DEFINITION OF CULTURE CONDITIONS FOR TANNASE PRODUCTION BY *Lactobacillus plantarum* CIR1

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Key words: Bacteria, Tannase, Taguchi DOE

Introduction. Tannase is an enzyme that catalyzes the hydrolysis of the ester bonds present in hydrolysable tannins (1).The major commercial application of tannase resides in the elaboration of instantaneous tea and in the production of gallic acid (2). Few bacteria are known to produce tannase and include certain species of *Bacilli, Corinebacterium, Lactobacillus* and *Serratia* (3-5). Taguchi method of orthogonal array design of experiment involves the study of any given system by a set of independent variables over a specific region of interest (6). The application of Taguchi methodology for microbial enzyme production is most suitable and superior over the other statistical approaches (7).

The present investigation deals with the application of Taguchi DOE to define the submerged culture conditions of *Lactobacillus plantarum* CIR1 on the tannase production.

Methods. Six most influential factors were considered to define the culture conditions. They were pH (5, and 6), temperature (30, 35 and 40 ºC), tannic acid (0.5, 1.0 and 1.5 g/L), KH_2PO_4 (0.15, 0.30 and 0.45 g/L), NH_4Cl (0.35, 0.7 and 1.05 g/L) and $MgSO_4$ (0.05, 0.10 and 0.15 g/L). An L18 (2^1 x 3^5) array was obtained (6). Fermentation was carried out during 36 h in 25 mL anaerobic flasks according to the experimental matrix. Tannase activity was assayed employing the methanolic rodanine method (7). Statistical analysis was performed using the software Statistica 7.

Results. Maximal tannase activity was 4055.8 U/L obtained in experimental matrix. The most influential factors were pH and tannic acid (Fig. 1). Optimal culture conditions are shown in table 1. Model prediction is near to experimental validation. Tannase production increase of 2.54-fold compared with unoptimized conditions.
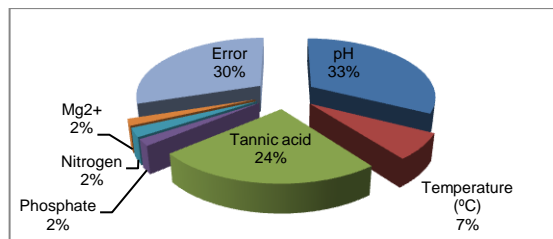

Fig.1 Relative influence of individual factors.

Table 1. Optimal culture conditions and predicted value.

Factors	Level	Value	Contribution U/L	Standard error
pH	2	6	522.529	290.002
Temperature	3	40 ºC	239.684	355.179
Tannic acid	3	1.50 g/100 mL	596.484	355.179
Phosphate	1	0.15 g/100 mL	174.371	355.179
Nitrogen	2	0.70 g/100 mL	158.076	355.179
Magnesium	3	0.10 g/100 mL	126.901	355.179
Predicted		3,272.065 U/L		

Initial tannase: 1,239 U/L
Average 1,454.02 U/L
Validation: 3, 143.09 U/L

Conclusions. Taguchi DOE allowed finding optimal culture conditions for *L. plantarum* CIR1. Model predicted is near to experimental data. Tannase production increases 2.54-fold respecting unoptimized conditions.

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