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PRODUCTION OF L-PHENYLALANINE FROM PHENYLPYRUVATE USING RESTING CELLS OF ESCHERICHIA COLI*

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SUMMARY

We describe the production of L-phenylalanine from phenylpyruvate using resting cells of a genetically modified strain of Escherichia coli. Fermentations were carried out by continuously raising the feeding rate of D-glucose. We reached a biomass of 10 g/l dry weight and an aminotransferase activity of 14000 U/l. The maximum phenylalanine concentration achieved was 173 mmol/l with a phenylpyruvate molar conversion yield of 95 %.

INTRODUCTION

Routes for the synthesis and breakdown of L-phenylalanine differ in mammals plants and microorganisms. Phenylalanine is synthesized exclusively via phenylpyruvate by transamination in Escherichia coli (Gelfand and Steinberg, 1977). An alternative pathway using arogenate was described in cyanobacteria by Stenmark et.al (1974). Phenylalanine dehydrogenase was discovered in Brevibacterium spec. by Hummel et. al. (1984). In plants and some

*Dedicated to Prof. Dr. Heinz Harnisch on the occasion of his 60th birthday

microorganisms phenylalanine is degraded to trans-cinnamic acid by phenylalanine ammonium lyase (Ogata et. al., 1967).
The terminal reaction in the biosynthesis of many amino acids including phenylalanine and tyrosine in E. coli involves the transfer of an amino group from glutamic acid (or another suitable amino donor) to the appropiate α-keto acid. In E. coli three major transaminases have been identified. These are the aromatic amino acid transaminase, the aspartate transaminase (Gelfand and Steinberg, 1977) and the branched-chain aminotransferase (Lawther et. al., 1979) .
The aromatic amino acid transaminase is the product of the tyrB gene and is active in the synthesis of phenylalanine, leucine tyrosine and aspartate. The transamination reactions are freely reversible and they play a major role in the amino acid metabolism.
In this paper we describe the use of a genetically modified E. coli with an overexpressed tyrB gene for the bioconversion of phenylpyruvate to phenylalanine.

MATERIALS AND METHODS

The microorganism used in this study was a genetically modified Escherichia coli strain B, Z1196, with high tyrB gene activity.

The fermentation medium contained, in one litre: Na_2HPO_4, 3.5g; KH_2PO_4, 1.8 g; $(NH_4)_2HPO_4$, 12 g; $(NH_4)_2SO_4$, 6 g; $MgSO_4$, 0.2 g; yeast extract, 1 g; glucose, 5 g; pH 7.0 . The strain was grown in a shake-flask at 37 C for 5 h at 200 rotations/min. and then inoculated into a 10 l fermenter (Braun, Melsungen, Germany). Exponential feeding with glucose was started four hours later.

Transaminase activity was assayed using the Sigma Test-Kit G-390 except that 2-oxoglutarate was replaced with 12 mmol/l phenylpyruvate. Enzyme activity was expressed as units (U) corresponding to 1 µmol of NADH oxidized/min.
Amino acids and phenylpyruvate were assayed by HPLC (Hill et. al. 1979)and using an UV-monitor for detection.
Glucose was determined using the Sigma Test-Kit 16-UV.

The cell mass was estimated by measuring dry weight after drying at 105° C overnight.
Phenylpyruvate Na-salt (Sigma, St. Louis, USA) was prepared as a stock solution at 60 g/l in 50 mmol/l carbonate buffer, pH 7.4

RESULTS AND DISCUSSION

A typical fed-batch culture of the Escherichia coli strain is shown in Figure 1. After 4 h of incubation the exponential glucose feeding of the culture was started. The feed-rate was increased from 0.4 to 14 g/l/h glucose over 10 hours. After a short lag period the cells grew exponentially. Biomass and transamination activity within the cells developed in a similar manner. Growth continued until limited by the availability of dissolved oxygen. Due to this limitation glucose concentrations rose in the fermenter. Transamination activity reached a maximum of 14000 U/l at the end of growth.
The cells from the fermenter were used directly without further washing for the bioconversion of phenylpyruvate to phenylalanine
A batch experiment with resting cells is shown in Fig. 2. After the addition of 182 mmol/l phenylpyruvate an initial transamination velocity of 11 g/l/h was determined. After 8 h a maximum concentration of 173 mmol/l phenylalanine was reached. The molar conversion yield was 95 % at the end of this bioconversion.

No significant amounts of oxaloacetate, which is one of the reaction products, appeared in the mixture. Similar results were obtained with Pseudomonas putida(Ziehr and Kula, 1985). No other reaction products than phenylalanine or pyruvate could be detected.
The maximum initial reaction velocity and molar conversion yield declined when the initial phenylpyruvate concentration exceeded 182 mmol/l (results not shown). This suggests that the

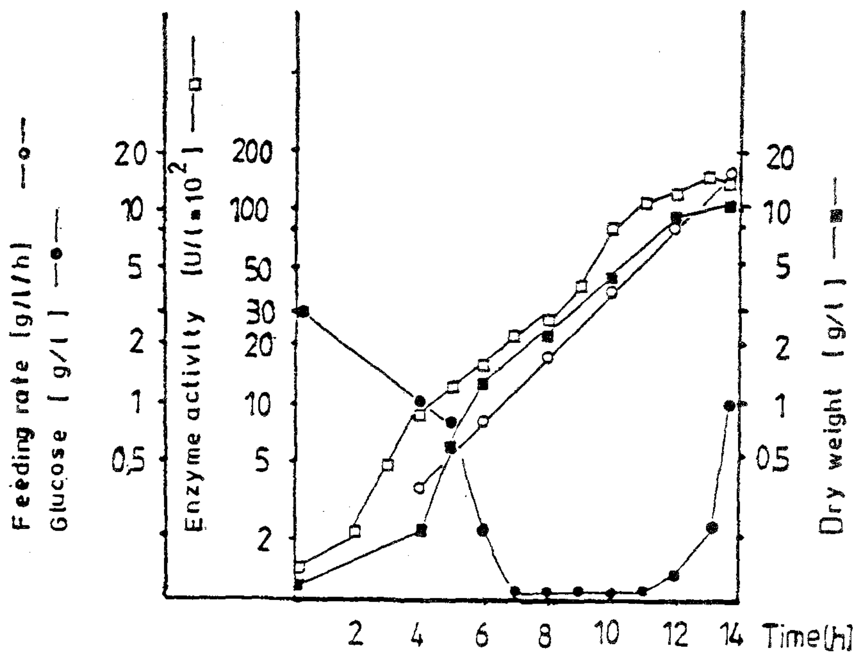

Figure 1. Fed batch culture of E. coli.
Glucose feeding started 4 h after inoculation.

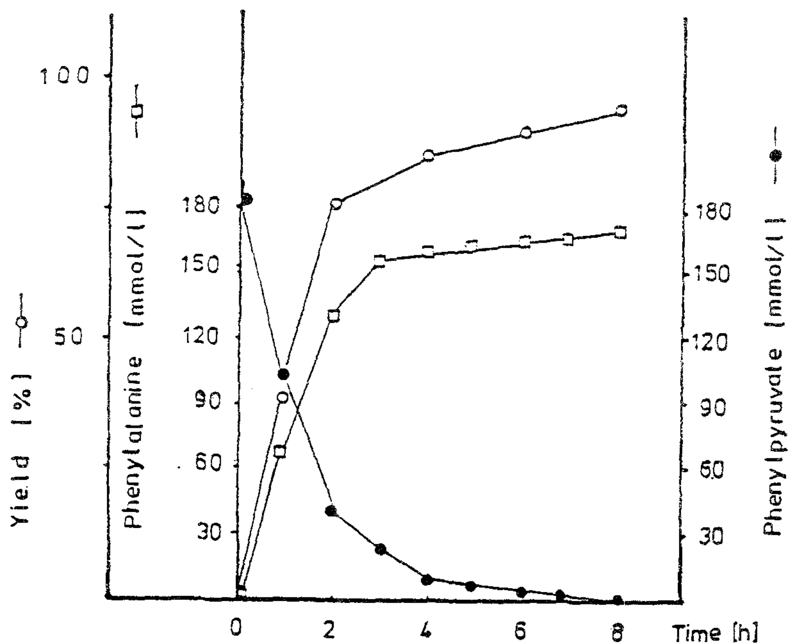

Figure 2. Time course of bioconversion of phenylpyruvate to phenylalanine.

A total of 30000 U transamination activity was added to a 10 l solution of 190 mmol/l aspartate and 182 mmol/l phenylpyruvate at pH 7.4 and 40 C. The reactor was aerated at 0.5 V/V m.

aromatic aminotransferase in this stain is subject to substrate inhibition at high phenylpyruvate concentrations.
Application of the biotransformation described in this paper to give a practical and economical process requires high molar conversion yields, high aminotransferase activities and an inexpensive source of phenylpyruvate. We have shown that, using a genetically engineered E. coli strain B, considerable progress can be made towards meeting the first two of these requirements.

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