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Production and properties of fibrinolytic enzyme from *Streptomyces* sp. NRC 411

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Of 16 *Streptomyces* spp. investigated for the production of extracellular fibrinolytic enzyme, one species was chosen as the most promising producer. Using shaken cultures grown for 7 days, optimal conditions for enzyme production were pH 6.0, 5% (w/v) starch as carbon source, $(NH_4)_2SO_4$ and soybean flour as nitrogen sources and KH_2PO_4 at 1.2 g/l. Maximal activity of the crude enzyme was at pH 6.0 and 45°C. Holding the enzyme at 37°C for 2 h decreased the activity by only 10%.

Key words: Enzyme, fibrinolysis, *Streptomyces*

Because of the significance of fibrinolytic enzymes in the treatment of thrombosis in man, microbial sources of such enzymes (Abdel-Fattah & Ismail 1983, 1984a,b Rudenskaya *et al.* 1987 Ismail *et al.* 1990) including *Streptomyces* (Egorov *et al.* 1976; Belyauskaite *et al.* 1986) have been investigated.

The present work deals with the production of fibrinolytic enzymes from *Streptomyces* spp. under different cultural conditions, particularly from *Streptomyces* sp. NRC 411. Some properties of the crude enzyme are also presented.

Materials and Methods

Organisms

Streptomyces strains used in the present work were from the culture collection of the National Research Centre (NRC), Dokki-Cairo, Egypt, and from the United States Department of Agriculture, NRRL, Peoria, IL, USA.

Media Composition and Cultivation

The medium for liquid cultures consisted of (g/l): starch, 70.0; soybean flour, 17.5; $(NH_4)_2SO_4$, 0.9; KH_2PO_4, 0.8; $CaCO_3$, 3.0; $ZnSO_4.7H_2O$, 0.01; $MnCl_2$, 0.01, with the pH adjusted to 6.5 with 0.1 M NaOH. Cultivation was in 250 ml Erlenmeyer flasks containing 50 ml of sterile medium, inoculated with 1 ml of spore suspension (4×10^7) spores from a 7-day slant. The cells were harvested by filtration through glass wool and the culture filtrates were assayed for enzyme activity.

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Fibrinolytic Activity

The fibrinolytic activity was measured by using a reaction mixture of 1.0 ml containing 10 mg human fibrin (Sigma), 7.5 μmol KH_2PO_4/Na_2HPO_4 buffer, pH 6.8 and an enzyme source. The mixture was incubated for 120 min at 37°C and then the reaction was stopped by adding 0.5 ml 15% (w/v) trichloro-acetic acid, centrifuged (34,000 × *g*) and the solubilized proteins in the supernatant assayed using the phenol method of Greenberg (1957). A unit of enzyme activity is defined as the amount of enzyme which releases 1.0 μg tyrosine per min.

For studying the properties of the fibrinolytic enzyme, the culture filtrate of *Streptomyces* sp. NRC 411 was concentrated by ultrafiltration through a membrane with a molecular weight cut off of 10,000 and then lyophilized. This preparation was used throughout the experiments.

Protein was determined by the method of Lowry. All the results of growth and assays are the means of three separate experiments.

Results and Discussion

Screening of Streptomyces *Strains for the Production of Fibrinolytic Enzyme*

Fibrinolytic activities in the culture filtrates of 16 *Streptomyces* isolates were determined (Table 1). *Streptomyces* sp. NRC 411 was the best producer and its activity (16 U/ml) is greater than those reported previously (Fayek *et al.* 1976; Abdel-Fattah & Ismail 1983; Ismail *et al.* 1990).

Fibrinolytic Enzyme Production by Streptomyces NRC 411 *Culture*

The cultural conditions were examined to obtain the highest production of fibrinolytic enzyme and the optimal

Table 1. Fibrinolytic enzyme activity in culture filtrates of *Streptomyces*.

Streptomyces isolates	Fibrinolytic activity	
	(Units/ml)	(Units/mg protein)
sp. NRC M 13	5.3	3.7
S. sp. NRC 1	4.0	2.2
S. sp. NRC 1 B	5.8	7.6
S. *albus* NRRL 3917	6.0	9.3
S. sp. NRC 6	4.4	7.1
S. sp. NRC 16 A	4.2	4.3
S. *achromogenus* NRRL 2021	6.2	4.2
S. *rimosus* NRRL 2234	6.4	4.1
S. sp. NRC 413	nil	nil
S. sp. NRC 412	1.3	2.3
S. sp. NRC 19	5.6	5.9
S. sp. NRC 718	3.9	9.2
S. *rimosus* IFO 12907	6.6	10.3
S. sp. NRC 411	15.6	15.0
S. sp. NRC 717	3.1	11.1
S. *rimosus* NRC 7	5.8	13.7

conditions (Table 1). Cultivation at pH 7.5 was the most favourable for fibrinolytic enzyme production, in accord with the results of Liginova *et al.* (1980) and Disler (1982).

Utilization of starch at different levels was evaluated (Table 2) and a concentration of 5% found to be best for synthesis of the enzyme. Substitution of starch in the basal medium by dextrin, sucrose, lactose, glucose or ribose led to a decrease in the synthesis of fibrinolytic enzyme (data not shown). Starch has previously been found to stimulate the biosynthesis of proteolytic enzymes from several bacterial and actinomycetes species (Dolidze *et al.* 1975; Valdimirova & Kornienko 1975; Lignova *et al.* 1980).

Table 3 shows the effect of various nitrogen sources on the production of fibrinolytic enzyme: $(NH_4)_2SO_4$ (with the basal medium's soybean) was the most favourable, as found by Geshkov & Tonkova (1974), Dolidze *et al.* (1975), Liginova *et al.* (1980) and Egorov *et al.* (1982).

Table 3. Effect of the nitrogen source in the basal medium on the production of fibrinolytic enzyme by *Streptomyces* sp. NRC 411.

Nitrogen source	Concentration (g/100 ml)	Final pH	Fibrinolytic activity	
			(Units/ml)	(Units/mg protein)
Soybean	2.4	6.5	7.8	7.6
Casein	0.5	8.0	3.6	1.4
Peptone	0.5	8.0	4.6	0.4
Yeast extract	0.6	8.0	2.3	7.7
Human fibrin	0.7	7.2	0.5	0.6
Bovine fibrin	0.7	7.3	1.0	0.9
Meat extract	0.6	8.3	1.7	4.1
$(NH_4)_2SO_4$	0.3	6.5	11.7	16.6
$NaNO_3$	0.4	6.5	7.7	11.4
NH_4Cl	0.2	6.5	10.4	16.4
Control*	–	6.8	18.0	18.2

* Basal medium, including soybean at 1.75 g/100 ml.

Increasing the inorganic phosphate (KH_2PO_4) from 0.05 to 1.2 g/l stimulated enzyme production (14.3 to 19.4 U/ml). Above this concentration, enzyme production decreased. Adding trace metals (Zn^{2+}, Fe^{2+}, Mn^{2+}), separately or combined, to the medium had no significant effect on the production of fibrinolytic enzyme.

Properties of the Fibrinolytic Enzyme

The enzyme was most active at pH 6.0 in phosphate buffer, with 50% activity at pH 5.0. Maximal enzyme activity was at 45°C.

Thermal denaturation of the enzyme in the absence of its substrate was determined by preincubating the enzyme in

Table 2. Effect of carbon source in basal medium on the production of fibrinolytic enzyme of *Streptomyces* sp. NRC 411.

Carbon source	Concentration (g/100 ml)	Suspended dry matter	Fibrinolytic activity**	
			(Units/ml)	(Units/mg protein)
Control (basal medium without starch)	–	105	3.1	3.1
Starch	1	166	10.5	11.8
	3	187	14.9	13.7
	5	203	17.9	18.2
	7	221	17.5	16.3
	9	239	13.3	14.3
	11	252	13.0	10.1

* See materials and methods section.

** Basal medium without starch.

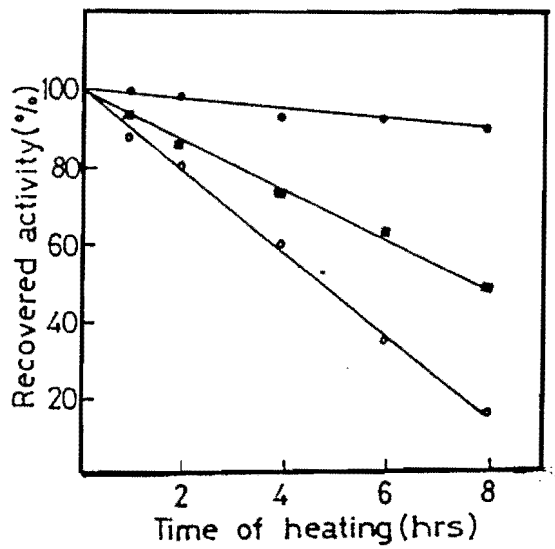

Figure 1. Thermal stability of the fibrinolytic enzyme produced by *Streptomyces* sp. NRC 411 at 37°C (●), 45°C (■) and 53°C (○).

phosphate buffer for different periods at 37°C, 45°C and 55°C (Figure 1). At 37°C, 90% of the activity was retained after 2 h, but after 8 h at 45°C and 55°C the enzyme lost 60 and 80% of its original activity, respectively. In terms of thermal stability, the enzyme is superior to fibrinolytic and proteolytic enzymes from *Actinomycetes fradiae* (Petrova *et al.* 1975), *Bacillus alvei* (Novikova *et al.* 1986); *Bacillus subtilis* (Treitskii *et al.* 1987) and *Cochliobolus lunatus* (Abdel-Fattah & Ismail 1984b).

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