

## Study on the Breeding of L-Valine Producing Mutants (Prototroph and Auxotroph) and Comparison of Their Properties

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NVS-137 and JVK-427 were isolated from mutagenesis on strain ZQM-246. Strain NVS-137 is prototroph, while strain JVK-427 is auxotroph. The results indicate strain NVS-137 is superior to JVK-427 in the fermentation process. Addition of biotin had an effect on L-valine fermentation and dry weight of cells.

**Key Words:** Prototroph, Auxotroph, L-Valine, Mutagenesis, Fermentation.

### INTRODUCTION

L-Valine is not only one of three branched chain amino acids (BCAAs), but also is an essential amino acid in animal nutrition. It is mainly used as precursor in chemical synthesis of many bioactive compounds (*e.g.*, antiviral drugs). L-Valine can be produced by extraction from animal raw materials, chemical synthesis and by fermentation. Because of a higher flexibility of produced amounts and the friendly process to the environment, the way of fermentative production is getting more important today.

Besides L-valine, L-leucine and L-isoleucine are also branched chain amino acids (BCAAs). Three branched chain amino acids share a common precursor that is pyruvate (Fig. 1). In order to breed a L-valine high-producing strain, pathways from pyruvate to L-leucine and from pyruvate to L-isoleucine should be cut. Katsurada<sup>1</sup>, Wang *et al.*<sup>2</sup> and Xiong *et al.*<sup>3</sup> have developed L-valine producing strains which can't produce L-leucine or L-isoleucine. They are all auxotrophic strains. However, Zhang *et al.*<sup>4</sup> have bred a L-valine high producing strain which produces some L-leucine and some L-isoleucine. The strain isn't auxotrophic and is referred to as a strain leaking L-leucine and L-isoleucine (Fig. 2).

Biotin is a water-soluble vitamin that is a member of the B-complex group of vitamins. Biotin is also referred to as vitamin H or vitamin B7. It plays an important role in the catalysis of essential metabolic reactions to synthesize proteins, fatty acids and polysaccharide<sup>5</sup>.

During the amino acids fermentation, the amount of biotin has direct effects on many aspects of producing strains, such

as the growth of cells, cell reproduction, cell metabolism, cell wall permeability, cell membrane permeability, amino acid yields<sup>6</sup>.

### EXPERIMENTAL

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

**Microorganisms and medium:** ZQM-246, an original producer of L-valine, was kept in our laboratory. NVS-137 is isolated from N-methyl-N'-nitrosoguanidine (NTG) mutagenesis on strain NVI-225 which is a mutant of ZQM-246 (Fig. 2). JVK-427 is isolated from N-methyl-N'-nitrosoguanidine (NTG) mutagenesis on strain JVN-345 which is a mutant of ZQM-246 (Fig. 2).

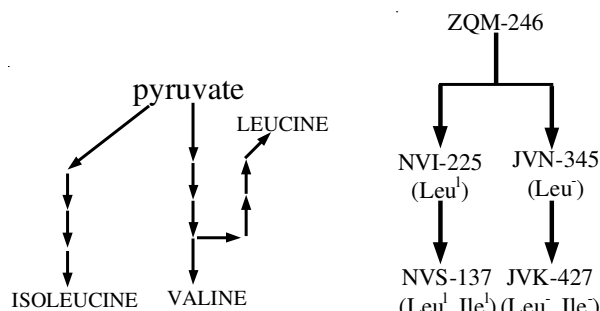


Fig. 1. Biosynthesis of L-valine and in the strain

Fig. 2. Mutagenesis route of NVS-137 and JVK-427

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<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5, KH<sub>2</sub>PO<sub>4</sub> 0.1, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.05, corn-steep (65 % dry wt.) 0.5, CaCO<sub>3</sub> 1.0 and adjusted to pH = 7.0 with 0.1 M of HCl or NaOH. The culture medium used for the the fermentation of strain NVS-137 contained (g/100mL): glucose 12.5, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 6, KH<sub>2</sub>PO<sub>4</sub> 0.2, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.08, corn-steep (65 % dry wt.) 0.6, CaCO<sub>3</sub> 3 and pH = 7.0. The culture medium used for the the fermentation of strain JVK-427 contained (g/100mL): glucose 12.5, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 4, KH<sub>2</sub>PO<sub>4</sub> 0.1, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.05, corn-steep (65 % dry wt.) 1.0, L-leucine 0.02, L-isoleucine 0.02, CaCO<sub>3</sub> 3 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)<sup>7</sup> and 721 spectrophotometer made in China with a filter (540 nm).

**Procedure of the fermentation:** For the fermentation, the first preculture was grown for 8-10 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 °C and the cultivations were finished after 72-100 h when glucose was totally consumed. Final extracellular L-valine concentrations were determined at that point.

## RESULTS AND DISCUSSION

**L-Valine producing strain NVS-137 (Leu<sup>+</sup>, Ile<sup>+</sup>) and strain JVK-427 (Leu<sup>-</sup>, Ile<sup>-</sup>):** Strain NVS-137 (Leu<sup>+</sup>, Ile<sup>+</sup>) was obtained, which could produce a very high yield without any addition of L-leucine or L-isoleucine in the medium. Pathways from pyruvate to isoleucine and to leucine weren't cut completely, so the strain could offer the two amino acids by itself during the fermentation process. Strain NVS-137 (Leu<sup>+</sup>, Ile<sup>+</sup>) is prototroph, which could synthesize all the compounds that the parent organism could.

Strain JVK-427 (Leu<sup>-</sup>, Ile<sup>-</sup>) was obtained, which could produce a very high yield with addition of L-leucine or L-isoleucine in the medium. Pathways from pyruvate to isoleucine and to leucine were cut completely and the strain couldn't offer the two amino acids by itself during the fermentation process. Strain JVK-427 (Leu<sup>-</sup>, Ile<sup>-</sup>) is auxotroph, which couldn't synthesize L-leucine or L-isoleucine required for its growth.

**Production capacity of strain NVS-137 and strain JVK-427:** Biomass of strain NVS-137 and strain JVK-427 in the medium was measured by OD<sub>625</sub> value. The OD<sub>625</sub> value of strain NVS-137 was up to 0.8 after 12 h, while the OD<sub>625</sub> value of strain JVK-137 was not up to after 15 h. Strain NVS-137 grows much faster than strain JVS-427 in seed culture medium.

The process of strain NVS-137 fermentation 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 47.4 g in Fig. 3(a). The process of strain JVK-427 fermentation continued for 95 h when the glucose was used up. At the end of the process, the yield of L-valine in g/L was 43.7 g in Fig. 3(b).

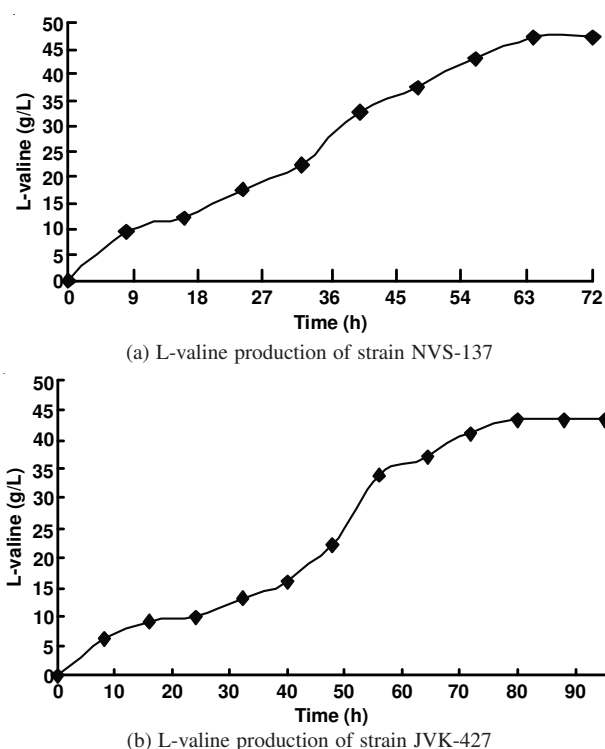
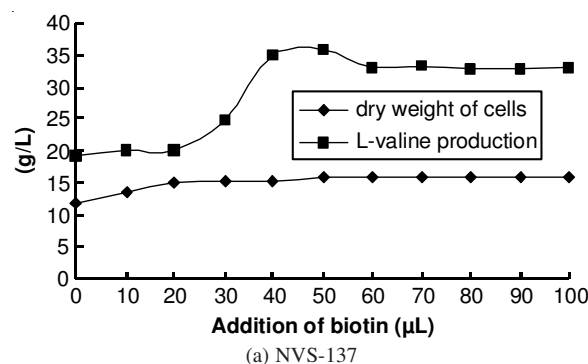


Fig. 3. L-valine production of Strain NVS-137 and Strain JVK-427

It is easy to conclude that JV(auxotroph) has a higher yield of L-valine than NV (prototroph) based on theory of metabolic engineering. However, our results indicated that NV (prototroph) had the higher yield of L-valine. In fact, Prof. Zhang Weiguo screened two strains: the yield of L-valine in g/L was 66.7g by one strain 4 and the yield of the other was more than 80 g L-valine in g/L. The latter yield is the highest level reported in the world. L-valine producers engineered by Blombach *et al.*<sup>8</sup> produced up to 48 g/L L-valine.

**Influence on L-valine fermentation by addition of biotin:** The dry weight of NVS-137 cells was greater as addition of biotin increased from 0 µg/L to 50 µg/L. And it became constant (about 15.71g/L) as the concentration of biotin was more than 50 µg/L. L-Valine production reached maximum (about 35.64/L) when the addition of biotin was 50 µg/L (Fig. 4a). The dry weight of JVK-427 cells was greater as addition of biotin increased from 0 to 50 µg/L. And it became constant (about 13.11g/L) as the concentration of biotin was more than 50 µg/L. L-Valine production reached maximum (about 29.56/L) when the addition of biotin was 60 µg/L (Fig. 4b).



(a) NVS-137

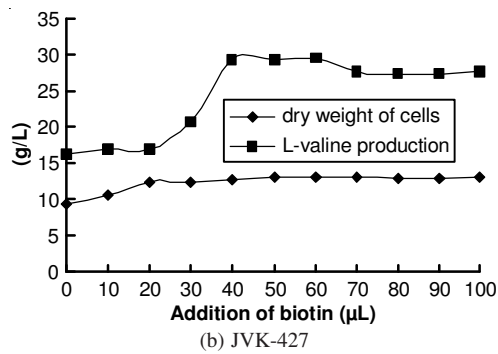


Fig. 4. Effect of biotin on cell growth and the biosynthesis of L-valine

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