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Process Biochemistry 40 (2005) 1559–1564

PROCESS BIOCHEMISTRY

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Comparison of fermentative capacities of lactobacilli in single and mixed culture in industrial media

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Received 19 February 2004; accepted 16 April 2004

Abstract

This paper is concerned with comparing the fermentative capacity of single and mixed cultures of lactobacilli on batch lactic acid fermentation. The fermentation characteristics of five strains of lactic acid bacteria, *Lactobacillus delbrueckii* subsp. *lactis* (ATCC 12315), *Lactobacillus casei* (NRRL-B1445), *L. delbrueckii* (NRRL-B445), *Lactobacillus helveticus* (NRRL-B1937), and *L. casei* (NRRL-B1922), were compared in de Man–Rogosa–Sharpe (MRS) media. Cell growth, glucose utilization, lactic acid synthesis, and free amino acid production were the main parameters studied. Strain B1445 was found to be the excellent lactic acid bacterium for the production of lactic acid with a high yield. Production of lactic acid from corn steep liquors by fermentation was studied using strain B1445 and mixed type of five lactobacilli. Even though nitrogen source consumption in mixed culture was lower than that of single culture, the cell density and lactic acid production were better in mixed culture than single culture. These results suggest that mixed culturing of lactobacilli may be more effective than single culturing of *Lactobacillus* for improving lactic acid production.

Keywords: Lactic acid fermentation; Single and mixed culturing; Corn steep liquor; Nitrogen source consumption

1. Introduction

Lactic acid (LAC) is an industrially important product due to its versatile chemical properties, used (1) as an acidulant, flavor, and preservative in the food, pharmaceutical, leather, and textile industries; (2) for the production of base chemicals; and (3) for polymerization to biodegradable poly LAC [1–4].

For lactic acid production complex, a basal medium is used. Basal medium satisfies the nutritional requirements of lactic acid bacteria that are commonly used in lactic acid fermentations, but the cost of this raw material contributes significantly to lactic acid production costs. Consequently, many studies have attempted to find cheaper industrial media for lactic acid fermentation. Use of corn steep liquor or cheese whey permeate or molasses as a sole nutritional supplement in large-scale fermentation operations represents an opportunity for apparent significant cost reduction.

The lactic acid bacteria are facultative anaerobes that are nutritionally fastidious [5,6]. Microbial growth also depends on environmental factors, such as pH, temperature, and accumulation of metabolic end products. Consequently, it is desirable to develop strains that are able to tolerate high acid concentration and temperatures, which allow the fermentation process to proceed almost free of contamination.

Mixed culture systems have been recognized to be effective for certain fermentations. Mixed cultures of lactic acid are currently used in the dairy industry for manufacturing cheeses and fermented milks. The existence of symbiotic relationship among various bacteria has been clearly demonstrated [7–10].

In this study, first, the growth, lactic acid production, and amino acid utilization/production of individual *Lactobacillus* strain were determined in MRS media. Then, the differences between single *Lactobacillus* cultures and mixed-type

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doi:10.1016/j.procbio.2004.04.017

lactobacilli cultures, by comparing their fermentative abilities, were investigated using industrial media.

2. Materials and methods

2.1. Bacterial strains

Five Lactobacillus strains and mixed-type strains were used in this study. These strains were identified as *Lactobacillus delbrueckii* subsp. *lactis* (ATCC 12315), *Lactobacillus casei* (NRRL-B1445), *L. delbrueckii* (NRRL-B445), *Lactobacillus helveticus* (NRRL-B1937), and *L. casei* (NRRL-B1922). The mixed-type strains were obtained from Argonne National Laboratory, The United State. For the sake of convenience, the above five bacterial strains and mixed-type strains were called strains 12315, B1445, B445, B1937, B1922, and LBM 5A, respectively, in this paper. The strains were stored at −80 °C in de Man–Rogosa–Sharpe (MRS) medium [11].

2.2. Media and culture conditions

In the first comparison of five Lactobacillus strains, the bacteria were grown under anaerobic conditions in MRS broth containing glucose for 48 h at 42 °C. The medium composition per litre was as follows: (a) peptone 10 g, beef extract 10 g, yeast extract 5 g, Tween 1 g, K_2HPO_4 2 g, $MgSO_4{\cdot}7H_2O$ 0.2 g, $MnSO_4{\cdot}4H_2O$ 0.2 g; and (b) glucose 20 g. Components (a) and (b) were autoclaved separately, and aseptically mixed together before starting the cultivation. Single or mixed culture experiments were conducted by using 500-ml Erlenmeyer flask containing 100 ml of the above medium (150 rpm, pH 6.6, 42 °C). Inoculum volume of each bacterial strain and mixed cultures was 1 ml. Fermentation runs were performed anaerobically in a fermentor (Bioengineering, Sweden, 3.71) with 21 working volume, agitation (120 rpm), and temperature (42 °C). The pH was maintained constant at pH 5.5 by automatic addition of 10N NaOH solution and the total time of fermentation was approximately 68 h. Unless otherwise stated, the fermentation medium was inoculated with 10% (v/v) of the seed MRS broth culture. The fermentation culture was grown in a 21 industrial media containing glucose 120 g/l and corn steep liquor (CSL) 3%, 5%, and 7.5% (v/v) for 68 h at 42 °C.

2.3. Analysis of amino acid concentration

Time-course culture samples were filtered onto a membrane (0.45 μm, GS; Millipore), and the filtrate was collected for amino acid analysis. The protein in the filtrate was removed by precipitation with trichloroacetic acid (TCA) (0.5 M, final concentration), according to the method described by Mansour [12], and then centrifuged at 5000 × *g* for 15 min. The supernatant was filtered on a Millipore membrane (0.45 μm) and subjected to a derivatization reaction for HPLC analysis with an ACCQ-Tag derivatization kit (Waters Corp., Milford, MA). The peak of each amino acid from the culture broth was integrated separately and the concentration determined by a standard calibration curve prepared with standard amino acids, the concentration of which ranged from 50 to 100 pM.

2.4. Other analytical methods

Bacteria growth was monitored by spectrophotometric measurement at 560 nm. The concentration of glucose was measured enzymatically with a glucose assay kit (Sigma, USA). Lactic acid was analyzed by HPLC equipped with an RI detector (Waters, USA). The column used was an Aminex HPX-87H (Bio-Rad Co., USA) operated at 50 °C, and the flow rate was 0.6 ml/min. The protein content was estimated by the bicinchoninic acid method [13].

3. Results and discussion

3.1. Cell growth, glucose utilization, and lactic acid synthesis in single culture

In this study, the characteristics of individual strain in mixed culture 12315, B1445, B445, B1937, and B1922 were compared in 500 ml flasks using a MRS medium containing 5 g/l yeast extract. Two replicates, at each condition, were carried out. There were greater differences among the single cultures with respect to growth arid lactic acid production. The single culture of B1445 and B1937 grew rapidly, while strains 12315, B445, and B1922 had a relatively slow rate of maximal fermentation. Strain 12315, B445, and B1922 entered a stationary phase despite the high concentration of residual glucose, whereas strains B1445 and B1937 had

Table 1
Maximum growth yield to glucose, lactic acid concentration and yield to glucose in the cultures of *Lactobacillus* strains used

	12315	B1445	B445	B1937	B1922
Process time (h)	32	24	32	20	32
Maximum culture turbidity (OD_{560})	17	24	18	28	16
Maximum lactic acid concentration (g/1)	13.5	14	13.9	8	13.5
Maximum yield of lactic acid to glucose (%)	69	70	70	42	67.5

General results of runs in media containing MRS medium and 5 g/l yeast extract (glucose concentration ≈ 20 g/l). The process time is the time necessary for the complete consumption of glucose.