

Glutamic Acid Production from Potato by *Brevibacterium linens*

Marzieh Moosavi-Nasab, Masoumeh Izadi, and Sara Hosseinpour

Abstract—In this study, the possibility of using potato as a substrate for glutamic acid production by *Brevibacterium linens* was investigated. For preparation of fermentation medium, potato was hydrolyzed by hydrochloridric acid. The medium contained potato hydrolysate, tween 80, mineral solution, glucose, and potassium hydrogen phosphate. The initial pH of the medium was adjusted to 7-7.5. For achieving the optimum time with maximum yield, the beakers containing the medium and the inoculums were incubated in a rotary water bath flask shaker for one to five days. Thin layer chromatography was used for quantitative and qualitative assay of the glutamic acid produced. The results revealed that as fermentation time increased, pH of the fermentation medium significantly decreased ($P<0.05$). Furthermore, glutamic acid concentration in fermentation medium increased significantly ($P<0.05$). The highest amount of the glutamic acid obtained was 5.6 g/l on the fourth day of fermentation.

Keywords—*Brevibacterium linens*, Fermentation, Glutamic acid, Thin layer chromatography

I. INTRODUCTION

MANUFACTURE of amino acids reflects the increased recognition of the nutritional and taste values of amino acids in many foods and beverages, such as fruit juice, cheese, beer, seafood, soup and tea.

Today, amino acids are used not only as food additive (~66%), but also feed additives (31%) and components in pharmaceuticals, cosmetics and others industrial raw materials (3%) [8], [14].

The history of the species *Corynebacterium* as amino acid producer started in the 1950s when Dr Kinoshita was the first to discover that *C. glutamicum* is a superior amino acid producer. Until this time amino acids were available exclusively by extraction methods or chemical synthesis. The increasing demand for L-glutamic acid as a flavour enhancer combined with the discovery of a microbial L-glutamic acid producer by Kinoshita from *C. glutamicum* [14].

M. Moosavi-Nasab is with the Department of Food Science and Technology, College of Agriculture, Shiraz University, Shiraz, Fars 7144165186 I.R.Iran (corresponding author to provide phone: +98-711-6138227; fax: +98-711-2287029; e-mail: marzieh.moosavi-nasab@mail.mcgill.ca).

M. Izadi, is a graduate student at the Department of Food Science and Technology, Shiraz University, Shiraz, Fars 7144165186 I.R.Iran (e-mail: mizadi@shirazu.ac.ir).

S. Hosseinpour is a graduate student at the Department of Food Science and Technology, Shiraz University, Shiraz, Fars 7144165186 I.R.Iran (e-mail: sarahoseinpour@yahoo.com).

Since this time, biotechnological processes using *Corynebacterium* species developed to be among the most important in terms of tonnage and economical value. Nowadays L-Glutamic acid and L-lysine are bulk products. L-Valine, L-isoleucine, L-threonine, L-aspartic acid and L-alanine are among other amino acids produced by *Corynebacteria* [1], [14]. Nowadays more than 2 million tons of amino acids are produced per year, around 1.5 million tons L-glutamic acid is produced per year using *Coryneform* bacteria, mainly in the USA and Japan [14], and the price of world trade on average is 1.2 \$ per Kilogram.

Monosodium glutamate (MSG), the sodium salt of L-glutamic acid is a popular flavour enhancer [10] and an additive for foods. It was used primarily in Asian foods but its use is now widespread [2]. MSG is used in foods as a taste enhancer because of its own unique flavour called “Umami” in Japanese. Prepared foods usually contain 0.1-0.8% MSG but especially in east Asian dishes a higher supplementation is common [14].

The early 1900s, MSG was extracted from natural protein rich foods, such as seaweed and it was also prepared by the acid hydrolysis of wheat gluten or soybean protein which were expensive materials [2]. L-glutamic acid is produced mainly through microbial means because chemical method produces a racemic mixture of DL-glutamic acid [9]. Certain strains of bacteria, such as *Corynebacterium* sp. and *Brevibacterium* sp. excrete glutamic acid when fed on ammonium rich diet. Fermentation of ammonia in this way is now the standard commercial method for MSG production. Glucose or hydrolysed starch is the usual carbon source [2], [12].

Optimum condition for production of glutamic acid by *Corynebacterium glutamicum* is obtained when the fermentation media contains low amount of biotin. The best condition for production of glutamate occurs when biotin is depleted. The addition of detergent compounds such as polyoxyethylene sorbitan monopalmitate (Tween 40) or polyoxyethylene sorbitan monostearate (Tween 60), or addition of one of the β -lactam antibiotics such as penicillin, or the addition of ethambutol or cerulenin enhances the over production of glutamate and are used when excess biotin is present [1], [13].

Brevibacterium sp. requires biotin for cell growth. Biotin is necessary for fatty acid synthesis. It was thought that the cell membrane permeability increased when biotin was depleted in the culture medium. Similarly, addition of detergent, penicillin, ethambutol or cerulenin changes the permeability of the cell membrane and cell wall [1].

Widespread researches have been performed on factors order to separate the cells; supernatant was stored in -18°C affecting glutamic acid production such as aeration on yield, performing the experiments. till performing the experiments.

biotin on production yield (a factor affecting membrane permeability), mutation of bacteria to increase production efficiency, extracted glutamic acid, etc [9], [12]. Cane and beet molasses, starch and hydrolysed starch, starchy tubers (potatoes, sweet potatoes, and cassava) and tapioca are used as main raw materials for production of glutamic acid [12].

The purpose of this study was using low-cost resources such as waste of potatoes for the production of glutamic acid by *Brevibacterium linens*.

II. MATERIALS AND METHODS

A. Organism

Stock cultures of *B. linens* (PTCC 1603) were obtained from the Persian Type Culture Collection, Karaj, Iran and maintained in nutrient broth.

B. Preparation of Seed Cultures

Activated bacteria incubated in the media consisting of glucose (20 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.25 g), KH_2PO_4 (1 g), peptone water (10 g), K_2HPO_4 (1 g), NaCl (2.5 g), $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (0.1 g) and yeast extract (10 g) per liter [2]. The culture was incubated in a rotary flask shaker (Gyrotory water bath shaker, model G76, USA) at 180 rpm and 30°C for 24 h.

C. Preparation of the Fermentation Medium

Fermentation medium were prepared from potato. The tubers were washed and cooked to gelatinize starch by steaming for 1 h, peeled and mashed using a meat homogenizer. To mash 1:1 distilled water was added, and filtered by mesh size 18. For hydrolysis of starch the pH of mashed tuber was adjusted to 2 with hydrochloric acid 1 N and was incubated in water bath at 100°C for 3 h, and then centrifuged (Surval, Superspeed refrigerated centrifuge, model RC-5, USA) in $5000 \times g$ [12].

Prior to fermentation, the hydrolyzed tuber was diluted to contain 5 percent total soluble solids using distilled water [12]. For enrichment of this medium potassium hydrogen phosphate (1.8 g), glucose (20 g), Tween 80 (0.1 ml), 10 ml of mineral solution ($\text{FeSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, NaCl, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ each 1 mg/ml) per liter were added and then, [2] pH was adjusted to 7 with 1 N NaOH solution [12].

100 ml of each medium was taken in 250 ml beakers and autoclaved at 15 psi for 15 min. 10 ml seed culture was inoculated to each flask and incubated in a rotary flask shaker (gyrotory water bath shaker model G76, USA) at 30°C and 200 rpm. Samples after fermentation were filtered and centrifuged in $8000 \times g$ to remove the cell mass and other residues and the amount of glutamic acid in the broth was estimated [2].

D. Chemical proximate analysis

In order to monitor the process of fermentation, samples were taken from the fermentation media in the incubator every 24 h. Instantly after sampling, pH of samples was measured and then samples were centrifuged in $8000 \times g$ in

The amount of ash [4], protein [6], lipid [3], fiber [5] and moisture [7] of the fermentation media were measured. All the experiences were performed in triplicates.

E. Qualitative and Quantitative Measurement of the Produced Glutamic Acid

In order to confirm the presence and measure the amount of glutamic acid produced, thin layer chromatography on silicagel plate was used. The solvent system used in this method was butanol, acetic acid and distilled water (60: 15: 25 v/v) (Fig. 1) [11]. Ninehydrine in ethanol (0.5%) was used to develop the color of spots [11]. To measure the amount of the produced glutamic acid, the spots on the plate were scraped and thrown in the micro tubes which contained 5 ml 75% ethanol [8]. After shaking for 5 minutes and 5000 rpm, the sample was centrifuged in $4000 \times g$ and its absorbance at wavelength of 560 nm [8] was measured using spectrophotometer (Perkin-Elmer, Junior model 35, USA). The concentration of glutamic acid was measured by standard curve and the amount of the produced glutamic acid was reported in g/l substrate. To draw standard curve, different concentrations of glutamic acid were prepared and the process was performed the same as for the samples.

F. Statistical Analysis

The data were analyzed using one way ANOVA and the means were compared using Duncan's multiple comparison test. The level of significance was set for $P < 0.05$.

III. RESULTS AND DISCUSSION

The amounts of ash, protein, carbohydrate, lipid, fiber and moisture of the fermentation medium are given in Table 1. The results revealed that as fermentation time increased, glutamic acid concentration in fermentation medium increased significantly ($P < 0.05$). The highest amount of the glutamic acid obtained was 5.6 g/l on the fourth day of fermentation (Table 2). The beneficial role of activated charcoal addition for glutamic acid production by *Micrococcus glutamicus* (ATCC 13032) using potato hydrolysate was demonstrated by Joseph and Rao, 1973. After 72 h the glutamate yield was $10.7 \text{ mg} \cdot \text{ml}^{-1}$ because addition of activated charcoal removed excess biotin from the tuber hydrolysate. Activated charcoal was added at 1.0 percent level to the diluted potato hydrolysate (5 percent total soluble solids) at pH 4.0. It was observed that urea addition at a concentration of 0.3 percent served as the best nitrogen source with a yield of $6.2 \text{ mg} \cdot \text{ml}^{-1}$ glutamate [12]. Furthermore, pH of fermentation medium significantly decreased during fermentation ($P < 0.05$). Considering the fact that glutamic acid is an acidic amino acid, pH variations indicate the production of glutamic acid in the fermentation medium (Table 3).

TABLE I
COMPOSITION OF POTATOES USED IN FERMENTATION MEDIUM

Composition	Amount (%)
ash	2.13
protein	2.8
lipid	0.3
carbohydrate	20.56
fiber	0.75
moisture	73.46

TABLE II
CHANGES IN GLUTAMIC ACID CONCENTRATION (g/l) DURING FERMENTATION

Time (day)	glutamic acid concentration(g/l)
0	0± 0 ^a
1	1.8± 0.26 ^b
2	3.7±0.2 ^c
3	4.3±0.05 ^d
4	5.6±0.1 ^e
5	5.1±0.27 ^f

Means marked by different letters show significant differences

TABLE III
CHANGES IN PH DURING FERMENTATION

Time (day)	Changes pH
0	7±0 ^d
1	5.58± 0.1 ^c
2	4.43± 0.1 ^b
3	4.25± 0.13 ^a
4	4.07± 0.09 ^a
5	4.1± 0.13 ^a

Means marked by different letters show significant differences

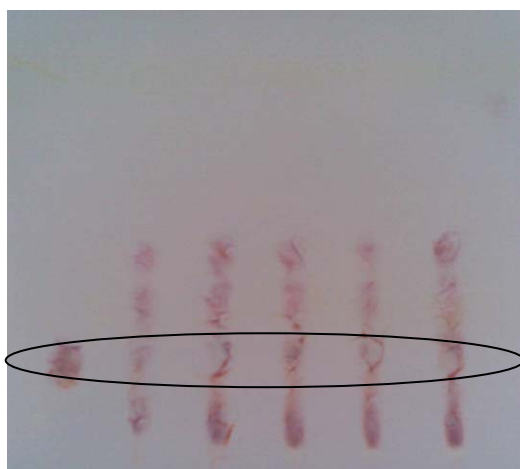


Fig. 1 Thin layer chromatography of the produced glutamic acid. Spots from left to right: standard, first day, second day, third day, fourth day, and the fifth day of fermentation.

IV. CONCLUSIONS

The results obtained from this investigation show that by use of *brevibacterium linens*, glutamic acid production from potato is possible. Glutamic acid is used to produce monosodium glutamate which is a flavor enhancer compound and its production from by-products of food industries using microorganisms is economically beneficial. Therefore, to increase the yield, the fermentation conditions must be optimized. In this research, in order to measure the quantity and quality of the produced glutamic acid, thin layer chromatography was used and the highest percentage of the produced glutamic acid occurred in the fourth day of fermentation.

REFERENCES

- [1] A. Steinbüchel, *Microbiology Monographs*. Berlin Heidelberg: Springer-Verlag, 2007, ch. 5.
- [2] A. N. Jyothi, K. Sasikiran, B. Nambisan, and C. Balagopalan, "Optimisation of glutamic acid production from cassava starch factory residues using *Brevibacterium divaricatum*". *Process. Biochem.* Vol. 40, 3576–3579, March.2005.
- [3] AOAC. Soxhlet analysis. Official Methods of Analysis of the Association of Official Analytical Chemists, 12nd ed. Washington DC., US., 1975. methods 14.035 and 14.036.

- [4] AOAC. Ash of flour. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17nd ed. Washington DC., US., 2000. method 923.03.
- [5] AOAC. Fiber (crude) in flour. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17nd ed. Washington DC., US., 2000. method 920.86.
- [6] AOAC. Protein (total) in flour. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17nd ed. Washington DC., US., 2000. method 920.87
- [7] AOAC. Solids (total) and moisture in flour. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17nd ed. Washington DC., US., 2000. method 39.1.21.
- [8] B. H. Lee, *Fundamentals of Food Biotechnology*. montreal: VHC publishers, Inc, 1996, pp. 324–327.
- [9] G.A. Amin, and A. Al-Talhi, "Production of L-glutamic acid by immobilized cell reactor of the bacterium *Corynebacterium glutamicum* entrapped into carageenan gel beads," *World Appl. Sci. J.*, vol. 2, no. 1, pp. 62–67. 2007.
- [10] G. Calik, F. Unlutabak, And T. H. Ozdamar, "Product and by product distributions in glutamic acid fermentation by *Brevibacterium flavum*: effect of the oxygen transfer," *Biochem. Eng. J.*, vol. 9, pp. 91–101. 2001.
- [11] J. Gasparic and J.Churacek, *Laboratory hand book of paper and thin-layer chromatography*. Newyork: Ellis Horwood Limited. 1978, pp. 175-178.
- [12] R. Joseph, and T. N. R. Rao, "Glutamic acid fermentation employing starchy tubers as raw material", *J. food. sci. technol.* pp. 160-164.1973.
- [13] S.-U. Choi, T. Nihira, and T. Yoshida, "Enhanced glutamic acid production of *Brevibacterium* sp. with temperature shift-up cultivation," *J. Biosci. Bioeng.*, Vol. 98, no. 3, pp. 211–213, May. 2004.
- [14] T. Hermann, "Industrial production of amino acids by coryneform bacteria," *J. Biotechnol.* ,vol. 104, pp.155–172, April. 2003.