Interactions between glycerol, PEG-200 and $(NH_4)_2SO_4$ in the stability of heterologous cutinase

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Cutinases (EC 3.1.1.74) are versatile enzymes that have hydrolytic activity on various esters [1]. The spacial structure and the catalytic site of the enzymes can be protected by chemical additives to promove the stability of the activity [2, 3]. The goal of this work was to improve the stability of a recombinant cutinase produced by *Escherichia coli*.

The production of cutinases CUT-N1 by a culture of *E. coli* genetically modified was carried out in the presence of Luria broth, supplemented with 0.2 mM IPTG at 150 rpm, for 24 h, at 37 °C.

The esterase activity was determined by spectrophotometry at 405 nm by the hydrolysis of p-nitrophenyl butyrate (p-NPB). One international unit (U) of cutinase was defined as the amount of enzyme required to convert 1 µmol of p-NPB into p-nitrophenol (p-NP) per minute. All activity assays were performed in triplicate.

The 2^3 full factorial design with four center point replicates was carried out to investigate the enzyme stability (Table 1). Aliquots were removed at 0, 30 and 60 days of storage at room temperature (28 °C) to determine esterase activity.

Table 1. Factors and levels of a full factorial design to investigate the stabilization of cutinase

Factors (%)	Full Factorial Design Levels		
	-	0	+
Glycerol	0	2.5	5
PEG-200	5	7.5	10
$(NH_4)_2SO_4$	5	7.5	10

The metabolic liquid free of cells was concentrated twice by ultrafiltration and used to investigate the stability of cutinase activity, according to the full factorial design (Table 1).

The activity of cutinase in the presence of glycerol, PEG-200 and $(NH_4)_2SO_4$ decreased during 60 days of storage at room temperature. Physicochemical interactions between these additives and the components of liquid metabolic free of cells destabilized the structure of the protein chain of the enzyme investigated.

The analysis of the full factorial design by Pareto chart of standardized effects illustrated different behaviors of the chemical additives interactions on the cutinase activity during the storage. The interaction between $(NH_4)_2SO_4$ and PEG-200 showed a negative effect on the activity of cutinase during 60 days of storage whose result was not shown with 30 days at room temperature.

Keywords: heterologous cutinase; enzyme stabilization

References

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