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L-Valine Fermentation by *Brevibacterium flavum* Strain NV246

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A L-valine hyper-producer *Brevibacterium flavum* strain NV246 (α-AB^{hr}, D-glucose^{hr}) was obtained, which could be resistant to high concentration of α-aminobutyric acid (α-AB) or D-glucose. The result indicates that the final L-valine concentration, 20 % glucose added in the culture medium at one time at the beginning of the fermentation process, is much higher than the one adding lower percentage of glucose in the medium at several times during the process. The influence of some alcohols on the L-valine production by strain NV246 was studied. The result indicates N-butyl alcohol and ethylene glycol enhance the production of L-valine.

Key Words: *Brevibacterium flavum*, L-Valine, Fermentation.

INTRODUCTION

L-Valine is an essential amino acid mainly used as precursor in chemical synthesis (*e.g.*, antiviral drugs). It has a market volume of approximately 500 tons/year with relatively high market prices[1,2]. L-Valine can be produced by chemical synthesis, fermentation and by extraction from animal raw materials. Nowadays, the way of fermentative production is getting more important due to a higher flexibility of produced amounts and the friendly process to the environment.

In the metabolism of *Brevibacterium flavum*, L-valine is formed in four steps beginning with the condensation of two pyruvate molecules to acetolactate by acetyl-hydroxy acid synthase (Fig. 1)[3]. Acetohydroxy acid synthase (AHAS), also called acetolactate synthase, is the first key enzyme in the pathway of L-valine biosynthesis (Fig. 1). Activity of *Brevibacterium flavum* with AHAS can be inhibited by any of the three branched-chain amino acids (isoleucine, leucine and valine), with valine as the strongest inhibitor. It is necessary to relieve the inhibition by valine in order to enhance the production.

α-Aminobutyric acid was used to relieve the inhibition at comparatively low concentration by Chinese Academy of Science[4-6]. Zhang *et al.*[7] have revealed the inhibition by high concentration of α-aminobutyric acid. The strain has also been given a property to be resistant to high concentration of D-glucose.

EXPERIMENTAL

All the chemicals used were of analytical grade and mainly purchased from Sigma Chemical Company.

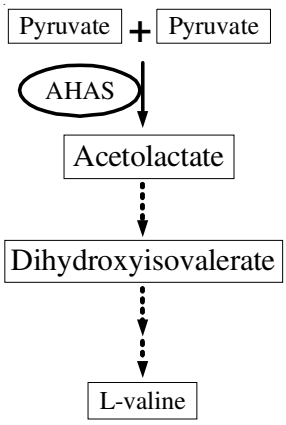


Fig. 1. Biosynthesis of L-valine in *Brevibacterium flavum*. Acetylhydroxy acid synthase (AHAS)

B. flavum NV001, an original producer of L-valine, was kept in our laboratory. The identification of this strain was performed according to the descriptions of Buchanan[8]. Mutant NV246, a producer of L-valine, is isolated from N-methyl-N-nitrosoguanidine (NTG) mutagenesis on strain *B. flavum* NV001.

The culture medium used for the storage contained (g/L): peptone 10, beef extract 10, yeast extract 5, NaCl 2.5, agar strip 20, adjusted to pH = 7.0 with 0.1 M of HCl or NaOH. The culture medium used for the seeds (g/100 mL): glucose 2.5, $(NH_4)_2SO_4$ 0.5, KH_2PO_4 0.1, $MgSO_4 \cdot 7H_2O$ 0.05, corn-steep (65 % dry wt.) 0.5, $CaCO_3$ 1.0 and adjusted to pH = 7.0 with 0.1 M of HCl or NaOH. The culture medium used for the

fermentation (g/100 mL): glucose 20, $(NH_4)_2SO_4$ 6, KH_2PO_4 0.2, $MgSO_4 \cdot 7H_2O$ 0.08, corn-steep (65 % dry wt.) 0.6 and pH = 7.0.

Determination of L-valine in the broth: As a quick and simple method to determine the concentration of L-valine in the broth, the paper chromatographic method of assay was applied, using a solvent system *n*-butanol-acetic acid-water (2:2:1 by volume)[9] and Klett-Summerson photo-electric colorimeter with a green filter (540 nm)[10].

Procedure of the fermentation: For the fermentation, the first preculture was grown for 10 h in 250 mL shaking flasks containing 15 mL seed medium. Afterwards, 2 mL was transferred to a 500 mL shaking flask with 40 mL of fermentation medium. Temperature was maintained at 30 ºC and the cultivations were finished after 72 h when glucose was totally consumed. Final extracellular L-valine concentrations were determined at that point.

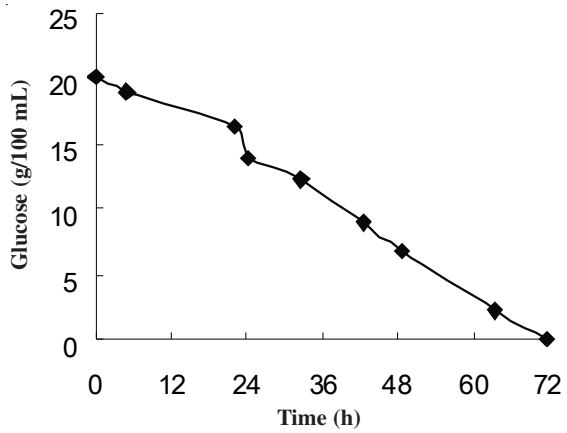
RESULTS AND DISCUSSION

***Brevibacterium flavum* strain NV246:** Strain NV246 was obtained, which could be resistant to medium containing 25 % α-amino butyric acid. The strain NV246's ability to resist α-amino butyric acid is much higher than the strain's ability breed by Xiong *et al.*[11]. Thus, the inhibition by valine to strain NV246 has been relieved in a deeper degree than the one breed by Zhao *et al.*[7]. Strain NV246 is a L-valine hyper-producer. Meanwhile, strain NV246 could be resistant to 20 % D-glucose, which was much higher[2].

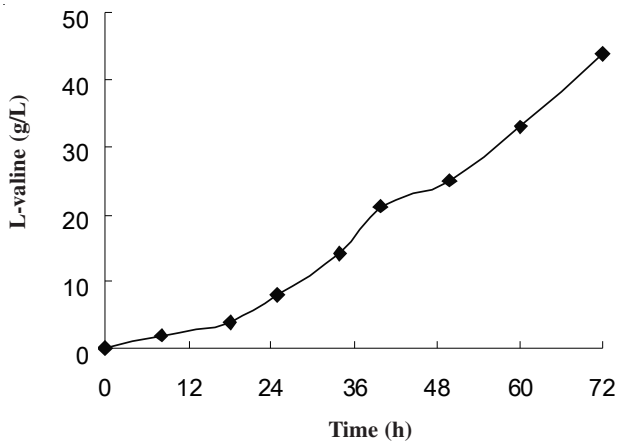
Production capacity of *Brevibacterium flavum* strain NV246: The initial glucose concentration was 20 g/100 mL in Fig. 2a. During the fermentation process, no more glucose was added. The process of fermention continued for 72 h when the glucose was used up. At the end of the process, the yield of L-valine in g/L was 44.1 g in Fig. 2b. The initial glucose concentration was 10 g/100 mL in Fig. 3a. When the glucose in the broth was used up afer about 50 h, glucose was added to make sure the concentration was 10 g/100 mL again. The process of fermention continued for 91 when the glucose was used up. At the end of the process, the yield of L-valine in g/L was 28.2 g in Fig. 3b.

Influence of some alcohols on L-valine fermentation by *Brevibacterium flavum* strain NV246: The data recorded in Table-1 shows that the presence of methanol has inhibitory effect on the fermentative production of L-valine by strain NV246. The maximum yield of L-valine was observed at 0.1 % concentration of methanol *i.e.,* 42.856 g/L in 72 h of optimum incubation period which was 2.9 % less than that of control. *n*-Propyl alcohol, *n*-butyl alcohol and propanetriol also have inhibitory effect on the production of L-valine.

The influence of alcohol on L-valine production by *Brevibacterium flavum* strain NV246 was found to be slightly

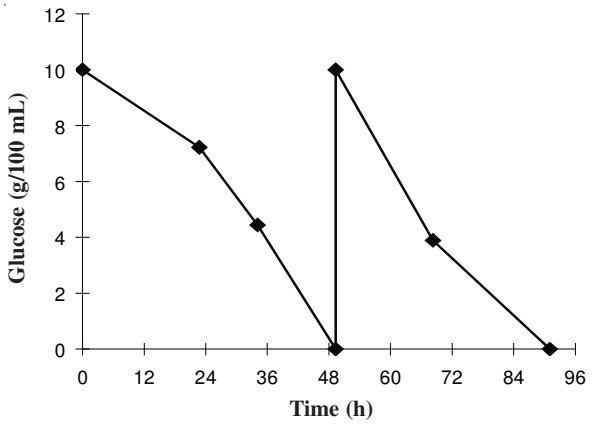


(a)

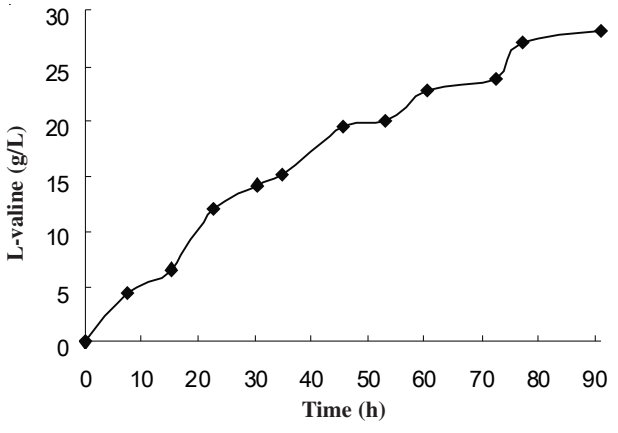


(b)

Fig. 2. Time-courses when the initial glucose concentration was 20 g/100 mL



(a)



(b)

Fig. 3. Time-courses when the initial glucose concentration was 10 g/100 mL

TABLE-1
INFLUENCE OF SOME ALCOHOLS ON L-VALINE FERMENTATION BY *Brevibacterium flavum* STRAIN NV246 IN 72 h OF OPTIMUM INCUBATION PERIOD

Alcohols used	Optimum concentration of alcohols used (%)	Max. yield* of L-valine in control (g/L)	Max. yield* of L-valine in the presence of different alcohols (g/L)	Residual glucose* (g/100 mL)	Percentage of L-valine increase (+)/decrease (–) in 72 h of optimum incubation period
Methanol	0.1	44.130	42.856	0.965	(–) 2.8869
Alcohol	0.1	44.136	45.283	0.583	(+) 2.5988
N-Propyl alcohol	0.1	44.133	41.339	1.023	(–) 6.3309
N-Butyl alcohol	0.2	44.134	43.837	0.736	(–) 0.5982
Propanetriol	0.1	44.147	43.647	0.835	(–) 1.1326
Ethylene glycol	0.3	44.122	46.735	0.223	(+) 5.9222

*Each value represents mean of three trials. (+)/(–) values indicate % increase/decrease in the yield of L-valine. Experimental deviation ± 2.0-2.5%.

favourable at the low concentration *i.e.,* at 0.1 % and could produce 45.283 g/L of L-valine in 72 h of optimum incubation period which was 2.60 % higher than that of control. The data recorded (Table-1) also shows that ethylene glycol has stimulatory effect on L-valine fermentation. The maximum yield of L-valine was found to be 46.735 g/L at 0.3 % concentration of ethylene glycol which was 5.92 % higher than that of control. The prodution of L-valine usually corresponded with the consumption of glucose.

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