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## Production of Transglutaminase by *Streptovorticillium ladakanum* NRRL-3191 Grown on Media Made from Hydrolysates of Sorghum Straw

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### Summary

The aim of this work was to elucidate the suitability of the biotechnological production of transglutaminase by *Streptovorticillium ladakanum* NRRL-3191 grown on media made from hydrolysates of sorghum straw. Transglutaminase activity was determined in fermentations on sorghum straw hydrolysates and commercial xylose with initial xylose 10, 20 or 30 g/L. Using media containing commercial xylose 20 g/L, transglutaminase activity up to 0.282 U/mL was obtained in 96 h. Using neutralized, charcoal-treated hydrolysates of sorghum straw with xylose 30 g/L sterilized in autoclave at 121 °C, up to 0.155 U/mL was obtained in 96 h. However, when the sterilization was performed by filtration, using the same hydrolysates with xylose 20 g/L, up to 0.348 U/mL was obtained in 72 h. It was demonstrated that hydrolysates of sorghum straw are suitable media for transglutaminase production by *Streptovorticillium ladakanum*.

*Key words:* transglutaminase, sorghum, hydrolysates, *Streptovorticillium ladakanum*

### Introduction

*Streptovorticillium ladakanum* NRRL-3191 is a source of transglutaminase (EC 2.3.2.13), an enzyme that catalyses an acyl transfer reaction between  $\gamma$ -carboxamide groups of glutamyl residues in proteins. The two important features of microbial TGase (MTG) are to be extra-cellular and  $\text{Ca}^{2+}$ -independent. These properties increased the food industry interest for this type of enzymes. MTG has been proposed in industrial processes for the formation of thermally stable gels (1–2). MTG catalyses the

formation of covalent bonds between adjacent proteins, and thereby improves the gel structure of proteins.

MTG has been employed to improve the mechanical and textural properties of different protein foods, including surimi products or restructured fish products (3). This enzyme is recognized as the one responsible for the setting phenomenon in fish proteins (4). However, MTG cannot be used with hydrocolloids such as low methoxyl pectin because a disruptive effect was reported (5).

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Fermentation media can represent almost 30 % of the cost for a microbial fermentation (6). General media employed for growth of *Streptovorticillium* are not economically attractive due to the high amount of expensive nutrients such as yeast extract and peptone. Xylose is a hemicellulosic sugar that can be used as carbon and energy source for growth of microorganisms. The interest in using xylose as a carbon source for microbial proliferation could be enhanced if the fermentation media were prepared from hemicellulosic hydrolysates of a cheap raw material such as sorghum straw or sugar cane bagasse (7–8).

The aim of this work was to elucidate if the biotechnological production of transglutaminase by *Streptovorticillium ladakanum* NRRL-3191 growing on media made from hydrolysates of sorghum straw is suitable.

## Materials and Methods

### Raw material

Sorghum straw was obtained from a local factory, milled to pass through a 1-mm screen, homogenized in a single lot, air-dried, and stored in polyethylene bags at room temperature. Aliquots from the homogenized lot were used for experimentation.

### Acid hydrolysis

Acid hydrolyses were performed under conditions selected previously (2 % of aqueous sulphuric acid, 122 °C, 70 min, liquor/solid ratio = 10/1/(g/g) (9). Hydrolysates were neutralized with CaCO<sub>3</sub> (pH=6) and the formed CaSO<sub>4</sub> was separated by filtration.

### Adsorption

CaCO<sub>3</sub>-neutralized hydrolysates were mixed with charcoal (Prolabo, Fontenay, France) for 90 min at room temperature in a stirred glass reactor using a hydrolysate/charcoal mass ratio of 50/1/(g/g).

### Concentration of hydrolysates

Charcoal-treated hydrolysates were concentrated by vacuum evaporation at 50 °C to obtain solutions with xylose mass concentrations in the range of 10–30 g/L.

### Microorganisms and culture conditions

Freeze-dried broths of wild *Streptovorticillium ladakanum* NRRL-3191 strain were obtained from the Agricultural Research Service Culture Collection (Peoria, Illinois, USA). Microorganisms were maintained on agar plates at 4 °C, and transferred monthly. Experiments were carried out for 120 h at 26 °C in orbital shakers (agitation speed, 250 rpm) using 250-mL Erlenmeyer flasks with 50 mL of culture medium. Media were hydrolysates or commercial xylose with xylose 10, 20 or 30 g/L which were supplemented with: yeast extract 2.5 g/L, peptone 10.5 g/L, MgSO<sub>4</sub> 0.5 g/L, KH<sub>2</sub>PO<sub>4</sub> 2 g/L, Na<sub>2</sub>HPO<sub>4</sub> 5 g/L and sodium caseinate 20 g/L.

### Analytical methods

Samples were withdrawn from the fermentation media at 72, 96 and 120 h. They were centrifuged (4500 × g, 10 min) and liquors were analyzed for xylose and acetic acid by HPLC. This was carried out using a Transgenomic ION-300 column (oven temperature 45 °C) with isocratic elution (flow rate 0.4 mL/min, mobile phase: 0.0025 M H<sub>2</sub>SO<sub>4</sub>) and a refraction index detector. Pellets were washed twice with a solution of sodium chloride 9 g/L in deionized water, centrifuged again and then dried at 102 °C for 48 h, in order to allow the calculation of the biomass concentration on dry weight basis.

The MTG activity was measured by a colorimetric procedure based on the formation of hydroxamate from N-carbobenzoxy-L-glutaminyglycine (10). One unit of activity (U) was defined as the amount that causes the formation of 1 μmol of hydroxamate in 1 min at 37 °C.

All experimental data were carried out in triplicate and the mean values are given. Microsoft PowerPoint 2000 (Microsoft Corporation, Redmond, WA, USA, 1999) was used to plot the experimental data and models.

The biomass yield and product yield were calculated as follows:

$$Y_{X/S} = \frac{X - X_0}{S_0 - S} \quad /1/$$

$$Y_{P/S} = \frac{P - P_0}{S_0 - S} \quad /2/$$

where  $X$  is biomass concentration (g/L);  $X_0$  is initial biomass concentration (g/L);  $S$  is xylose concentration (g/L);  $S_0$  is initial xylose concentration;  $P$  is MTG activity (units);  $P_0$  is initial MTG activity (units);  $Y_{X/S}$  is biomass yield coefficient ( $m(\text{cell dry weight})/m(\text{xylose consumed})/(\text{g/g})$ ), and  $Y_{P/S}$  is product yield coefficient ( $(\text{MTG/U})/(\text{xylose consumed/g})$ ).

## Results and Discussion

In order to assess the possibility of using neutralized, charcoal-treated hydrolysates of sorghum straw for culturing *Streptovorticillium ladakanum* NRRL-3191, a set of experiments was carried out taking into consideration the concentration of hydrolysates with xylose 10, 20 and 30 g/L. Fig. 1 shows a general idea of this process. The initial composition of hydrolysates was: xylose (12.13±0.32) g/L, glucose (3.27±0.56) g/L, arabinose (1.99±0.15) g/L, acetic acid (3.19±0.28) g/L, which varied with the concentration process.

For comparative purpose, a set of experiments with dissolutions of commercial xylose were also carried out. The growth of *Streptovorticillium ladakanum* in xylose was faster than in the hydrolysates. At 72 h, only residual xylose (4.5 g/L) was detected in the experiments with commercial xylose 30 g/L. Using hydrolysates at the same time (72 h), xylose 5 g/L remained in the media of initial xylose 10 g/L, xylose 8.75 g/L in media of 20 g/L and xylose 9.9 g/L in media of 30 g/L. This could be due to the slow growth caused by the presence of inhibitors such as acetic acid. The other sugars of the hydrolysates (glucose and arabinose) were depleted at 72 h.

The MTG activities obtained in commercial xylose media are shown in Fig. 2. Using xylose 10 g/L, 0.170 U/mL were obtained in 72 h, and then it was decreased until 0.108 U/mL were reached at 120 h. These results are contradictory to those obtained by Junqua *et al.* (11), which did not detect MTG activity in media containing xylose 10 g/L. The highest activity in media containing commercial xylose was 0.282 U/mL, obtained using xylose 20 g/L during 96 h. This indicates that the cells continue generating the extra-cellular enzyme after the complete depletion of the carbon source, which occurred at 72 h. Increasing the fermentation time, lower activity was detected, 0.099 U/mL, suggesting the activity of proteases. The increase of xylose concentration (30 g/L) did not give higher activity, being 0.254 U/mL at 96 h, the highest for this xylose concentration. In all cases, a decrease in MTG activity at 120 h was observed, which suggests that proteolysis occurred.

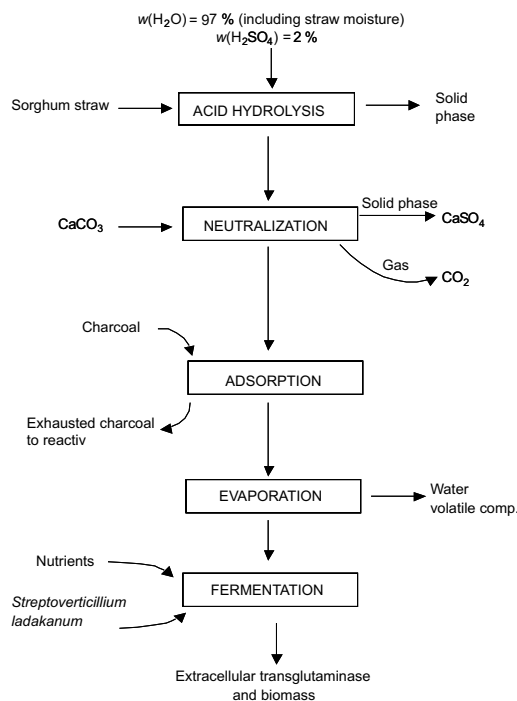


Fig. 1. General idea concerning the studied process

During the culturing of *Streptovercillium ladakanum* on hydrolysates of sorghum straw sterilized in autoclave at 121 °C MTG activities were observed (Fig. 3). In this case, the maximum activity (0.155 U/mL) was obtained using hydrolysates with xylose 30 g/L at 96 h, with a  $Y_{P/S}$  of 5 U/g and a  $Y_{X/S}$  of 0.39 g/g. In these conditions, 8 U/g and 0.19 g/g were obtained in the synthetic media, suggesting that in the hydrolysates the cell metabolism is oriented more towards the increase of biomass than towards the increase of MTG.

Therefore, the MTG activity obtained was lower in hydrolysates than in commercial xylose. The reason for this could be the breakdown of the sorghum straw constituents in the autoclave sterilization. Therefore, a new set of experiments was carried out using hydrolysates sterilized by filtration through membranes of 0.2  $\mu\text{m}$ . The results are shown in Fig. 4. In comparison with hy-

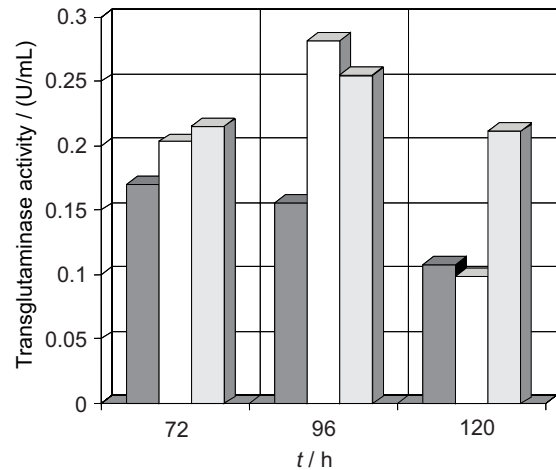


Fig. 2. Transglutaminase activities obtained by the growth of *Streptovercillium ladakanum* in commercial xylose media  
■  $\gamma$ (xylose) = 10 g/L, □  $\gamma$ (xylose) = 20 g/L, ▨  $\gamma$ (xylose) = 30 g/L

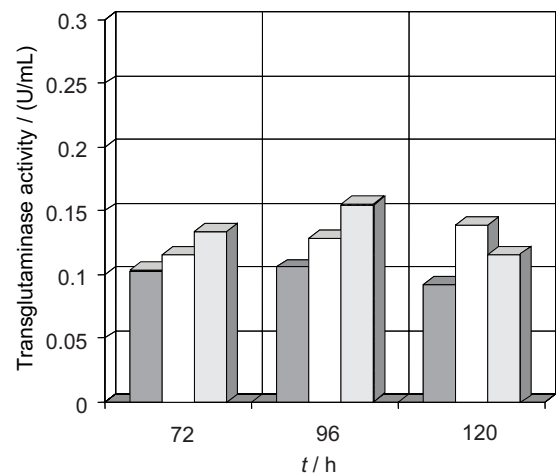


Fig. 3. Transglutaminase activities obtained by the growth of *Streptovercillium ladakanum* in media based on hydrolysates of sorghum straw sterilized by heat for different initial xylose concentrations  
■  $\gamma$ (xylose) = 10 g/L, □  $\gamma$ (xylose) = 20 g/L, ▨  $\gamma$ (xylose) = 30 g/L

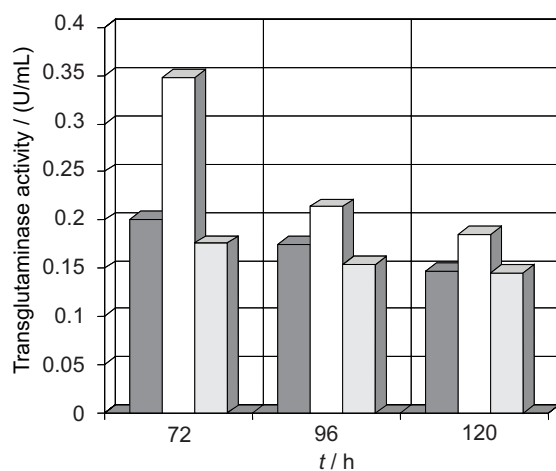


Fig. 4. Transglutaminase activities obtained by the growth of *Streptovercillium ladakanum* in media based on hydrolysates of sorghum straw sterilized by filtration  
■  $\gamma$ (xylose) = 10 g/L, □  $\gamma$ (xylose) = 20 g/L, ▨  $\gamma$ (xylose) = 30 g/L

drolysates sterilized by heat the MTG activity was increased in all fermentations. Up to 0.348 U/mL was obtained using filtrated hydrolysates with xylose 20 g/L at 72 h. This MTG activity was also higher than the maximum obtained using commercial xylose (0.282 U/mL). This suggests that the hydrolysates may contain inducers of the MTG production that are degraded by heat.

The lower MTG activity in filtrated hydrolysates of xylose 30 g/L could be caused by the increase of growth inhibitors (furfural and acetic acid) present in the hydrolysates: in filtrated hydrolysates with xylose 30 g/L, 2.2 g/L of acetic acid was detected and in sterilized hydrolysates with the same xylose concentration 1.8 g/L of acetic acid was found. The MTG activities obtained in hydrolysates are in the range reported in other studies with different substrates (12–14). It was demonstrated that hydrolysates sterilized by filtration of sorghum straw are suitable media for MTG production by *Streptovorticillium ladakanum*.

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## Proizvodnja transglutaminaze s pomoću *Streptovorticillium ladakanum* NRRL-3191 uzgojenom u mediju dobivenom od hidrolizata slame sirka

#### Sažetak

U radu je ispitana mogućnost biotehnoške proizvodnje transglutaminaze s pomoću *Streptovorticillium ladakanum* NRRL-3191 uzgojenom u mediju dobivenom od hidrolizata slame sirka. Određena je aktivnost transglutaminaze dobivene fermentacijom na hidrolizatu slame sirka uz dodatak 10, 20 ili 30 g/L tehničke ksiloze. U podlozi koja je sadržavala 20 g/L tehničke ksiloze nakon 96 h dobivena je aktivnost transglutaminaze iznosila 0,282 U/mL. Koristeći neutralizirani hidrolizat sirka, obrađen aktivnim ugljenom uz dodatak 30 g/L ksiloze sterilizirane u autoklavu pri 121 °C, nakon 96 sati dobivena je aktivnost od 0,155 U/mL. Međutim, kada se sterilizacija provela filtracijom, koristeći isti hidrolizat s 20 g/L ksiloze, nakon 72 sata dobiveno je 0,348 U/mL. Utvrđeno je da su hidrolizati slame sirka pogodan medij za proizvodnju transglutaminaze s pomoću *S. ladakanum*.