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Note

Enhanced Production of L-Aspartate β-Decarboxylase by Nitrogen Source in *Pseudomonas dacunhae*

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Improvement of L-asparate β-decarboxylase production from *Pseudomonas dacunhae* ATCC 21192 was attempted by optimizing fermentation conditions. Optimum carbon and nitrogen sources for cell growth and enzyme production were determined. L-Glutamate (2%) was the most suitable carbon source, and D-glucose, D-glycerol and fumarate repressed enzyme production. Yeast extract (2%) was the most effective as nitrogen source. A slight change of pH to 6.5 from medium pH resulted in a meaningful increase in the production of enzyme. The production of the enzyme was highly improved by using 2% yeast extract and 2% L-glutamate in culture media. Maximum L-asparate β-decarboxylase activity reached up to over 24 U/mL-broth by 15 h flask fermentation.

Key words: Pseudomonas dacunhae, *L-asparate β-decarboxylase, L-glutamate, yeast extract*

L-alanine, one of the 20 amino acids most widely used in protein biosynthesis, is a non-essential α-amino acid and yet an important amino acid for the liver because it can serve as the substrate for gluconeogenesis.[1,2] It is particularly important in pharmaceuticals and food industry as food acidulant. Industrial production of L-alanine is accomplished from L-aspartic acid by biotransformation using immobilized *Pseudomonas dacunhae* cells containing L-aspartate β-decarboxylase (EC 4.1.1.12).[3] This manufacturing process has the advantage of high yield, selectivity and continuous production because of immobilization technique. Therefore, there is much information in the literature about the immobilization methods of *P. dacunhae* cells.[4-6] The productivity and activity of immobilized enzyme can be enhanced by increasing L-aspartate β-decarboxylase activity in the course of enzyme fermentation; nevertheless, only a few reports are available on the *P. dacunhae* cell growth and L-aspartate β-decarboxylase production.[7,8] The optimization of cultivation conditions can enhance the production of L-aspartate β-decarboxylase from *P. dacunhae*.

Since fermentation conditions are critical for the production of L-aspartate β-decarboxylase, we investigated to what extent both enzyme production and cell growth is affected by various parameters of cell cultivation, such as pH and medium compositions. In this work, the effect of nitrogen sources on the bacterial growth and enzyme production was especially investigated.

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Materials and Methods

Cultivation of microorganism. *Pseudomonas dacunhae* ATCC 21192 was used as the microbial source of the L-aspartate β-decarboxylase enzyme in this work. The stock culture was maintained on Nutrient Agar slant and subcultured monthly. The preculture was grown in a 250 m*l* baffled flask containing 50 mL of basal medium (2% L-glutamate, 0.9% peptone, 0.2% casein hydrolysate, 0.05% K_2HPO_4 and 0.05% $MgSO_4 \cdot 7H_2O$; pH 7.2) at 30°C for 24 h on a rotary shaker at 200 rpm.[7] The culture medium for main cultivation was inoculated with 2% (v/v) seed culture. The main cultivation for the production of enzyme was performed for 15 h under the same conditions as with the preculture. Cell growth was measured spectrometrically at 600 nm. All experiments were carried out at least twice until the optimal culture conditions were found; as such, the data shown in this paper are representative.

Enzyme activity assay. The L-aspartate β-decarboxylase activity of cells was determined according to the modified method of Shibatani *et al.*[7] For the enzyme assays, 0.2 mL of culture broth and 0.2 mL of 10% (w/v) SDS were incubated with reaction buffer for 60 min at 30°C. The reaction buffer contained 100 mM L-aspartic acid, 1.3 mM pyridoxal-5-phosphate, 1.7 mM α-ketoglutaric acid, 0.5% (w/v) bovine serum albumin and 200 mM acetate buffer (pH 5.3) in a final volume of 3 mL. The reaction was stopped by boiling the reaction mixtures, and 1 mL of the reaction mixture was centrifuged at 10,000 × *g* for 5 min. After centrifugation, L-alanine concentrations of the supernatants were measured with a Waters HPLC system using the OPA method.[9] One unit of L-aspartate β-decarboxylase was equivalent to the

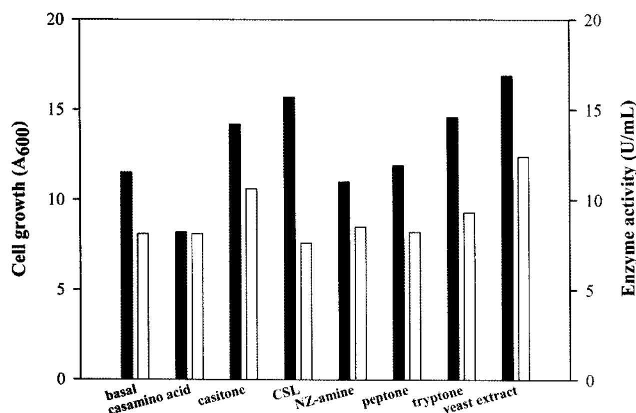

Fig. 1. Effect of nitrogen sources on cell growth (closed bar) and L-aspartate β-decarboxylase activity (open bar) by *P. dacunhae* ATCC 21192 in shake flask culture. Abbreviation CSL means corn steep liquor.

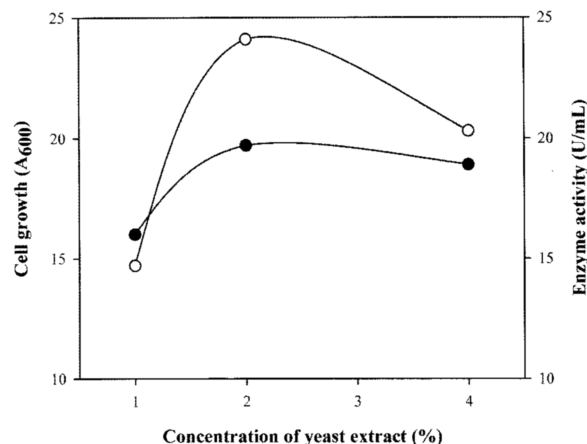

Fig. 2. Effect of concentration of yeast extract on cell growth (closed circle) and L-aspartate β-decarboxylase activity (open circle) by *P. dacunhae* ATCC 21192. Fermentations were carried out for 15 h at 30°C with basal medium containing 2.0% L-glutamate.

amount of enzyme required to produce 1 μmol of L-alanine per min under the assay conditions.

Results and Discussion

To determine the basal medium, some reported media compositions were tested for cell growth and L-aspartate β-decarboxylase production in ATCC 21192. Since the media composition of Shibatani *et al.*[7] with peptone, casein hydrolysate and L-glutamate was found to be effective in enzyme production and *P. dacunhae* growth (data not shown), this was used as basal medium in this work.

The effects of carbon sources on the bacterial growth and production of L-aspartate β-decarboxylase by *P. dacunhae* ATCC 21192 were investigated. Various carbon sources (1%) were supplemented in the basal medium. After 15 h cultivation, enzyme production and bacterial growth were severely repressed when glucose, fumarate and glycerol were added in the media (data not shown). The highest cell growth (A_{600} = 11.1) and enzyme production (7.9 U/mL) were obtained in the basal medium containing L-glutamate as the carbon source. To investigate the effect of nitrogen sources on the growth and enzyme production, various organic nitrogen sources (1%) were used instead of peptone and casein hydrolysate in the basal medium. Among the seven nitrogen sources examined, the highest cell growth and enzyme production was obtained when yeast extract was used in the media (A_{600} = 16.9 and 12.4 U/mL), followed by casitone, tryptone, NZ-amine and peptone (Fig. 1).

The effects of concentration of yeast extract and L-glutamate on cell growth and enzyme production were investigated. Concentration of yeast extract was changed from 1.0 to 4.0% in the basal media with 2% L-glutamate. Addition of 2.0% yeast extract resulted in an increase in the enzyme production by more than 60% (Fig. 2), but a further increase in the nitrogen source appeared to be no more effective. To determine the optimum concentration of L-glutamate, its

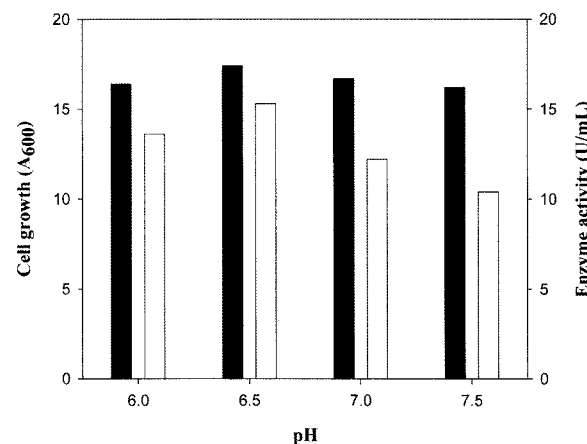

Fig. 3. Effect of medium pH on cell growth (closed bar) and L-aspartate β-decarboxylase activity (open bar) by *P. dacunhae* ATCC 21192.

concentration in the basal medium with 2.0% yeast extract was varied from 0 to 4.0% as 24.1 U/mL, 2.0% L-glutamate concentration gave the best production of enzyme (data not shown). This result was similar with the previous reports.[7,8] As enzyme production from microorganisms is strongly influenced by media components in the presence of some easily metabolizable sugars and nitrogen sources in medium,[10,11] the evaluation of the effects of suitable carbon and nitrogen sources of culture medium is of interest. The optimal carbon source for the production of L-aspartate β-decarboxylase by *P. dacunhae* ATCC 21192 was L-glutamic acid. As L-glutamic acid is central in the biosynthesis of amino acid,[12] the necessary component for L-aspartate β-decarboxylase production in *P. dacunhae* and optimum concentration of initial glutamic acid was 2.0%.

Since enzyme production and cell growth were enhanced by changing from medium pH to a slightly lower value, the effect of medium pH to cell growth and enzyme activity were

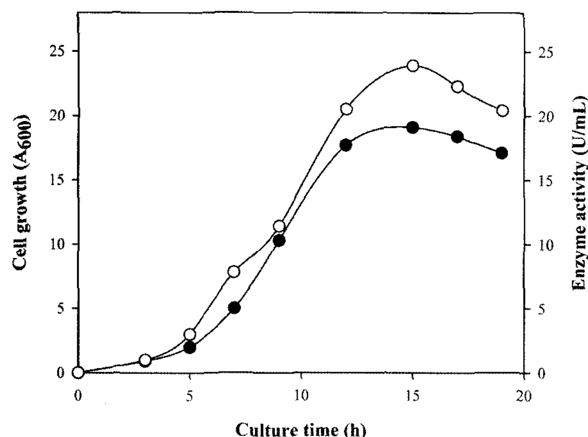

Fig. 4. Time courses of cell growth (closed circle) and L-aspartate β-decarboxylase activity (open circle) by *P. dacunhae* ATCC 21192. Fermentation conditions were; temperature, 30°C; pH, 6.5; 2% yeast extract supplemented with 2% L-glutamate; buffer system, K-phosphate solution with $MgSO_4 \cdot 7H_2O$.

investigated in basal medium excluding casein hydrolysate. This was confirmed by carrying out fermentation under the same conditions except for medium pH: that is, when the cells were cultivated at pH 6.5, the activity of L-aspartate β-decarboxylase was found to be significantly higher (by ca. 25%) compared with culturing at pH 7.0, as shown in Fig. 3. However, Yamamoto *et al.*[4] and Çalık *et al.*[13] reported that they made the cultivation of the *P. dacunhae* cells at initial pH 7.3 and pH 7.5 in shake flasks, respectively.

As a result of cultivation using the above results, the activity showed a maximum at 15 h which corresponds to the stationary growth phase of cells and reached up to 23.6 U/mL (Fig. 4). However, Çalık *et al.*[13] and Chibata *et al.*[14] stated the highest L-asparate β-decarboxylase activity had been obtained at 20 h and 26~28 h of fermentation performed in flask culture which corresponds to the stationary phase of the cells. Consequently, the optimization of culture conditions such as nitrogen source and pH could be enhanced the productivity of L-asparate β-decarboxylase fermentation via shortening of cultivation time and improving of enzyme activity thereby significantly increasing the specific production. (Table 1) Particularly, enhancement of the specific production shows that increase of cell growth is not the only reason for improvement of enzyme activity.

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Table 1. Effect of medium optimization on L-aspartate β-decarboxylase production by *P. dacunhae* ATCC 21192

Medium	Culture time (h)	Cell growth (A_{600}) [A]	Enzyme activity (U/mL) [B]	Specific production [B/A]	Ref.
Basal medium[a]	24	10.3	7.7	0.75	7
Medium A[b]	16	11.3	7.4	0.65	5
Medium B[c]	15	18.9	23.6	1.25	This work

[a]Basal medium: 2.0% L-glutamate, 0.9% peptone, 0.2% casein hydrolysate, 0.05% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$ (pH 7.2).
[b]Medium A: 4.0% NZ-amine, 0.1% KH_2PO_4, 0.03% $MgSO_4 \cdot 7H_2O$ (pH 6.8).
[c]Medium B: 2.0% L-glutamate, 2.0% yeast extract, 0.05% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$ (pH 6.5).

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