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Production of Fibrinolytic Enzyme by Streptomyces Rimosus at Conditions of Nitrogen Limitation

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Abstract

The kinetic of the enzyme production of *Streptomyces rimosus,* a producer of exoproteases was investigated in conditions of nitrogen limitation.The maxima of fibrinolytic and caseinolytic activities by *Streptomyces rimosus* were reached at 84 h, respectively 96 h. Values of activities were increased 5-fold with those on initial medium. The ultrastructure changes were followed. In the earlier hours an aggregation of ribosomes in cells was established. Large membranes and numerous electron-transparent structures were found. The obtained results indicated close connection between cell status of the producer, its enzyme productivity and an ability of the strain to survive at conditions of nitrogen limitation.

Keywords: Proteolytic enzymes; *Streptomyces rimosus*; Ultrastructural changes; Nitrogen limitation; Stress response

Introduction

Permanent increase of heart-related deseases require effective medicine drugs for their theraphy. Different enzymes as urokinase, streptokinase and staphylokinase were widely applied at thrombosis but these agents have some disadvantages as thermolability, ability to cause haemorrhagie side effects and high cost which restrict their use. Usually the attention of researchers has been devoted to conditions of biosynthesis of fibrinolytic enzymes by bacteria and actinomycetes and little is known about detail characteristics of the strains and cell changes during biosynthesis especially at nutrient limitations (Abdel-Nabbi et al., 1992; Kim et al., 1996; Shao et al., 1998; Chitte and Dey, 2000; Chitte and Dey, 2003).

The present work deals with ultrastructural cell changes of *S.rimosus* producer of proteases with caseinolytic and fibrinolytic actions in conditions of nitrogen limitation during increased enzyme biosynthesis.

Materials and Methods

Microorganism, cultivation and media composition

S.rimosus is used in the present work. The production nitrogen-limited medium for liquid culture consisted of (g/l):glycerine, 10; K_2HPO_4, 0.2; NH_4Cl, 0.2; $NaNO_3$, 0.11. Cultivation was carried out in 750ml Erlenmeyer flasks with 100ml medium, inoculated with 5% of preculture grown 40 h at 28°C on rotary shaker 220 rpm. Inoculation medium contained (g/l): glucose,10; soy bean flour, 10; NaCl, 0.5, $CaCO_3$, 1.0.

Electron microscopy

The harvested biomasses from *S. rimosus* in dynamic, 24-96 h were centrifuged with phosphate buffer, pH 7.0 and fixed in glutaraldehyde (5% , v/v in the same buffer) for 2.5 h. Specimens were then transferred to a sucrose solution (0.2 M) at 4°C for 12 h. Postfixation was carried out in osmium tetraoxide (3 % w/v, phosphate buffer). Samples were dehydrated in a graded alchohol series and acetone and embedded in Epon. Ultrathin sections were prepared with LKB 4800 microtome and stained according to Reinolds, (1963). Samples were examined with a JEM-100C electron microscope.

Enzyme assays

The fibrinolytic activity was determined by fuibrin plates assay (Astrup and Müllertz, 1952). The caseinolytic activity was measured by the method described by Anson, (1979) in term μg tyrosine/ml min. Biomasses were determined by weighing to constant dry weight after drying at 105°C.

Results and Discussion

Culture *S. rimosis* has grown in conditions of nitrogen limitation on production medium. The biomass increased rapidly during 72 h and declined after 84 h (Table 1). It is known that the nitrogen or carbon limitation favoured the onset of antibiotic biosynthesis and significantly increased the yields of products (Sanchez and Demain, 2002; Gesheva et al., 2005; Filipova et al., 2005). As a stress response to nitrogen limitation *S.rimosus* began to produce increased amounts of proteolytic enzymes.The maxima of caseinolytic and fibrinolytic activities of *S.rimosus* were reached at 96 and 84 h which indicated that the fibrinolytic enzyme is a strong–fibrin specific. The values of fibrinolytic and caseinolytic activities were increased 5-fold in comparison with those on initial medium, 800 U/ml and 48 μg tyrosine/ml min.

Hours	pH	Biomass (g/l)	Activity	
			Caseinolytic (μg tyrosine/ml min)	Fibrinolytic
24	6.8	2.0	80	1000
48	7.0	6.0	120	2500
72	7.2	12.2	200	3800
84	7.2	5.0	230	4200
96	7.2	2.0	300	3500
120	7.2	1.5	100	2800

Table 1: Kinetics of fermentation parameters of *S. rimosus*.

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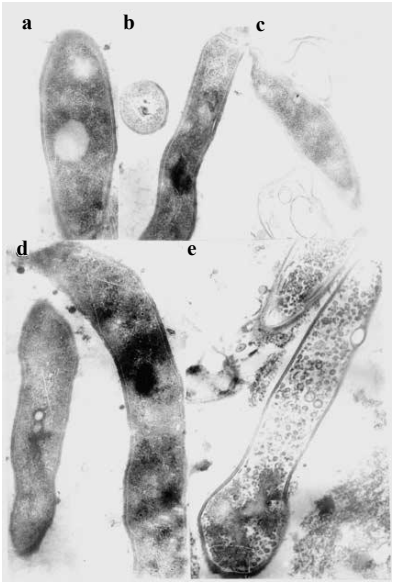

Figure 1: Ultrathin sections of cells of *S. rimosus*. (a), 24 h, 50 000x; (b), 48 h, 30 000x; (c), (d), 72 h, 30 000x; (e), 96 h, 60 000x.

Observations on cell ultrastructure of *S.rimosus* (Figure 1) showed that cells had typical streptomycete cell wall. It was homogenic and consisted from 3 layers as the outside and inner ones were more osmiophilic which points the increased contents of proteins as enzymes, antigens and others. In different cells periplasmic spaces lacked or varied by sizes. Cytoplasmic membrane with thickness 7.5-8 mm was formed of 3 layers and divided cytoplasm from periplasm. In cytoplasm of young cell hypha there were ribosomes aggregated in polyribosomes. They were observed in other actinomycete strains and authors explained their occurrence as a toxic action of own antibiotic or increase of proteins following antibiotic production (Kurylowich et al., 1974; Zaslavskaya et al., 1977; Kuimova et al., 1978). In our case, polyribosomes indicated about perfect protein-producing system of *S. rimosus*. Vesicular or tubular inner-cell membranes were situated in different places of cells-in periphery, center or around the septa. In some cases the membranes were very large. Usually membranes were connected with the intensive biosynthesis of antibiotics or other metabolites (Kurylowich et al., 1974; Zaslavskaya et al., 1977; Spassova et al., 1991). The mesosomes, nucleosomes, electron-transparent structures were clearly visible. Filipova et al., (2005) have studied *S. avermitilis*, a producer of antibiotic at nitrogen limitation and showed that the strain forms numerous electron-transparent structures which are connected with the acceleration. Other authors explained the formation of electron-transparent structures by phage infection, specific influence of antibiotic on own producer or as a place of store of metabolites with next exudation in medium after cells' destruction (Kurylowich et al., 1974; Zaslavskaya et al., 1977; Kuimova et al., 1978; Spassova et al., 1997). In our case, globular substance covered with osmiophilic membrane was detected in environment which perhaps is a material from electron-transparent structures, excreted by life of dead cells. On 48 h some of cells were polymorphic with properties of degeneration, others contained polyphosphates and numerous membranes. During the growth ribosomes decreased of the reason that protein biosynthesis declined. Many cells were lysed. This event is correlated with data about kinetics of fermentation parameters (Table 1). The obtained results showed the close connection between cell status of the producer *S. rimosus* and its extracellular enzyme activities in conditions of nutrient limitation.Increase of protease activities is a stress response to the deteriorated conditions of nitrogen limitation and may be used as a tool for improvement of yield of fibrinolytic enzyme.

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