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PURIFICATION AND CHARACTERIZATION OF PROTEASES FROM STREPTOMYCES STRAINS

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Abstract

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Proteolytic enzymes are essential for cell growth, physiology and differentiation in genus *Streptomyces*. The objective of this work was to study the conditions for production and characteristics of proteases secreted by three newly isolated *Streptomyces* strains. The assayed *Streptomyces* strains produced proteases that degrade casein and beta-lactoglobulin. Proteolytic activity of extracellular proteases was higher on casein and was significantly reduced when the substrate was beta-lactoglobulin. One of the secreted proteases showed specific activity against beta-lactoglobulin. This could be perspective for future research as anti-allergic substance.

Key words: Streptomyces, protease, protease activity

Introduction

Proteases play essential role in metabolic and regulatory functions in many biological processes and are often subject of regulation by small molecules and proteins through activation and inactivation of proteases. Proteases can be obtained from several sources, including plant, animal and microorganisms. Microbial proteases are preferred in view of their rapid growth, ease of cultivation, purification and genetic manipulation. Microbial proteases can be produced using many processes like solid-state fermentation and submerged fermentation. Cultural conditions play significant role in the production of extra cellular proteases by microorganisms (Akhtar et al., 2013). Proteolytic enzymes are essential for cell growth, physiology and differentiation in genus *Streptomyces* as they regulate localization and activity of many proteins. On the other hand, protein protease inhibitors are also widely distributed in the in *Streptomyces* sp. and are important for proteolytic activity regulation.

The extra cellular proteases and protease inhibitors from *Streptomyces* find multiple applications in biotechnological, medicinal, and agricultural fields. They are used in tanning industry, manufacturing of biological detergents, meat tenderization, peptide synthesis, food industry, pharmaceutical industry, and other (Akhtar et al., 2013; Sabotič and Kos, 2012).

The objective of this work was to study the conditions for production and characteristics of proteases secreted by three newly isolated *Streptomyces* strains.

Materials and Methods

Microorganisms and culture conditions

The producers' strains of *Streptomyces* 3-146-K, *Streptomyces* M4 and, Streptomyces M3, were isolated from soil. The strains were cultivated in medium Gause I, and medium with peptone (Egorov, 1965; Taguchi et al., 1992). The cultivation consisted in batch fermentation in 500 ml Erlenmeyer flasks stirred at 220 rpm for 168 h at 28°C.

Purification and protein determination

Partial purification was performed according to Hiraga et al. (2000). After centrifugation, 40 min, 12 000 rpm/min at 4°C, the supernatants were precipitated with 80% $(NH_4)_2SO_4$ for two days at 4°C, centrifuged for 40 min at 4°C, 12 000

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rpm. The precipitates were re-suspended in 20 mM Tris HCl (pH 8.0) and dialyzed against the same buffer for two days. Extract of crude protein samples were then separated and concentrated by centrifugation for 15min at 3000rpm using membranes (Amicon Ultra-15, Millipore, cutoff 30 kDa, and 10 kDa pH 7.8). Permeate and retentate of each stage of filtration were collected and stored at –20°C. 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 proteolytic activity

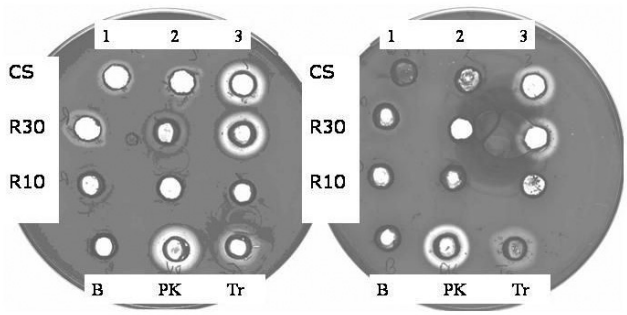
Proteolytic activity was detected by two methods. Method I: Proteolytic activity was determined using the radial diffusion assay in plates (Wikstrom et al., 1981) with modifications. The agar gels containing 1% casein or 1% beta-lactoglobulin in 50 mM Tris (pH 7 and 8) were used as substrates. The plates were incubated 4 h at 37°C. Protease activity was estimated by observing the zone of hydrolysis around small wells cut in agar plates. In both assays, trypsin and proteinase K were used as positive control.

Method II was an estimation of the degree and pattern of degradation of casein and beta-lactoglobulin after incubation 4 h at 37°C (Hill and Gasson, 1986). Samples contained 5 µl of crude enzyme preparation, 2 mg/ml of casein or beta-lactoglobulin, and 50 mM Tris (pH 8) in a total volume of 100 µl. The substrates and degradation products were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis on 12% gels by the method of Laemmli (Laemmli, 1970). The protein markers and substrates as control, were run alongside of the sample (10 µg protein sample was used). Protein bands on the gel were stained with Coomassie blue R250. After staining of the gels with Coomassie blue, the degree of substrate degradation was assessed.

Results and Discussions

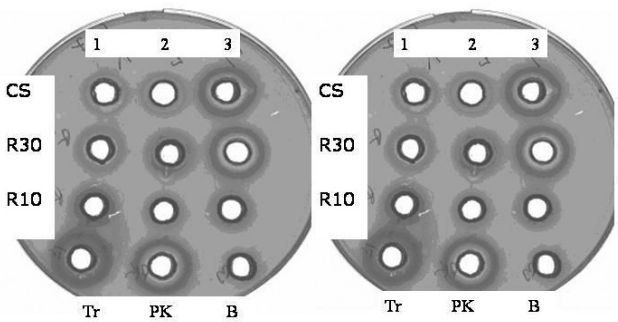
Proteolytic activity was examined in the culture medium at 144 hour of cultivation. The received concentrated samples were assayed for proteolytic activity. Proteolytic activity was analyzed by the plate method. Results are presented in Figures 1 and 2.

From the presented results is clear that the proteolytic activity against casein and beta-lactoglobulin was observed by culture supernatants and 30 kDa retentate. No activity was observed at 10 kDa retentate. Presented results in Figure 1 showed more distinct and larger clear zones for strain M3. The observed clear zone with substrate casein was comparable to clear zone of proteinase K. Smaller clear zones could be seen at strains 3-146-K and M4.



a ***b***

Fig. 1. Protelytic activity of culture supernatants (CS) retentate 30 kDa and 10 kDa of obtained from *Streptomyces* strains 1 – 3-146-K, 2 – M4, 3 – M3 on *a* – casein and *b* – beta-lactoglobulin; B – buffer phosphate pH 8; PK – proteinase K; Tr – trypsin



a ***b***

Fig. 2. Protelytic activity of culture supernatants (CS) retentate 30 kDa and 10 kDa of obtained from *Streptomyces* strains 1 – 3-146-K, 2 – M4, 3 – M3 on *a* – casein and *b* – beta-lactoglobulin; B – buffer phosphate pH 8; PK – proteinase K; Tr – trypsin.; Tr – trypsin

The proteolytic activity of samples received from *Streptomyces* strains was influenced by pH .A decrease of pH of the reaction medium to 7 caused a decrease in the protease activity.

Electrophoretic patterns of the samples obtained after incubation of the crude extracts with beta-lactoglobulin and casein for 4 h at 37°C are shown in Figure 3. The assayed *Streptomyces* strains (3-146-K, M4, M3) produced proteases that degraded casein and beta-lactoglobulin. The examined samples of the three strains in the presence of beta-lactoglobulin as substrate had protease activity. It was highest for strain M3 in comparison with strains 3-146-K and M4.

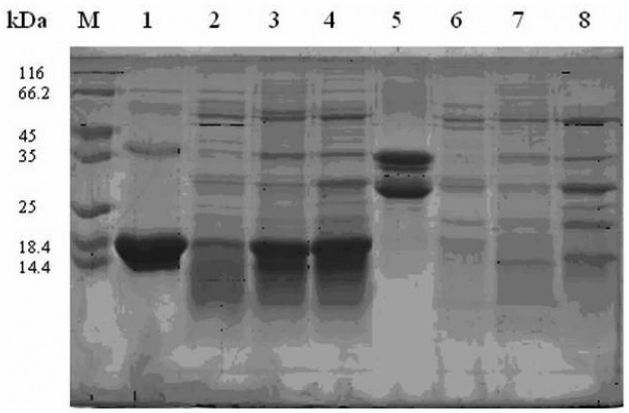


Fig. 3. Patterns of degradation of beta-lactoglobulin (bLg) after incubation 24 h at 37°C by polyacrylamide gel electrophoresis and Coomassie blue staining. M – The standard protein markers; *1* – bLg; *2* – bLg +3-146-K; *3* – bLg +M4, *4* – bLg + M3; *5* – casein, *6* – casein +3-146-K; *7* – casein +M4, *8* – casein + M3

When casein was used as substrate the electophoretic partern showed total degradation of the latter. The protease activity of strain M3 was again higher.

Conclusions

Strain M3 produce protease with high activity against beta-lactoglobulin. This strain could be perspective for future analysis.

Acknowledgements

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