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

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The active of the compound creation of Streptomyces rimosus, a maker of exoproteases was examined in states of nitrogen limitation.The maxima of fibrinolytic and caseinolytic exercises by Streptomyces rimosus were reached at 84 h, separately 96 h. Qualities of exercises were expanded 5-overlay with those on beginning medium. The ultrastructure changes were followed. In the prior hours a total of ribosomes in cells was built up. Huge films and various electron-straightforward structures were found. The acquired outcomes demonstrated close association between cell status of the maker, its protein profitability and a capacity of the strain to make due at states of nitrogen constraint.

Introduction

Permanent increase of heart-related deseases require effectivemedicine drugs for their theraphy. Different enzymes as urokinase, streptokinase and staphylokinase were widely applied atthrombosis but these agents have some disadvantages as thermolability, ability to cause haemorrhagie side effects and highcost which restrict their use. Usually the attention of researchershas been devoted to conditions of biosynthesis of fibrinolyticenzymes by bacteria and actinomycetes and little is known aboutdetail characteristics of the strains and cell changes during biosynthesis especially at nutrient limitations. The present work deals with ultrastructural cell changes ofS.rimosus producer of proteases with caseinolytic and fibrinolytic actions in conditions of nitrogen limitation during increasedenzyme biosynthesis.

Materials and Methods

Microorganism, cultivation and media compositionS. rimosus is used in the present work. The production nitrogen-limited medium for liquid culture consisted of (g/l):glycerine,10; K2HPO4, 0.2; NH4Cl, 0.2; NaNO3, 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 rotaryshaker 220 rpm. Inoculation medium contained (g/l): glucose,10 soy bean flour, 10; NaCl, 0.5, CaCO3, 1.0.Electron microscopyThe harvested biomasses from S. rimosus in dynamic, 24-96 hwere centrifuged with phosphate buffer, pH 7.0 and fixed in glutaraldehyde (5% , v/v in the same buffer) for 2.5 h. Specimenswere then transferred to a sucrose solution (0.2 M) at 4°C for 12h. Postfixation was carried out in osmium tetraoxide (3 % w/vphosphate buffer). Samples were dehydrated in a graded alchohol series and acetone and embedded in Epon. Ultrathin sectionswere prepared with LKB 4800 microtome and stained according to Reinolds, (1963). Samples were examined with a JEM100C electron microscope.Enzyme assaysThe fibrinolytic activity was determined by fuibrin plates assay. The caseinolytic activity wasmeasured by the method described by Anson, (1979) in term µgtyrosine/ml min. Biomasses were determined by weighing toconstant 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. It is known that thenitrogen or carbon limitation favoured the onset of antibioticbiosynthesis and significantly increased the yields of products. As a stress response to nitrogen limitation S.rimosusbegan to produce increased amounts of proteolytic enzymes. Themaxima of caseinolytic and fibrinolytic activities of S.rimosuswere reached at 96 and 84 h which indicated that the fibrinolyticenzyme is a strong–fibrin specific. The values of fibrinolytic andcaseinolytic activities were increased 5-fold in comparison withthose on initial medium, 800 U/ml and 48 µg tyrosine/ml min. Observations on cell ultrastructure of S.rimosus showed that cells had typical streptomycete cell wall. It washomogenic and consisted from 3 layers as the outside and innerones were more osmiophilic which points the increased contentsof proteins as enzymes, antigens and

others. In different cellsperiplasmic 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 cellhypha there were ribosomes aggregated in polyribosomes. Theywere observed in other actinomycete strains and authors explained their occurrence as a toxicaction of own antibiotic orincrease of proteins following antibiotic production Inour 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 werevery large. Usually membranes were connected with the intensive biosynthesis of antibiotics or other metabolites. The mesosomes, nucleosomes, electron-transparent structureswere clearly visible. We have studied S.avermitilis, a producer of antibiotic at nitrogen limitation andshowed that the strain forms numerous electron-transparent structures which are connected with the acceleration. Other authorsexplained the formation of electron-transparent structures byphage infection, specific influence of antibiotic on own produceror as a place of store of metabolites with next exudation in medium after cells' destruction In our case, globular substance covered with osmiophilicmembrane was detected in environment which perhaps is a material from electron-transparent structures, excreted by life ofdead cells. On 48 h some of cells were polymorphic with properties of degeneration, otherscontained polyphosphates and numerous membranes. During the growth ribosomes decreased ofthe reason that protein biosynthesis declined. Many cells werelysed. This event is correlated with data about kinetics of fermentation parameters. The obtained results showedthe 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 fibrinolyticenzyme.