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Stimulating effect of B-vitamins and bivalent metals on lysine accumulation by *Microbacterium lacticum*

C.C. Ezemba*, I.A. Ekwealor , C.A. Ozokpo, C.E. Chukwujekwu, V.N. Anakwenze

Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Nigeria

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Abstract

The stimulating effect of B-vitamins and bivalent metals on lysine production by *Microbacterium lacticum* was investigated. Four of the bivalent metals, Ca^{2+}, Sr^{2+}, Co^{2+}, Zn^{2+}, stimulated lysine accumulation and Sr^{2+} at 5.0µg/ml level, gave the highest lysine yield. The effect of vitamins on lysine accumulation by *Microbacterium lacticum* showed that biotin, folic acid and riboflavin enchanced lysine production. Biotin at all lysine level improved lysine accumulation while Sr^{2+} at 1.0µg/ml concentration gave a maximum lysine yield of 1.95mg/ml.

* **Corresponding Author:** Ezemba C.C. ✉ constancechinyere790@yahoo.com

Introduction

Out of the twenty naturally occurring amino acids, L-Lysine is one of the 9 essential and commercially important amino acids, found in naturally occurring proteins of all living organisms (Wikipedia 2007). Its major commercial form is L-lysine Monohydrochloride (L-lysine-HCL). Good sources of lysine are foods rich in protein like meats, cheese, certain fish, nuts, eggs, soyabeans.

L-Lysine is nutritionally important to man and animals and can be used to supplement food and food materials especially cereal products to improve protein quality (Dutta and Ottaway, 1976). Tosaka *et al.* (1993) reported that since these cereal products contain only small quantities of lysine, poultry, cattle and other livestock unable to synthesize this amino acids must have lysine added to their feed stuff to provide adequate diet. Children and growing animals require high levels of lysine, for bone formation, adequate milk production and proper growth. Lysine also has some pharmaceutical application in the formulation of diets with balanced amino acid composition and in amino acid infusion (Shan *et al.*, 2002).

L-Lysine is the common protein-forming form of lysine and has, by far, the highestcommercial value of the different lysine forms. L-Lysine is one of the essential amino acids for higher animals and is widely used as a feed additive for swine and poultry. The protein in traditional feedstuffs such as corn, wheat, and barley has low lysine content, and in order to increase feed efficiency, pure L-lysine is added (Brautaset and Ellingsen, 2011).

Chemical, enzymatic and microbiological fermentation processes have been used to synthesize amino acids but fermentation is more advantageous than other methods. Anastassiadis (2007) revealed that fermentation process is more economical, optical active and the sterospecificity (the L-isomer) make it more advantageous compared with synthetic processes. In biotechnology, little work have been devoted to the fermentation process development and optimization, still leaving large opportunities for further improvement.

Accordingly, the bulk of research has focused on development of efficient methods for production of L-lysine, in particular on understanding and improving production capacities of L-lysine-producing bacteria, as well as development of efficient fermentation and downstream processes (Schrumpf *et al.*, 1992; Eggeling, 1994; Ekwealor and Orafu, 2003).

This work was therefore carried out to determine the effect of vitamins and bivalent metals on lysine productivity by *Microbacterium lacticum* previously isolated from different oil contaminated soil ecovars in Nigeria.

Materials and methods

Microorganism

Microbacterium lacticum was isolated from oil contaminated soil in Delta, Rivers and Anambra, 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 I N of NaOH. One loopful of a 24 h slant culture was used to inoculate a 100 ml 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.

Effects of Different Concentration of Vitamins on Growth and Lysine Production

The influence of varying concentrations (0.10, 1.00, 10.0, 100µg/ml) of biotin, riboflavin, nicotinic acid, folic acid and thiamine HCL on growth and lysine production were examined. Erlenmeyer flasks (100ml) containing 25ml of the basal medium: KH_2PO_4 ,1.0g; $MgSO_4.7H_2O$,0.4g; $MnSO_4.H_2O$, 2.0mg; $FeSO_4.7H_2O$,2.0mg; $CaCO_3$,50.0g; glucose ,40.0g; $(NH_4)_2SO_4$,10.0g; H_2O ,1 L, pH adjusted 7.2 and sterilized at 115°C for 10 min. were used. Fermentation was carried out for 72h 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 as previously described by acid ninhydrin method of Chinard (1952).

Effects of Varying Concentrations of Bivalent Metals on Growth and Lysine Production

The effects of varying concentration (0.10, 1.00, 5.00, 10.00µg/ml) of $CaCl_2$, $CoCl_2$, $CuCl_2$, $SrCl_2$ on growth and lysine production were production were studied. A basal medium consisting of Erlenmeyer flasks (100ml) containing 25ml of the basal medium: KH_2PO_4 ,1.0g; $MgSO_4.7H_2O$,0.4g; $MnSO_4.H_2O$, 2.0mg; $FeSO_4.7H_2O$,2.0mg; $CaCO_3$,50.0g; glucose ,40.0g; $(NH_4)_2SO_4$,10.0g; H_2O ,1 L, pH adjusted 7.2 and sterilized at 115°Cfor 10 min. were used. Fermentation was carried out for 72h at 30°C. 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).

Results

Influence of Different Concentrations of B -Vitamins on Growth and Lysine Production

The influence of different concentrations of B-vitamin on growth and lysine production by the isolate (Table 1), showed that biotin, folic acid and riboflavin enhanced lysine production while nicotinic acid did not stimulate lysine production. Biotin at all levels improved lysine accumulation and at 1.0µg/ml concentration, folic acid gave a maximum lysine yield of 1.95mg/ml.

Table 1. Influence of Different Concentration of B-Vitamins on Growth and Lysine Production.

Vitamins	Concentration (µg/ml)	Growth (OD_{660nm})	Lysine (mg/ml)
Biotin	0.1	0.88	1.66
	1.0	0.89	1.74
	10.0	0.79	1.53
	100.0	0.75	1.47
Folic acid	0.1	0.66	1.58
	1.0	1.20	1.95
	10.0	1.00	1.85
	100.0	0.75	1.75
Nicotinic acid	0.1	0.64	1.36
	1.0	0.45	0.96
	10.0	0.50	0.98
	100.0	0.43	0.92
Riboflavin	0.1	0.75	1.43
	1.0	0.76	1.51
	10.0	0.69	1.27
	100.0	0.69	1.22
Control	No B-vitamin added	0.70	1.44

Effects of Varying Concentrations of Bivalent Metals on Growth and Lysine Production.

The influence of varying concentrations of bivalent metals on growth and lysine productions are shown in Table 2. Four of the bivalent metals, Ca^{2+}, Sr^{2+}, Co^{2+}, Zn^{2+}, stimulated lysine accumulation while Cu^{2+} and Ni^{2+} did not increase lysine production. Sr^{2+} at 5.0µg/ml level, gave the highest lysine yield of 1.87mg/ml.

Discussion

The effect of vitamins on lysine production by *Microbacterium lacticum*, presented in Table 1, shows folic acid, biotin and riboflavin have significant

influence on growth and lysine accumulation while nicotinic acid did not stimulate lysine production. Biotin at all levels improved lysine accumulation and at 1.0µg/ml concentration, folic acid gave a maximum lysine yield of 1.95mg/ml. This is in line with the work of Tosaka *et al.,*(1979) and Young and Chipley., (1984) who reported that presence of biotin induced the growth and production of lysine. The roles of vitamins on lysine production is not yet known, but have been reported to play catalytic roles within microbial cells which are components of coenzymes or prosthetic group of enzymes. Abou-Zeid, A.Z. *et al.*, (1976), studied the influence of some compounds on antibiotics production and reported increased gentamicin production by *Micromonospora purpurea* with the addition of cobalamin, folic acid, riboflavin, vitamin B_1, vitamin B_6 and biotin. Ekwealor and Obeta (2007) studied the effects of vitamins on lysine yield in *Bacillus megaterium* and reported an enhanced lysine yields in *B. megaterium* SP 86 and *B. megaterium 76* with the addition of 10µg/ml and 100µg/ml of folic acid and riboflavin.

Table 2. Effect of Varying Concentrations Bivalent Metals on Growth and Lysine Production.

Bivalent metal	Concentration (µg/ml)	Growth (OD_{660nm})	Lysine (mg/ml)
$CaCl_2$	0.10	0.52	1.45
	1.00	0.49	1.25
	5.00	0.59	1.58
	10.00	0.47	1.25
$CuCl_2$	0.10	0.50	1.44
	1.00	0.45	1.25
	5.00	0.43	1.04
	10.00	0.47	1.03
$SrCl_2$	0.10	0.50	1.37
	1.00	0.56	1.45
	5.00	0.64	1.87
	10.00	0.57	1.58
$NiCl_2$	0.10	0.49	1.00
	1.00	0.65	1.00
	5.00	0.59	1.00
	10.00	0.50	0.80
$CoCl_2$	0.10	0.62	1.76
	1.00	0.49	1.44
	5.00	0.51	1.41
	10.00	0.42	1.25
$ZnCl_2$	0.10	0.45	1.16
	1.00	0.50	1.29
	5.00	0.65	1.58
	10.00	0.61	1.65
Control	No metal added	0.70	1.44

The influence of varying concentrations of bivalent metals on growth and lysine productions is shown in Table 2. Four of the bivalent metals, Ca^{2+}, Sr^{2+}, Co^{2+}, Zn^{2+}, stimulated lysine accumulation while Cu^{2+} and Ni^{2+} did not increase lysine production. Scorium chloride exhibited a good effect on growth and lysine production at a 5.0µg/ml giving a lysine concentration of 1.87mg/ml. Regarding the role of bivalent metals in microorganisms, Martin and McDaniel (1977); Hughes and Poole (1989), suggested that metal ions probably acted as activators or inhibitors of metabolites. The actual mechanism of stimulation or inhibition of growth and metabolite formation is still unknown.

This result is supported by the works of Ekwealor and Obeta (2007).They reported improved lysine production in a broth supplemented with Zn^{2+}. Weinberg, (1970) studied the role of trace elements in microorganism and reported that zinc is one of the key metals for growth of certain microorganism. Sigel (1983) also reported the role of zinc ion in the synthesis of industrially and medically significant microbial secondary metabolites.

The stimulatory effect of Ca^{2+} observed in this work agrees with the findings of Schafee, *et al.* (2005) who reported an increase in protease production from *Bacillus cereus* with the addition of Ca^{2+}. Harol (1986) reported that the role of Ca^{2+} in growth and product formation in microbes is not yet known but has been implicated in the stabilization of cell wall, activation of extra cellular enzymes and in the regulation or triggering of a range of cell functions (Banik and Majumdar (1974).

Morinaga *et al.* (1982) and Roy *et al.* (1989) reported the effects of metal ions on growth and methionine production by *Bacillus megaterium*. They reported maximum methionine production using Fe^{2+} and Mn^{2+} as metal ions.

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