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STABILIZATION OF PHENYLALANINE AMMONIA LYASE ACTIVITY IN THE YEAST

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L-Phenylalanine (L-Phe) is an aromatic ammonia acid with physiological activity. It is one of the essential amino acids in human beings. *L*-Phe has been widely used in the food, health protection, and pharmaceutical industry [1].

Phenylalanine ammonia lyase (E.C.4.3.1.5-PAL) converts *trans*-cinnamic acid (*t*-CA) to L-Phe, which has been identified as a major route to L-Phe production [2]. PAL is the key enzyme.

Availability of a rich enzyme source is a prerequisite for a biocatalyst in industry. Although PAL has several potential clinical and industrial applications, including the production of L-Phe, the stabilization of PAL is still a challenge for researchers. There has been some reports on stabilization of Yeast PAL activity [3, 4]. However, previous procedures were lengthy and gave lower yields. We try to develop a simple and rapid method for stabilizing PAL. The present work reports the results of such a study.

The effects of the following parameters on the PAL stabilization of the strain JM432 were tested: (a) inducers; (b) [NH_4^+] (0.05–0.3%); (c) metal ion. Experimental analysis shows that malt extract medium supplemented with inducers, NH_4^+, or metal ion, can affect the stabilization of PAL activity (Table 1).

Table 1 show that different inducers have no remarkable effect on the stabilization of PAL activity. The addition of L-Ile gave a higher PAL activity, by only 0.21-fold as compared to the control (untreated). The medium used in the study may contain certain inducers, only a few of which could increase the production of PAL. However, too much inducer may cause its decrease. Table 2 shows that 0.1% NH_4^+ gives higher PAL activity. For cells, a higher concentration of NH_4^+ may impact the absorbance of L-Phe, which results in a decrease in PAL activity [5].

Interestingly, when Cu^{2+} was added to the medium, the PAL activity was enhanced by about one fold in comparison to the control (without addition). This has not been reported before.

Under biotransformation reaction conditions, the strain was used to evaluate their usefulness in producing L-Phe from *trans*-cinnamic acid. It is noteworthy that high PAL activity does not necessarily translate to a rapid reversal of PAL-catalyzed reaction and high conversion yield [6]. During the reaction from *t*-CA to L-Phe, we treat the strain JM432 with different methods. This is summarized in Fig. 1. The combination of *D*-sorbital and glutaraldehyde was found to be the most effective method of stabilizing PAL activity. The yield of L-Phe was enhanced by one fold in comparison to the control (untreated cells).

Microorganism. *Rhodotorula glutinis*, JM432 was provided by the Food Fermentation Institute of Guangxi University.

Chemicals. The chemicals and solvents used were of analytical grade.

PAL Forward Assay. PAL forward activity of JM432 was monitored by following the formation of *t*-CA from L-Phe of 290 nm [7]. A reaction mixture containing 25 mM Tris-HCl buffer pH 8.8, 25 mM L-Phe and enough cell was incubated at 30° for 10 min. The reaction was then terminated with 6 M HCl and the 290 nm band of the clear solution was measured. The reaction mixture to which the substrate was added after termination of the reaction served as control. The enzyme activity is expressed as units U: one unit of enzyme is defined as the amount required to convert L-Phe to one mmol *t*-CA per min at 30°.

***L*-Phe Assay**. The whole cells of JM432 were reacted with *t*-CA and ammonia at 30° for 8 hours. The supernatant was tested spectrophotometrically by the production of L-Phe [8].

Data Analysis. The values reported were the mean of at least three independent determination.

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TABLE 1. Effect of Inducers, [NH_4^+], and Metal Ion on PAL Activity

Inducer					[NH_4^+]/w/v, %				
L-Phe	L-Tyr	L-Ile	L-Phe+L-Ile	L-Tyr+L-Ile	0.05	0.1	0.15	0.2	0.3
Relative activity, %									
111.1	110.0	121.1	117.4	113.9	107.2	128.5	110.2	84.3	80.5
Metal ion									
Fe^{2+}	Cu^{2+}	Pb^{2+}	Co^{2+}	Ca^{2+}	Mg^{2+}	Zn^{2+}	Mn^{2+}	Na^+	
Relative activity, %									
95.0	190.1	105.6	86.6	52.3	59.3	39.6	59.2	44.6	

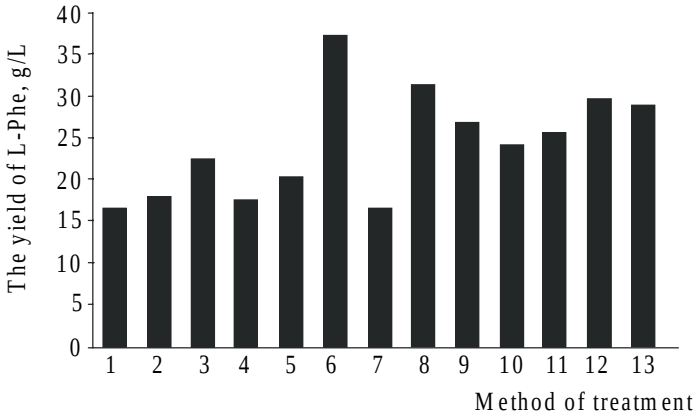

Fig. 1. The yield of *L*-Phe of JM432 treated by the following method: 1 – control; 2 – Tween-20; 3 – Tween-80; 4 – toluene; 5 – Span-60; 6 – D-sorbital + glutaraldehyde; 7 – glutaraldehyde; 8 – Tween-80 + glutaraldehyde; 9 – glycerol; 10 – glutamic acid; 11 – glycerol; 12 – PEG; 13 – CTAB

REFERENCES

1. M. Wang, *Chemical Production and Technology*, **10** (1), 23 (2003).
2. G. J. Calton, L. Wood, M. H. Updike, L. Lantz, and J. P. Hamman, *Biotechnology*, **4**, 317 (1986).
3. Q. Yuan and H. Yue, *Beijing Huadong Daxue Xuebao*, Ziran Kexueban, **30**, 1–3 (2003).
4. A. I. El-Batal, *Acta Microbiol. Pol.*, **51**, 139 (2002).
5. C. T. Evans, K. Hanna, D. Conrad, W. Peterson, and M. Misawa, *Appl. Microbiol. Biotechnol.*, **25,** No. 5, 406 (1987).
6. C. T. Evans, C. Payne, D. Conrad, K. Hanna, and M. Misawa, *Can. Microbiol.*, **33**, 636 (1987).
7. Godwin B. D Cunha, Satynayan Vaduvatha, and P. Madhusudanan Nair, *Phytochemistry*, **42,** No. 1, 17 (1996).
8. L. W. Xiang and J. B. Zheng, *Amino Acids & Biotic Resources*, **24,** No. 3, 69 (2002).