FERMENTATION CONDITIONS FOR L-LYSINE SYNTHESIS BY BREVIBACTERIUM LACTOFERMENTUM HS918

C.C. Chang, W.L. Cheng, J.S. Wang & Y.T. Liu
Taiwan Sugar Research Institute, Tainan

ABSTRACT

The effects of component concentrations of raw sugar fermentation medium (RSPM) and physical culture parameters on HS918 were studied by flask fermentation. L-lysine accumulated by HS918 gradually increased as HPP concn. in media decreased from 4 to 1% (v/v). The addition of molasses at 0.5 to 1% (w/v) significantly improved the L-lysine.HCl production to 30 g/l. When ammonium sulfate was increased to 4% in the medium, HS918 accumulated 35 g/l of L-lysine.HCl during a 45-h fermentation period at 30°C. Elevating temperature to 33°C and using three-baffle Erlenmeyer flasks to increase aeration shortened fermentation time dramatically. Final L-lysine.HCl concn. and yield were 38 g/l and 40%, resp.

Keywords: Sugarcane, L-lysine, Brevibacterium lactofermentum, raw sugar fermentation medium, Taiwan.

INTRODUCTION

Amino acid fermentation is an important branch of the fermentation industry. Most amino acids are commercially produced by specific bacterial strains that have been genetically induced to become auxotrophic and/or are regulatory mutants. L-lysine is one of the most important amino acids produced by fermentative process in which two genera—Corynebacterium and Brevibacterium—are frequently used as producers. L-lysine producers are generally selected from lysine-analogue resistant mutants. This property is closely related to feedback-resistant aspartokinase, a key enzyme for synthesizing L-lysine (Adia et al. 1986, Sano & Shiio 1970). Sometimes, L-lysine producers possess other auxotrophic properties such as His- and/or Ala- to enhance L-lysine production (Tosaka et al. 1978). A new lysine-producing strain (HS918) derived from Strain A357 was developed without the properties of Arg- and His- but rather to reinforce raw sugar metabolic rate by successive mutation steps (Chang et al. 1994). In this work we studied the influence of the composite concn. of raw sugar fermentation medium (RSFM) on L-lysine accumulation of HS918 by the flask fermentation process. Two physical parameters—temperature and aeration—were also considered.

MATERIALS AND METHODS

Microorganism

Strain HS918, which was constructed stepwise from A357 (Chang et al. 1994), was employed throughout this study.

Media

Trypticase soy broth (TSB) was used as the first seed medium and its agar plates as renew media. The second seed medium contained 5% raw sugar, 1% (w/v) molasses, 4% CaCO₃, 3% (NH₄)₂SO₄, 0.05% KH₂PO₄, 2% (v/v) HPP, 20 μg/dl thamine.HCl and 40 μg/dl d-biotin (pH 7.0). Composition of the raw sugar fermentation medium (RSFM) was 10% raw sugar, 4% CaCO₃, 3% (NH₄)₂SO₄, 0.05% KH₂PO₄, 4% (v/v) HPP, 20 μg/dl thamine.HCl and 40 μg/dl d-biotin (pH 7.0).

Cultivation

A loopful of cells grown on TSB agar plates at 30°C for 24 h were inoculated into a 500-ml Hinton flasks containing 50 ml TSB as the first seed culture. After 20 h cultivation, 2 ml of the first seed broth was transferred to 50 ml of the fermentation medium or 5 ml to the same vol of the second seed medium. If the second seed medium was employed, another 24-h cultivation followed that of the first one; then 5 ml of the second culture was transferred to 50 ml of the fermentation medium for lysine production.

Analysis

The analytical methods for L-lysine.HCl concn., residual sugar (RS) and dry cell weight (DCW) were described in a previous report (Wang et al. 1993).
RESULTS AND DISCUSSION

Effects of molasses and HPP concentrations

The effects of molasses and HPP on the production of L-lysine by HS918 were examined although the former was not a member of the RSFM. Addition of molasses alone improved the accumulation of L-lysine in the RSFM. When the amount of molasses in the RSFM was 2% (w/v), the final concn. of L-lysine.HCl reached 19 mg/ml, which is much more than that in the RSFM without molasses. If, however, HPP in the RSFM was 1% (v/v), the influence of molasses became ambiguous. On the other hand, no matter how much molasses was present, HS918-accumulated L-lysine increased as HPP concn. decreased. This phenomenon corresponds to previous reports and was very different from the fermentation patterns of Strains HA92Y and PI-13 (Liu 1986, Wang 1991, Wang et al 1993).

Based on these observations, we suspected that molasses has some nutritional factors that benefit L-lysine production; while the inhibitory effect at high concn. of HPP may be due to the fact that HS918 is not completely free of amino acid feedback inhibition. Thereafter, we added 0.5% (w/v) molasses to the RSFM, and the HPP concn. was adjusted to 1% (v/v).

Effect of ammonium sulfate

When the HPP concn. dropped to 1% (v/v), we observed the effect of another inorganic nitrogen source (ammonium sulfate) on the production of L-lysine. From 1-5% (w/v) of ammonium sulfate was tested (Fig. 2). The maximum concn. of L-lysine.HCl was 35 mg/ml when the RSFM contained 4% ammonium sulfate. This level was higher than that obtained originally with 3%. The increment of 1% ammonium sulfate may compensate for the N lost from the decreased HPP concn. and may also maintain the optimal N/C ratio for L-lysine production.

Effects of thiamine, d-biotin and potassium dihydrogen phosphate (PDP)

*B. lactofermentum* ATCC13869, the source for HS918, is always a glutamic producer. This suggested that addition of thiamine to the medium might facilitate cell growth; on the other hand, d-biotin had been reported as an effector for L-lysine biosynthesis (Tosaka et al 1979). Thus 3 levels of these two vitamins and 6 levels of PDP were tested. If neither of the two vitamins was added to the RSFM, HS918 produced only 8 mg/ml of L-lysine.HCl after 48-h fermentation, no matter how much PDP was present (Fig. 3). If the concn. of thiamine.HCl and d-biotin reached the original levels (20 and 40 µg/dl, resp.), L-lysine.HCl was evidently excreted into the medium as the PDP concn. were from 0.05-0.07%. Doubling the amount of these two vitamins in the RSFM did not apparently favor L-lysine production. Thus the concn. of these three factors were retained at the original levels to maintain excellent L-lysine synthesis by HS918. After making these adjustments, HS918 accumulated 35 mg/ml L-lysine in the new RSFM after 48-h fermentation. In addition, if the second seed culture was introduced to the fermentation process and inoculum size was elevated from 4 to 10% (v/v), only 40 h
Figure 3. Effects of thiamine, d-biotin and PDP on Strain HS918.

Figure 4. Time-course curves of L-lysine fermentation of HS918 using two-step seed inoculum process.
C.C. Chang, W.L. Cheng, J.S. Wang & Y.T. Liu

were needed for HS918 to produce the same amount of L-lysine (Fig. 4). Thus further tests were conducted under these new conditions.

Effects of aeration and temperature

Two physical parameters—aeration and temperature—were evaluated for their effect on L-lysine biosynthesis by HS918. With respect to aeration, we employed three-baffle Erlenmeyer (TBE) flasks to create higher aeration than in the Hinton flasks used in this study. Temperatures of 33 and 35°C were chosen. Fermentation performed in TBE flasks at 33°C required only 30 h to reach the maximum value of 38 mg/ml L-lysine.HCl (Table 1). The time needed here was much shorter at 30°C with Hinton flasks (Fig. 5). Although sugar was totally consumed after 30-h fermentation at 33°C, only 22 mg/ml L-lysine.HCl accumulated in the medium with Hinton flasks; therefore aeration must match elevated temperature to facilitate lysine production. If temperature increased to 35°C, however, both lysine yield and sugar consumption rate dropped in both TBE and Hinton flasks. Evidently optimal temperature for lysine production by HS918 was approx. 33°C, and aeration must be higher than that obtained with Hinton flasks.

CONCLUSIONS

The lysine-producing ability of HS918 increased substantially when the component concn. of RSFM and the physical parameters of culture conditions were adjusted. The adjustments included adding extra molasses (0.5% w/v), decreasing HPP concn. to 1% (v/v), increasing ammonium sulfate to 4%, raising temperature to 33°C and using TBE flasks to increase aeration. Consequently the final L-lysine.HCl concn. reached 35-38 mg/ml, which is threefold that obtained in the original media. Furthermore the fermentation process was shortened by 10 h. These results highlight the potential superiority of HS918 as an industrial lysine-producer.

ACKNOWLEDGMENTS

We are grateful to J. Liu and L.I. Su for their assistance in the analyses.
REFERENCES


MEJORAS EN LA SINTESIS DE L-LISINA POR BREVIBACTERIUM LACTOFERMENTUM HS918 MEDIANTE EL AJUSTE DE LAS CONDICIONES DE LA FERMENTACION

C.C. Chang, W.L. Cheng, J.S. Wang & Y.T. Liu
Instituto de Investigaciones Azucareras de Taiwan,
Taiwan, ROC.

RESUMEN

Se estudiaron los efectos de la concentración de los diferentes componentes de medios fermentativos a base de azúcar crudo (MFAC), así como los parámetros físicos del cultivo de HS918 en frasco. La L-Lisina acumulada por la cepa HS918, aumentó gradualmente según las concentraciones de HPP disminuyeron de 4 a 1 % (v/v). La adición extra de miel, de 0.5 a 1.0 % (w/v) hizo mejorar significativamente la producción de L-lisina.HCL en fermentaciones de períodos de 45 horas alcanzando 30 g/l. Si se aumentaba el contenido de sulfato de amonio al 4 %, la HS918 podía acumular hasta 35 g/l en un período de fermentación de 45 horas a 30 C. Adicionalmente, elevando la temperatura hasta 33 C, y utilizando Erlenmeyers con tres lamínas deflectoras, para aumentar la aereación, se disminuye dramáticamente el tiempo de fermentación, llegando el contenido de L-lisina.HCL hasta niveles de 38 g/l, con rendimientos del 40 %.

Palabras clave: L-lisina, Brevibacterium lactofermentum, medio de cultivo de azúcar crudo.