African Journal of Biotechnology Vol. 6 (11), pp. 1348-1351, 4 June 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Effect of vitamins and bivalent metals on lysine yield in *Bacillus megaterium*

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Accepted 2 February, 2007

The effects of vitamins and bivalent metals on lysine accumulation in *Bacillus* strains were investigated. Biotin enhanced lysine production in all the *Bacillus* strains, while folic acid and riboflavin stimulated lysine yields in *Bacillus megaterium* SP 86 only. All bivalent metals stimulated lysine accumulation in *B. megaterium* SP 86 and *B. megaterium* SP 14, while Co^{2+} and Zn^{2+} improved lysine levels in all the strains.

Key words: Vitamins, bivalent metals, L-lysine, *Bacillus,* accumulation.

INTRODUCTION

The amino acids represent the major fraction of primary metabolites produced by microbes. About 4.5 x 10^5 tonnes of L-lysine are produced per year, and the amino acids are used as food and feed additives (Leuchtenberger, 1996; Eggeling and Sahm, 1999). Extracellular lysine production is not restricted to any particular group of microorganisms, though the high yielding strains are mostly the species of *Arthrobacter, Corynebacterium* and *Brevibacterium* (Sen and Chatterjee, 1989). Very good producer strains have also been developed from *Bacillus subtilis* and *Escherichia coli* (Leuchtenberger, 1996; Eggeling et al., 2001).

However, Nakayama, (1985) noted that while good production strains have been obtained, the character required for high productivity remain largely unknown. The aim of this work, therefore, is to study the effect of vitamins and bivalent metals on lysine yields in *Bacillus strains*.

MATERIALS AND METHODS

Effect of vitamins on lysine accumulation in *Bacillus* strains

The effects of thiamine, nicotinic acid, biotin, pyridoxal HCL, folic acid and riboflavin, at varying concentrations (0.01 – 100 µg/ml) on lysine accumulation in *Bacilus megaterium* SP 86 *B. megaterium* SP 76 and *B. megaterium* SP 14 were studied. A 250 ml Erlenmeyer flask containing 25 ml of a basal medium (KH_2P0_4,1.0 g; $MgS0_4.7H_20$, 0.4 g; $MnS0_4.H_20$, 2.0 mg; $FeS0_4.7H_20$, 2.0 mg; $CaCO_3$, 50.0 g; H_20, lL, pH 7.2) was inoculated with 10% (v/v) of a 24 h seed culture of the *Bacillus* strain. The carbon and nitrogen sources of the fermentation medium were sucrose, 80.0 g and NH_4CL, 40.0 g, respectively, for *B. megaterium* SP 86. For *B. megaterium* SP 76 and *B. megaterium* SP 14, glucose, 80.0 g and $(NH_4)_2So_4$, 40.0 g were used. The flask was incubated for 72 h at 30°C on a rotary shaker (160 rpm). All experiments were performed in duplicate, with uninoculated flasks serving as control. Lysine accumulation was assayed from the broth culture as earlier described (Ekwealor and Obeta, 2005).

Effect of bivalent metals on lysine production by *Bacillus* strains

The effects of varying concentrations (0.10 – 10.0 µg/ml) of $NiCl_2$, $CoCl_2$, $ZnCl_2$, $CdCl_2$, $CuCl_2$, and $CaCl_2$, on lysine yields in *B. megateruim* SP 86, *B. megaterium* Sp 76 and *B. megateruim* SP 14 were examined. Fermentation medium and fermentation process were as previously described.

RESULTS AND DISCUSSION

The results of the effects of vitamins on lysine accumulation in *Bacillus* strains are presented on Table 1. Biotin at 0.10 µg/ml stimulated lysine accumulation in all the *Bacillus* strains, although the highest lysine yields in

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Table 1. Effect of vitamins on lysine production by *Bacillus* strains.

Vitamins	Concentration (µg/ml)	Lysine (mg/ml)		
		***B. megaterium* SP 86**	***B. megaterium* SP76**	***B. megaterium* SP14**
Thiamine HCl	0.10	3.40	1.59	3.10
	1.00	5.04	1.42	2.54
	10.00	5.08	1.34	2.00
	100.00	5.16	1.13	1.72
Nicotinic acid	0.10	3.86	1.33	1.54
	1.00	4.12	1.40	1.69
	10.00	3.84	1.24	1.80
	100.00	3.70	1.19	1.51
Biotin	0.01	6.52	1.84	2.48
	0.10	6.32	1.75	3.32
	1.00	4.04	1.74	2.06
	10.00	3.98	1.68	2.00
Pyridoxine HCl	0.10	3.80	1.16	1.70
	1.00	3.96	1.30	1.80
	10.00	4.04	1.28	1.72
	100.00	4.12	1.10	1.66
Folic acid	0.10	5.16	1.11	1.80
	1.00	6.00	1.34	1.90
	10.00	6.20	1.55	2.40
	100.00	7.68	1.32	1.64
Riboflavin	0.10	4.12	1.53	1.88
	1.00	5.88	1.41	1.72
	10.00	6.04	1.37	1.70
	100.00	7.40	1.35	1.54
Control		5.96[a]	1.62[b]	3.28[c]

a, b, c = Controls (without metals).

B. megaterium SP 86 and *B. megaterium* SP 76 were obtained at a concentration of 0.01 µg/ml. Folic acid and riboflavin at 10 µglml and 100 µglml enhanced lysine yields in *B. megaterium* SP 86 (Table 1). All bivalent metals stimulated lysine accumulation in *B. megaterium* SP 86 and *B. megaterium* SP 14 (Table 2). Co^{2+} and Zn^{2+} improved lysine yields in all the *Bacillus* strains.

The effect of vitamins on lysine production by *Bacillus* strains (Table 1) indicates strain differences in vitamin utilization, but show that biotin is a requirement for lysine accumulation in all the strains. Similar observation was reported by Young and Chipley, (1984) and Sen and Chatterjee (1989), on the role of biotin in lysine production by *Brevibacterium lactofermentum* ATCC 21086 and *Micrococcus varians*_2fa, respectively. The very high lysine level produced by *B. megaterium* SP 86 and *B. megaterium* SP 76, was as a result of the low biotin level (0.01 µg/ml) used. This view is supported by the work of Sen and Chatterjee (1989) but contradicts the report of Tosaka et al. (1979). The latter noted that lysine accumulation in *B. lactofermentum* was stimulated considerably by increasing the biotin level (0.05 - 0.5 µg/ml), supporting the improved lysine yield observed in *B. megaterium* SP 14, at a biotin concentration of 0.1 µg/ml.

Biotin is known to play characteristic roles in glucose oxidation (Oishi and Aida, 1965), protein synthesis (Kimura et al., 1963), cell permeability (Shiio et al., 1963; Takinami et al., 1968) as well as covalent-bound Co^{2+} carrier (Ochra and Kaziro, 1961; Moss and Lane, 1977) but its exact role in amino acid-producing microorganisms is not definite. While Tanaka et al. (1966) reported that biotin limits growth, thus providing carbon and nitrogen sources for amino acid production, Shiiio et al. (1962) and Otsuka et al. (1965), are of the view that low biotin concentration increases cell permeability and allows for the release of high amino acid into the medium. Neither of these hypotheses, however, was supported by the work of Chatterjee and Banerjee (1973) on *B. megaterium* C119 and

Table 2. Effect of bivalent metals on lysine production by *Bacillus* strains.

Bivalent Metal	Concentration (µg/ml)	Lysine (mg/ml)		
		***B. megaterium* SP 86**	***B. megaterium* SP 76**	***B. megaterium* SP 14**
$NiCl_2$	0.10	4.12	1.44	4.14
	1.00	4.20	1.45	4.36
	5.00	4.24	1.34	3.96
	10.00	5.08	1.32	3.51
$CoCl_2$	0.01	3.98	1.63	4.60
	1.00	4.14	1.65	4.42
	5.00	4.12	1.57	3.47
	10.00	2.80	1.47	3.37
$ZnCl_2$	0.10	4.06	1.46	4.22
	1.00	4.08	1.63	4.38
	5.00	4.10	1.58	2.84
	10.00	6.40	1.50	2.39
$CdCl_2$	0.10	3.74	1.49	3.49
	1.00	3.98	1.50	3.46
	5.00	1.74	1.46	3.36
	10.00	0.81	1.44	3.34
$CaCl_2$	0.10	3.10	1.41	3.44
	1.00	3.76	1.42	3.54
	5.00	3.32	1.52	3.37
	10.00	3.02	1.39	3.09
$CuCl_2$	0.10	3.72	1.45	3.66
	1.00	3.18	1.45	3.64
	5.00	3.04	1.35	2.92
	10.00	1.60	1.33	2.89
Control		3.70^{d}	1.56^{e}	3.20^{f}

d, e, f = Controls (without metals).

Bacillus coagulans.

Folic acid and riboflavin stimulated lysine accumulation in *B. megaterium* SP 86 (Table 1). Like other vitamins, they are known to play catalytic roles within the bacterial cells, either as components of coenzymes or as prosthetic groups of enzymes, but their role in amino acid production are not yet known.

On the effect of bivalent metals on lysine yields in *Bacillus* strains (Table 2), all the metals had promoting effect to varying extent in *B. megaterium* SP 86 and *B. megaterium* SP 14. Lysine accumulation was stimulated by Ni^{2+} in the two strains, thus establishing the new role of Ni^{2+} as an essential metal for several enzyme-catalyzed reactions in microorganisms as observed by Thauer et al. (1980) and Lancaster (1988). The inhibitory effect of Ni^{2+} on lysine accumulation in *B. megaterium* SP 76 is similar to that reported by Welward et al. (1971) and Sen and Chatterjee (1989) in *Micrococcus glutamicus* and *M. varians* 2fa, respectively. According to Welward et al. (1971), this may be as a result of the inhibition of diaminopimelic acid decarboxylase activity of the microoranism by the metal ion.

Lysine yields in all the *Bacillus* strains were enhanced by Co^{2+}, but the role of this metal in amino acid production is not known. However, Jasper and Silver (1978) in their study on divalent cation transport system in *Rhodopseudomonas capsulata,* found cobalt to be essential for growth.

The stimulation of lysine in all the strains by Zn^{2+} is in line with the findings of Sen and Chatterjee (1989), implicating the possible role of this metal in lysine production. While Winberg (1970) noted the importance of the metal in growth of certain microorganisms, Sigel (1983) reported its role in the synthesis of industrially and medically significant microbial secondary metabolites. Cadmium like nickel improved lysine yields in *B. megaterium* SP 86

and *B. megaterium* SP 14. The reason for the stimulatory effect of such toxic metal on lysine production is not known, but Hughes and Poole (1989) reported that metals notorious for their toxicity exert beneficial effects at low concentration level. The enhanced lysine accumulation in *B. megaterium* SP 14 at all levels of Cd^{2+} concentration indicates the possibility of this metal ion playing a significant role in lysine production in this strain.

Calcium and Copper ions increased lysine production in *B. megaterium SP* 86 and *B. megaterium* SP 14 but their role in product formation in microorganisms is not yet known. However, Ca^{2+} has been implicated in stabilization of cell walls, activation of intracellular enzymes and in the regulation or triggering of a range of cell functions (Harol, 1986).

With regard to the role of bivalent metals in microorganisms, Martin and McDaniel (1977) and Hughes and Poole (1989) suggested that metal ions probably acted as activators or inhibitors of enzymes involved in the synthetic steps of metabolites. The actual mechanism of stimulation or inhibition of metabolites is still not known.

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