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RESEARCH ARTICLE

PRELIMINARY STUDIES REGARDING TRANSGLUTAMINASE SYNTHESIS BY POLAR FILAMENTOUS BACTERIA OF THE GENUS *STREPTOMYCES SP.*

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Abstract

Transglutaminase (TG-ase, EC 2.3.2.13), was obtained in a crude state (unpurified extract), using a *Streptomyces sp.* selected strain isolated from a polar soil sample. The present study emphasizes on the fermentation conditions assay using a *Streptomyces* sp. strain to obtain a novel transglutaminase. The experiments clearly show the possibility of obtaining transglutaminase by using polar *Streptomyces sp*. selected strains

Key words: *Streptomyces* sp., polar strains, transglutaminase, MTG-ase

Introduction

Transglutaminase (TG; protein-glutamine glutamiltransferase, EC 2.3.2.13) is an enzyme which catalizes an acyl-transfer reaction between the c-carboxyamide group of peptide or protein-bound glutaminyl residues, and primary amines. TG action over protein molecules causes a cross-linking and polymerizing effect of these latter, through ε-(c-glutamyl) lysine bonds (Kuraishi *et al.*, 1998). The great interest bear towards the production of transglutaminase is due to the efficiency with which the commercial enzyme is used in food processing, in pharmaceutics, in textile industry and even for biosensors manufacturing.

Transglutaminase has been found in animal and plant tissues (Folk, 1980) and microorganisms (Ando *et al*., 1989).

Until not so long ago, the only source of transglutaminase used in applications was guinea pig liver and in this matter the marked had little to offer. TG source was limited and insufficient, the separation and purification processes were complicated and expensive and this was affecting the final product's price, which was considerably high. For all these reasons and not only, the researchers' attention focused on finding a new source of TG, paying special attention on obtaining this enzyme with the use of microorganisms through biotechnological methods as they were to be continuously improved. In 1989 was first reported that strains from *Streptomyces sp.*

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screened from several thousands microorganisms had the ability to produce transglutaminase using the hydroxamate assay (Ando *et al.*, 1992). Motoki et al. reported that other *Streptoverticillium* strains, such as *S.griseocarneum*, *S.cinnamoneum* subsp. *cinnanoneum* and *S.mobaraense*, also had the ability to produce transglutaminase. These microorganisms excreted the enzyme, but just *Streptoverticillium mobaraensis* produced a high activity (Washizu *et al.*, 1994)

As far as MTG synthesis is concerned microorganisms are considered to be highly productive. Today, the food industry uses MTG produced by selected *Streptomyces sp.* strains, capable to synthesize it at an industrial scale, due to the fact that MTG is an extracellular enzyme and acts under no influence of $Ca2^{+}$ so, it can be used in producing protein rich food because in shortage of Ca^{2+} the enzyme doesn't precipitate (Zhu *et al.*, 1995).

Various researches aimed so far the isolation and screening of microorganisms for transglutaminase activity but also the purification and the characterization of novel enzymes.

The aim of this study was to find a novel actinomicetae strain which produced a large amount of transglutaminase. Influence of submerged cultivation period regarding transglutaminase's yield was also studied.

Materials and methods

Bacterial strains

There have been used five *Streptomyces sp.* polar (isolated from soils of Antarctica land) strains belonging to M.I.U.G (Collection of industrial Microbiology Laboratory) of the "Dunarea de Jos" University Galati, coded 6p, 7p, 11p, 12p and 13p. Two of the actinomyces strains used in this study were newly isolated from polar soils sampled from East and West Antarctica and they were coded *3K* and *P2C4.*

Sampling and isolation of filamentous bacteria

Approximately five grams of soil were suspended in $10cm^3$ sterile water and then stirred for an efficient homogenization. This suspension was then used to inoculate the starch-casein medium spread on Petri plates (Kuster and Williams, 1964) and then incubated at 25°C for 5 up to 12 days until visible characteristic sporulated colonies appear. The colonies were periodically streaked onto fresh agar test tubes slants with ISP2 media containing (%): 0.4 yeast extract, 1 malt extract, 0.4 glucose and 1.5 agar.

Culture conditions

An amount of 2 mL of spore suspension of selected microorganism was transferred into 250 mL Erlenmeyer flasks containing 50 mL of seed medium composed of (%): 2 peptone; 0.2 KH_2PO_4; 0.1 $MgSO_4 \cdot 7H_2O$; 2 potato starch; 0.2 glucose and 2 soybean flour (pH 7.0) in order to determine the ability to produce MTGase (Macedo, J. *et al.*, 2007). The cultivation was made using submerged batch fermentation at a temperature of 30°C on an orbital shaker with an agitation rate of 200 rpm, for 5-7 days.

MTGase activity assay

The culture broth was centrifuged at 9000 rpm for 15 minutes and the cell-free supernatant was used in the assay of enzyme activity. MTGase activity was determined by the hydroxamate procedure with N-carbo-benzoxy-L-glycine (Macedo *et al.*, 2007).

A reaction cocktail was prepared by combining the following reagents in suitable containers: crude enzyme (unpurified enzyme) 2ml, CBZ-Gln-Gly (Carbo-benzoxy-L-glycine) 120 mg, Tris buffer pH 6 2ml, hydroxylamine solution with reduced glutathione 5 ml and calcium chloride ($CaCl_2$) solution 0.05 ml. The pH was adjusted to pH 6.0 with 100 mM NaOH. The final volume was brought at 10.0 ml by adding deionized water.

This reaction mixture was homogenized by inversion and centrifuged for 5 minutes at 9000 rpm. Reaction mixture was transferred into suitable cuvettes and the optical density was recorded at a wavelength of A_{525nm} for the Standard, Test and Blanks samples (Sigma-Aldrich protocol).

One unit of MTGase activity was defined as the amount of enzyme, which caused the formation of 1.0 µmole of hydroxamate per min by catalyzing the reaction between N-CBZ-Glutamylglycine and hydroxylamine at pH 6.0 at 37ºC. L-glutamic acid

γ-monohydroxamate was used as the standard probe.

Results and discussion

Screening for MTG-ase producers

Various series of actinomycetes strains from the *Streptomyces* genus, from the newly isolated and the ones from MIUG collection, were tested. To each one was given an identification code: 6p, 7p, 11p, 12p, 13p the ones taken from the MIUG collection, and 3K respectively P2C4 the newly isolated.

After 5 days of submerged fermentation, only one strain showed increased transglutaminase activity - the strain coded 13p. The strains 3K and P2C4 presented low, similar transglutaminase activity values (Figure 1).

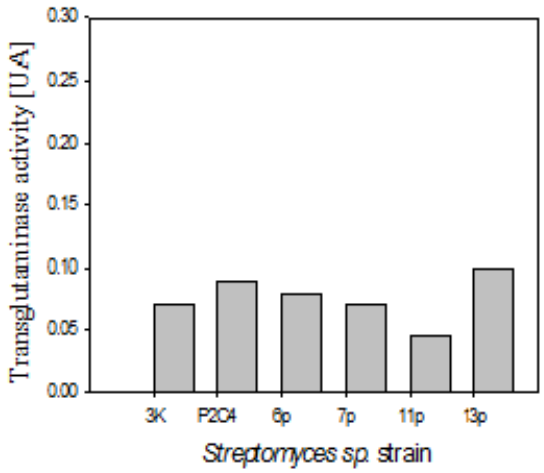

Figure 1. *The activity of crude extract transglutaminase from the Streptomyces sp. strains tested after 5 days of submerged cultivation*

If fermentation time was prolonged after 7 days the strain 13p showed strongest enzyme activity, approximately 0.22 UA/ml (Macedo *et al.*, 2007), meaning double its transglutaminase activity, comparing to the one measured after 5 days of fermentation.

In this assay the newly isolated strains also showed higher transglutaminase potentials and a significant improvement concerning its activity was noted in the case of *Streptomyces* P2C4 (Figure 2).

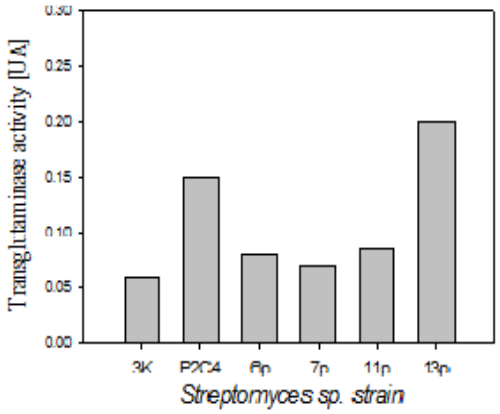

Figure 2. *The activity of crude transglutaminase from the Streptomyces strains, tested after 7 days by submerged cultivation*

The enzyme accumulation rate during submerged fermentation

In order to determine the exact stage of the fermentation process in which the maximum quantity of enzyme was accumulated, *Streptomyces* 13p was inoculated on the medium described in the chapter "Material and methods" and then the crude transglutaminase activity was assayed at different time periods.

The process involves 12 days submerged fermentation. Samples of this fermentation broth were taken once every 48 hours and then the enzymatic activity was assayed as described above. The selected strain exhibited metabolic stability during this 12 days long period as transglutaminase activity was concerned (Figure 3).

The transglutaminase synthesis began 96 hours after the inoculation; its activity gradually increases from the forth day of fermentation till the twelfth, the optimum fermentation period for a biotechnological process being between the eighth and the tenth day.

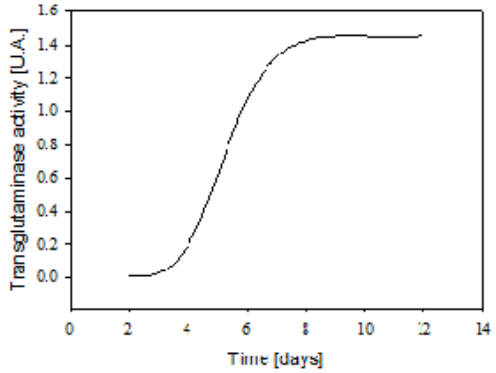

Figure 3. *The variation of the transglutaminase activity of Streptomyces MIUG 13P strain, during the submerged fermentation process*

Conclusions

The studies presented in this paper made possible the identification of *Streptomyces* 13p, which belongs to the M.I.U.G. collection, as an active strain, with a high transglutaminase synthesis potential. The strains 3K and P2C4 also showed a good transglutaminase activity but not relevant for the purpose of this research.

As far as strain MIUG 13p is concerned, transglutaminase synthesis begins after 96 hours of submerse fermentation and its highest yield is achieved in the eighth to the tenth day of fermentation.

Acknowledgements

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