STUDY OF PROTEASES AND PROTEASE INHIBITORS FROM *STREPTOMYCES* STRAINS

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Abstract


Proteases and protease inhibitors are important enzymes in *Streptomyces* physiology and differentiation. The objective of this paper was to investigate the optimal conditions for production of proteases and protease inhibitors secreted by three newly isolated *Streptomyces* strains. The optimal growth and inhibition activities were detected after cultivation on different media and the maximum of protease inhibition activities were specific for the examined strains. The extracellular proteins were purified by ammonium sulfate precipitation and SDS-PAGE. One of the examined strains showed protease inhibitory activity perspective for future research.

Key words: *Streptomyces*, protease, protease inhibitors

Abbreviations: BAPNA – Na-benzoyl-DL-arginine-p-nitro anilide, p-NA – p-nitro anilide

Introduction

*Streptomyces* are known, as producers of antibiotics. In recent years, some other products such as protease inhibitors were examined with regard to their pharmaceutical application as immunostimulators, anticancer and antiviral agents (Bentley et al., 2002; Lozitsky et al., 2002). Protease inhibitors are frequently found in *Streptomyces* spp. and are classified based on the similarity of their structures and protease inhibitory specificities as members of the *Streptomyces subtilisin* inhibitor (SSI) family. SSI share several common physicochemical properties: they have small molecular masses and are stable at low pH and at high temperatures. Protease inhibitors are widely distributed in the *Streptomyces* genus. They are classified into two families groups: *Streptomyces* subtilisin inhibitor and metalloprotease inhibitors. The mode of action of protease inhibitors on their target enzymes is diverse. It is possible different proteases to be inhibited by the same mechanism or structurally similar inhibitors to act by different mechanisms. Two basic types interaction were determined: reactions of irreversible binding loop and reversible reactions of binding (Rawlings et al., 2004).

The expression of protease inhibitor activity with *Streptomyces* displayed an important role in their physiology. Protease inhibitors are molecules, which take part in regulation of cellular metabolism, through activation and inactivation of proteases. On the other hand, they offer potential medicinal, biotechnological and agriculture applications (Sabotič and Kos, 2012).

The aim of the present report was a preliminary study of protease inhibitors production by three newly isolated *Streptomyces* strains from soil.

Materials and Methods

Microorganisms and culture conditions

The producers’ strains of *Streptomyces M3, Streptomyces M4, Streptomyces 3-146-K* were isolated from soil. The strains were cultivated in medium Gause I, with two carbon sources (starch and glucose) and medium (with peptone),
The cultivation consisted in batch fermentation in 500 ml Erlenmeyer flasks stirred at 220 rpm for 240 h at 28°C.

The mycelia in submerged cultures were harvested aseptically by centrifugation at 10,000×g for 10 min, washed twice with physiological saline solution and once with distilled water, and then collected by vacuum filtration (Whatman filter paper GF/C) and dried at 105°C for 24 h for measurements of growth. The obtained biomass was measured.

**Purification and protein determination**

Partial purification was performed according to Hiraga et al. (Hiraga et al., 2000). After centrifugation, 40 min, 12 000 rpm/min at 4°C, the supernatants were precipitated with 80% (NH₄)₂SO₄ for two days at 4°C, centrifuged for 40 min at 4°C, 12 000 rpm. The precipitates were re-suspended in 20 mM Tris HCl (pH 8.0) and dialyzed against the same buffer for two days. The protein content was determined by the BCA (bicinchoninic acid) assay (Pierce) using bovine serum albumin as a standard, according to the method of Smith et al. (Smith et al., 1985).

**Assay of inhibitory activity**

Inhibitory activity against trypsin was measured by hydrolysis of BAPNA, during the reaction in the forming p-nitroanilide equivalent of hydrolysed BAPNA. Optical density at 405 nm was measured during 5 min on a spectrophotometer (BOECO 522). Each sample contains – 50 mM Tris-HCl, 10 mM KCl, 80-100 μmol BAPNA, pH 8. Reaction was started with 0.25 mg/ml trypsin (Sigma) (0.1 mg/ml) dissolved in 100 mM Tris-HCl, pH 8.

**Polyacrylamide gel electrophoresis (PAGE)**

SDS-PAGE was performed using acrylamide gel (12%) by the method of Laemmli (Laemmli, 1970). The protein markers were run along side of the sample (10 μg protein sample was used). Protein bands were stained with Coomassie blue R250.

**Results and Discussions**

Produced biomass declined without a distinctive stationary phase. The production of protease inhibitor, determined by hydrolysis of BAPNA, was closely related to mycelia growth, beginning at the early hours and remaining at its maximum value until the end of the batch culture. The characteristics of growth were analyzed and biomass was determined in batch culture during the growth of *Streptomyces strains* cultivated on glucose as carbon source. Results are presented in Figure 1.

![Fig. 1. Growth kinetics of the studied strains in Gause 1 medium with glucose as a carbon source](image1)

The obtained results revealed that the highest protease in-
hibitory activity was detected in the case of cultivation on peptone. Strains M4 and 3-146-K showed the highest activity. Results in the induction of synthesis of inhibitors of Streptomyces in the medium specified by Taguchi et al. (1992) medium other authors (Nita et al., 2002) confirm (growth on peptone) too. Glucose is the preferred carbon source for Streptomyces. The obtained results showed a weak inhibitory activity on the synthesis of protease inhibitors in the tested strains. However, it can be assumed that they are constitutive inhibitors, which are required for basic metabolism of Streptomycetes.

The electrophoresis pattern of culture supernatants obtained from the three studied strains is presented on Figure 3. As it is seen, the pattern differed between the strains and relatively small numbers of bands were found. Probably the molecular weight of the protease inhibitors varies between 14 to 35 kDa for strain 3-146-K and M4 respectively. These results were confirmed by the inhibitory activity assay.

Conclusions

From the obtained preliminary results, one could conclude that one of the examined strain 3-146-K is perspective for future analysis.

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References


