteolytic activity was measured by assaying in the absence of inhibitors.

The strain produces a mixture of serine protease and metalloprotease, the former being present in excess of the latter. Serine proteases have serine as an essential amino acid of the active centre. They are endoproteases and have an alkaline pH optimum. They have a strong proteolytic activity and generally exhibit low specificity. Metalloproteases are endoproteases, the pH optimum is close to neutral, and stability in general is not as good as in the serine proteases. The activities of the two enzymes can be separately measured by using selective inhibitors. Phenylmethylsulphonyl fluoride selectively inhibits serine protease[2, 10]; at 10 mM it brings about 98% inhibition. Sequestering agents such as EDTA inhibit metalloprotease[2, 10]; they bring about 98% inhibition at 20 mM.

The strain was tested with 14 carbon sources for protease production. The carbon sources used were mannose, galactose, arabinose, melibiose, sucrose, inositol, sorbitol, fructose, glucose, xylose, soluble starch, mannitol, glycerol and maltose. Mannitol was selected as the most appropriate for a high level of protease production. Similarly casein hydrolysate, yeast extract, peptone and beef extract were tested and peptone was chosen as the best protein source for enzyme production.

Tables 1 and 2 explain the selection of 2.5% mannitol and 0.5% peptone as the optimum percentage of carbon and protein sources respectively for a high rate of protease production. Under the above conditions the organism produced 106 units of protease per ml of crude enzyme.

Table 1 *Effect of concentration of mannitol on extracellular protease production by Nocardia sp.- TP-8*

Conc. of mannitol (%)	Serine protease activity (U/ml)	Metalloprotease activity (U/ml)	Total activity (U/ml)
0.2	19.0	1.6	22.4
0.5	19.1	2.0	21.6
1.0	20.2	3.2	24.8
1.5	20.0	2.0	22.4
2.0	23.0	2.4	26.4
2.5	26.4	3.2	30.4
3.0	24.8	0.5	25.6
4.0	25.2	2.0	27.2

Table 2 *Effect of concentration of peptone on extracellular protease production by Nocardia sp.- TP-8*

Peptone conc. (%)	Serine protease activity (U/ml)	Metalloprotease activity (U/ml)	Total protease activity (U/ml)
0.1	53	5	58
0.2	68	6	73
0.5	80	22	106
1.0	27	11	39
1.5	28	11	41

The organism has a rapid growth rate at elevated temperatures and produces an abundant amount of protease. As described earlier enzymes from a thermophilic source have good commercial value. The *Nocardia* sp. isolated by us may prove worthy of industrial exploitation, attempts for which are in progress.

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