



Original Research Article

Influence of Amino Acids and Growth Promoting Factors on Lysine Production by *Microbacterium lacticum*

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A b s t r a c t	K e y w o r d s
The effect of amino acids and growth promoting factors on lysine production by <i>Microbacterium lacticum</i> was examined. All the amino acids at 0.01% (w/v) enhanced lysine accumulation with L-methionine giving a lysine yield of 1.89mg/ml. The growth stimulators at 0.1% (w/v) improved lysine accumulation in the culture medium of <i>Microbacterium lacticum</i> . A mixture of yeast extract, peptone and casein gave a maximum yield of 2.68 mg/ml.	Amino acids Casein Fermentation Growth promoting factors <i>Microbacterium lacticum</i>

Introduction

Proteins play key roles in cell structure, physiology, and metabolism, and the amino acids that build up proteins are therefore nutritionally important compounds for all living organisms. Together, the 20 common protein-forming L-amino acids represent a variety of different characteristics and chemical properties, and almost all of them have commercial interest (Eggeling and Bott, 2005; Kumagai, 2000).

Nine of the amino acids cannot be synthesized by higher animals and humans and must therefore be supplied in food or feed. These are the so-called essential amino acids, and lysine belongs to this class (Brautaset and Ellingsen, 2011). In addition to

the worldwide demand for L-lysine as a feed supplement for the pig and poultry industries, there is an ongoing exploitation of new applications for this amino acid, including in pharmaceuticals and cosmetics. L-Lysine has also been used for preventing atherosclerosis and to treat certain viral infections (Sanchez and Demain, 2008).

Since L-lysine is of great importance and the worldwide market is growing, there is constant effort aimed at improving the L-lysine fermentation processes, including development of better production strains, better fermentation processes, media optimization and downstream processing (product separation and purification).

The present report is on the effect of amino acids and growth promoting factors on lysine produced in a culture broth by a bacterial strain recovered from different oil contaminated soil in Nigeria.

Materials and methods

Microorganism

Microbacterium lacticum was isolated from oil contaminated soils in Delta, Rivers and Anambra State, Nigeria. It was maintained on Nutrient agar (Oxoid) slants at 4°C. The medium for seed culture consists of peptone, 10.0 g; yeast extract, 10.0 g; NaCl, 5.0 g; distilled water, 1 L; pH adjusted to 7.0 with 1 N NaOH. One loopful of a 24 h slant culture was used to inoculate a 100ml Erlenmeyer flask containing 25 ml of seed medium. The flask was incubated for 16-18 h on a rotary shaker at 120 rpm and 30°C.

Influence of different concentration of amino acids on growth and lysine production

The influence of 0.01% (w/v) concentration of alanine, butyric acid, asparagine, aspartic acid, cystine, glutamic acid, lysine, leucine, methionine, ornithine, phenylalanine, threonine, tryptophan and tyrosine on growth and lysine production were studied. Erlenmeyer flask (100ml) containing 25ml of the basal medium: KH₂PO₄, 1.0g; MgSO₄.7H₂O, 0.4g; MnSO₄.H₂O, 2.0mg; FeSO₄.7H₂O, 2.0mg; CaCO₃, 50.0g; Glucose, 20.0g; (NH₄)₂SO₄, 10.0g; H₂O, 1L, pH adjusted 7.2 and sterilized at 115°C for 10 min. Fermentation was carried out at 30°C for 72h. After 72 h incubation on a rotary shaker at 160 rpm and 30°C, growth and lysine accumulation were determined from the broth culture. Uninoculated flasks were kept as control. All values reported are an average of at least duplicates which agreed closely. Bacteria growth was determined turbidimetrically using JENWAY Spectrophotometer (Model 6405 UV/Vis) at 660nm. Quantitative estimation of L-lysine in the supernatant was carried out by acid ninhydrin method of Chinard (1952).

Influence of growth promoting factors on growth and lysine production

The effect of 0.1%(w/v) concentration of yeast extract, peptone, casein, a mixture of yeast extract

and peptone and another mixture of yeast extract, peptone and casein on growth and lysine production by isolate G₁ was studied. Erlenmeyer flask (100ml) containing 25ml of the basal medium: KH₂PO₄, 1.0g; MgSO₄.7H₂O, 0.4g; MnSO₄.H₂O, 2.0mg; FeSO₄.7H₂O, 2.0mg; CaCO₃, 50.0g; glucose, 20.0g; (NH₄)₂SO₄, 10.0g; H₂O, 1 L, pH adjusted 7.2 and sterilized at 115°C for 10 min. were used.

Fermentation was carried out for 72 h at 30°C. Growth and methionine production were determined from the broth culture at the end of fermentation period. All experiments were performed in duplicate, with uninoculated flasks serving as control. Growth was determined turbidimetrically using JENWAY Spectrophotometer (Model 6405 UV/Vis) at 660nm while lysine accumulation was assayed from the broth culture by acid ninhydrin method of Chinard (1952).

Results and discussion

The influence of 0.01% (w/v) of various amino acids on lysine accumulation by *Microbacterium lacticum* is presented in Table 1. All the amino acids at 0.01% (w/v) enhanced lysine accumulation, with L-methionine giving a lysine yield of 1.89mg/ml. As seen in Table 1, all amino-acid, especially L-methionine enhanced the L-lysine accumulation in the culture medium of *Microbacterium lacticum*. This is in line with the work of Shio and Uchio (1969), who noted that L-methionine enhanced the L-glutamic acid production. Similar findings were reported by Ekwealor and Obeta (2005) on studies on lysine production by *Bacillus megaterium*.

Table 2 shows the influence of growth promoting factors on growth and lysine production by *Microbacterium lacticum*. All the growth stimulators at 0.1% (w/v) improved lysine production in *Microbacterium* sp. A mixture of yeast extract, peptone and casein gave a maximum yield of 2.68mg/ml. The combination of all the growth-promoting factors at 0.1% (w/v) enhanced lysine production (Table 2). This is in line with the work done Ekwealor and Obeta (2005) who noted that growth promoting factor at 0.1% (w/v) enhanced lysine accumulation. Chao and Foster (1959); Tauro et al. (1963), however reported a retardation in glutamic acid production and lysine accumulation by *Bacillus subtilis*, when growth stimulators are used respectively.

Table 1. Influence of different concentrations of amino acids on growth and lysine production by *Microbacterium lacticum*

Amino acids [0.01% (w/v)]	Growth (OD _{660nm})	Lysine (mg/ml)
L- Methionine	1.04	1.89
L-Lysine	0.76	1.52
DL-Phenylalanine	0.99	1.82
L-Cystine	0.84	1.72
L-Alanine	0.64	1.50
Butric acid	0.67	1.54
DL-Aspartic acid	0.65	1.50
L-Glutamic acid	0.68	1.52
L-Leucine	0.78	1.60
DL-Ornithine	0.79	1.60
DL-Threonine	1.09	1.88
L-Tryptophan	0.63	1.52
L-Tyrosine	0.84	1.74
L-Asparagine	0.60	1.50
Control*	0.70	1.44
(* No amino acid added)		

Table 2. Influence of growth promoting factors on growth and lysine production by *Microbacterium lacticum*.

Growth factor [0.1%(w/v)]	Growth (OD _{660nm})	Lysine (mg/ml)
Yeast extract	1.07	2.24
Peptone	1.02	2.03
Casein	1.00	2.32
Yeast extract and Peptone	1.05	2.12
Yeast extract /Peptone/ Casein	1.07	2.68
Control*	0.70	1.44
(* No growth factor added)		

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