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Breeding of Producing L-Valine Strain *Brevibacterium flavum* NJ112 by Protoplast Fusion Technology

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With *Brevibacterium flavum* NVT1103 (Leu^{l}, α-AB^{hr}, 2-TA^{hr}, SG^{r}) and JVHK597 (Leu^{-}, Ile^{-}, Met^{l}, α-AB^{r}, 2-TA^{r}) as parental strains, conditions of two parental strains' protoplast formation and regeneration were groped about. On this basis, a series of protoplast fusion experiment were conducted to investigated the influence of fusion temperature in the fusion process, poly(ethylene glycol) concentration and the fusion time. Under unoptimized conditions, the L-valine production of the strain NJ112 derivative in flasks was 45.6 g/L.

Key Words: *Brevibacterium flavum*, L-valine, Fermentation, Protoplast fusion.

INTRODUCTION

L-valine is one of the eight essential amino acids for human and animal bodies. Protoplast fusion technology is a technology system, which is composed of a variety of independent techniques[1].

The genetic materials can be exchanged completely and the parental strians could have a high hybridization frequency and high rate of genetic recombination[2,3]. In the late 19th century, Hungarian scientist used protoplast fusion technology in the microbial successfully for the first time[4]. Scientists in Japan applied protoplast fusion technology in the breeding of amino acid industrial strains for the first time in the late 1980s[5].

In this paper, *B. flavum* NVT1103 (Leu^{l}, α-AB^{hr}, 2-TA^{hr}, SG^{r}) and *B. flavum* JVHK597 (Leu^{-}, Ile^{-}, Met^{l}, α-AB^{r}, 2-TA^{r}) were parental strains and they had different genetic markers. α-AB^{hr} and 2-TA^{hr} were unique genetic markers in our laboratory[6]. Leu^{l} and Met^{l} were genetic markers which could improve acid production remarkably[7]. Various factors which influence formation rate and regeneration rate of protoplasts of the two parental strains were studied. And a series of fusion strains were breed successfully.

EXPERIMENTAL

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

B. flavum ZGH6128 was kept in our laboratory and the identification of this strain was performed according to the descriptions of Buchanan[8] *B.flavum* NVT1103 and *B. flavum* JVHK597 were mutants derived from *B. flavum* ZGH6128. Object strain *B. flavum* NJ112 was a fusion strain and strain *B. flavum* NJv61 was derivative of strain *B. flavum* NJ112.

Culture medium used for the seeds (g/100mL): Glucose 2.5, $(NH_4)_2SO_4$ 0.5, KH_2PO_4 0.1, $MgSO_4·7H_2O$ 0.05, cornsteep (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, $MgSO_4·7H_2O$ 0.08, corn-steep (65 % dry wt.) 0.6 and adjusted to 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: by volume)[9] and Klett-Summerson photo-electric colourimeter with a green filter[10]. The procedure of the fermentation was according to Zhang *et al.*[11].

RESULTS AND DISCUSSION

Determination of the lysozyme concentration: Enzyme concentration associated with enzymatic hydrolysis time and hydrolysis temperature. Enzyme concentration data was indicated in the abscissa and the corresponding formation rate and regeneration rate was taken in the vertical axis. The results were shown in Fig. 1. Based on data in Fig. 1, 2 g/L lysozyme was used to treat the parental strain JVHK597, a higher formation rate (93 %) and regeneration rate (19.5 %) of the protoplast could be reached. In the same way, 1 g/L lysozyme was used to treat the parental strain NVT1103.

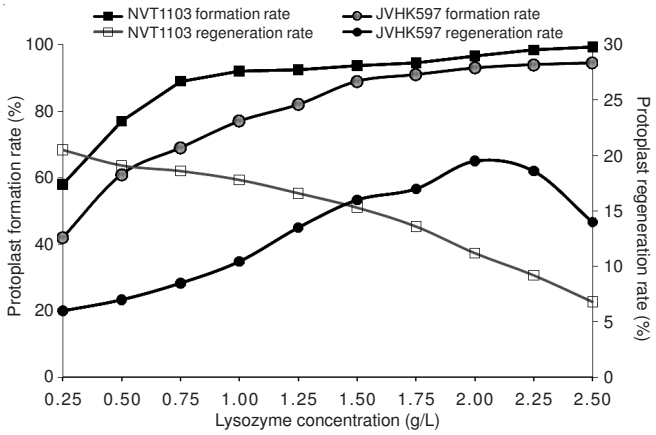

Fig. 1. Influence of different lysozyme concentration on formation rate and regeneration rate

Determination of enzymolysis time: The process of protoplast formation and the cell wall regeneration to form a complete bacterial cell was influenced by enzymolysis time[12]. In this paper, parental strains were treated for 6 to 14 h and the variation of protoplast formation rate and regeneration rate was studied in order to determine the optimal processing time and the results were shown in Fig. 2. Based on data in Fig. 2, when the parental strain NVT1103 was treated for 9 h, the harvest of the protoplast had a higher generation rate and regeneration rate. The parental strain JVHK597 was enzymolyzed for 11 h.

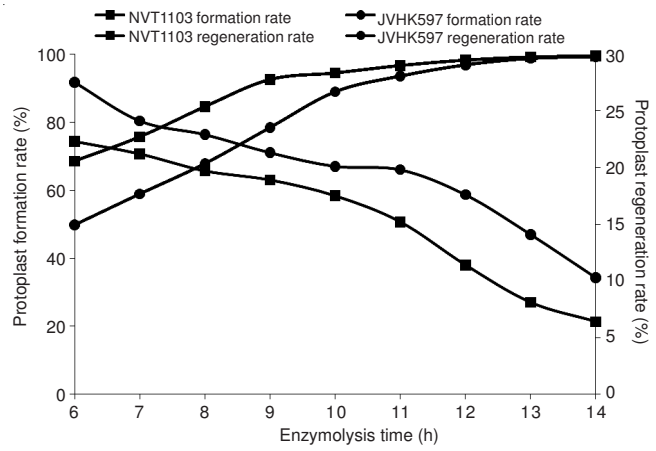

Fig. 2. Influence of enzymolysis time on formation rate and regeneration rate

Protoplast fusion process: There are many factors affecting the protoplast fusion process, such as the choice of cell fusion agent, concentration of cell fusion agent, length of treating time, fusion temperature, *etc*.

Influences of concentrations and treating time of poly(ethylene glycol) (PEG) on fusion rate: The two parental strains were treated with different concentrations of poly(ethylene glycol) and poly(ethylene glycol) mediated the fusion. As shown in Fig. 3, the best effect could be obtained under 400 g/L poly(ethylene glycol), that's to say, the highest fusion rate could be obtained under the same conditions. Based on data in Fig. 4, the optimum treating time is 25 min and the highest parental fusion rate (2.43×10^{-5}) was available.

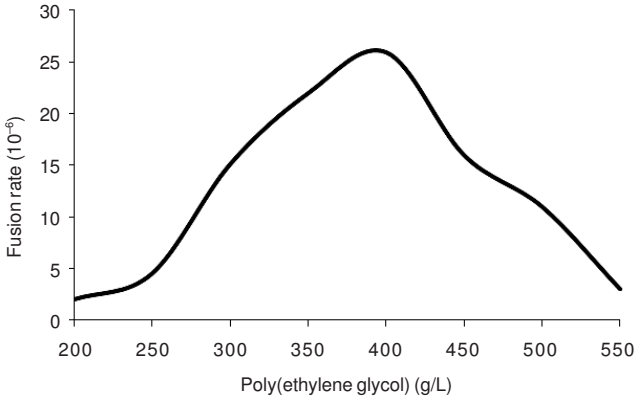

Fig. 3. Influence of poly(ethylene glycol) concentration on fusion rate

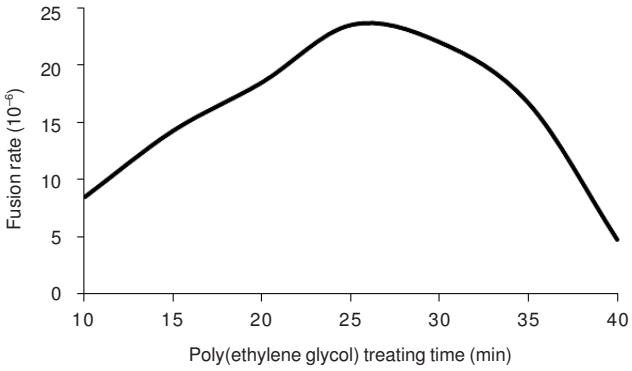

Fig. 4. Influence of poly(ethylene glycol) treating time on fusion rate

Influence of temperature on fusion rate: During the process of protoplast fusion, the temperature of the fusion buffer system was an important factor and it couldn't be ignored[13]. As shown in Fig. 5, when the temperature was kept at 34 ºC in the integration process,a high parental fusion rate (2.54×10^{-5}) was available.

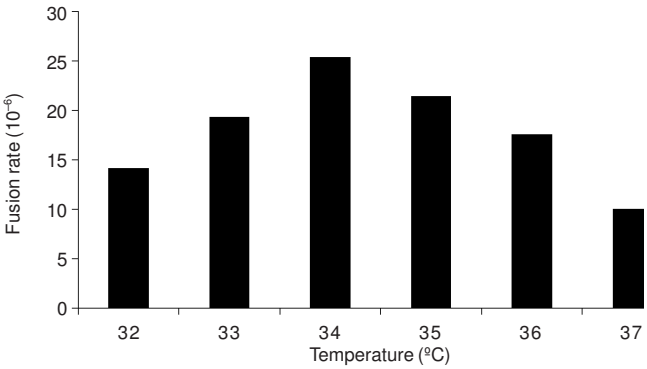

Fig. 5. Influence of temperature on fusion rate

Conclusion

1 g/L Lysozyme was used to treat the parental strain NVT1103 for 9 h, ideal results could be got. 2 g/L lysozyme was used to enzymolyse the parental strain JVHK597 for 11 h, the harvest of the protoplast had a higher formation rate and regeneration rate. The two parental strains were treated with 400 g/L poly(ethylene glycol) for 25 min at 34 ºC and a high parental fusion rate was available. Through protoplast fusion, object strain NJ112 were screened and its derivatives were

verified to have parental strains' genetic markers. Eventually, under unoptimized conditions, the L-valine production of the strain NJ112 derivative in flasks was 45.6 g/L.

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REFERENCES

1. C. Sanfeng and L. Dehu, Moden Microbial Genetics, Chemical Industry Press, Beijing, pp. 308-316 (2003).
2. Z.J. Tan, Q.M. Xiao and K.Y. Xiao, *Biotechnology*, **2**, 35 (2003).
3. D. Lianxiang and L. Fuping, Microbiology Experimental Techniques, China Light Industry Press, Beijing, pp. 210-211 (2005).
4. L. Ferenczy, F. Kevei and J. Zsolt, *Nature*, **248**, 793 (1974).
5. R. Uegaki, T. Fuiimori, H. Kaneko, K. Kato and M. Noguchi, *Agric. Biol. Chem.*, **43**, 1149 (1979).
6. H.M. Liu and W.G. Zhang, *Afr. J. Biotechnol.*, **9**, 21 (2010).
7. H.M. Liu, X.Y. Ge and W.G. Zhang, *Asian J. Chem.*, **23**, 4483 (2011).
8. R.E. Buchanan, Bergey's Manual of Srytematic Bacteriology, Science Press, Beijing, p. 869 (1984).
9. K. Fink, R.E. Cline and R.M. Fink, *Anal. Chem.*, **35**, 389 (1963).
10. S.K. Mandal and S.K. Majumdar, *Sci. Culture*, **36**, 556 (1970).
11. H.M. Liu, X.Y. Ge and W.G. Zhang, *Asian J. Chem.*, **23**, 2151 (2011).
12. L. Zaizi, Fundamental Theory of Biochemical Engineering, Chemical Industry Press, Beijing, pp. 18-19 (1999).
13. Z. Yingfu, K. Zhenrong and D. Shengmiao, *China Brewing*, **2**, 16 (2006).