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Full Length Research Paper

Antibiotic and surfactant effects on lysine accumulation by *Bacillus megaterium*

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The effects of antibiotics and surfactants on lysine accumulation in the culture broth of three strains of *Bacillus megaterium* (*B. megaterium* SP 86, *B. megaterium* SP 76 and *B. megaterium* SP 14) were investigated. Lincomycin, neomycin and tetracycline stimulated lysine increase in *B. megaterium* SP 76 and *B. megaterium* SP 14, while erythromycin enhanced lysine yield in all three strains. Palmitic acid improved lysine accumulation in *B. megaterium* SP 86 and *B. megaterium* SP 14. However, Tween 80 and linoleic acid stimulated lysine increase in *B. megaterium* SP 76.

Key words: L-Lysine, antibiotics, surfactants, *Bacillus megaterium*, culture broth.

INTRODUCTION

Lysine is an essential, economically important amino acid used as food and feed supplement. It has also some pharmaceutical application in the formulation of diets with balanced amino acid composition and in amino acid infusion (Shah et al., 2002). Chemical, enzymatic and fermentation processes have been used to synthesize amino acids, and the advantage of microbial methods is that the amino acid are purely optically active (Kinoshita et al., 1957a). Research on the possible utilization of wild strain revealed that many microorganisms such as bacteria, yeasts and filamentous fungi accumulated amino acids in culture fluid, but only bacteria have sufficient productivity to warrant the commercial production (Soda et al., 1983).

Most natural strains cannot produce industrially significant amounts of L-lysine in the culture broth due to various metabolic regulation mechanisms. However, alteration of these mechanisms can lead to L-lysine accumulation (Nakayama, 1972). The influences of antibiotics and surfactants on amino acid production by bacterial organisms have been reported (Smekal et al., 1982; Sen and Chatterjee, 1983; Israilides et al*.,* 1989; Konicek et al*.,* 1991). The present study, therefore, seeks to investigate the effects of antibiotics and surfactants on lysine accumulation in the culture broth of *B. megaterium*.

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MATERIALS AND METHODS

Microorganisms used

Bacillus megaterium SP 14, *Bacillus megaterium* SP 76 and *Bacillus megaterium* SP 86.

Effects of antibiotics on lysine accumulation by *Bacillus* strains

The effects of erythromycin, lincomycin, chloramphenicol, neomycin and tetracycline, at varying concentrations (0.01 - 1.0 µg/ml), on lysine accumulation by *Bacillus* strains were examined. A 25 ml basal medium [KH_2PO_4, 1.0 g; $MgSO_4.7H_20$, 0.4 g; $MnSO_4.H_20$, 2.0 mg; $FeSO_4.7H_20$, 2.0 mg; $CaCO_3$, 50.0 g; H_20, 1 L; pH 7.2, glucose, 80.0 g; $(NH_4)_2SO_4$, 40.0 g] for *B. megaterium* SP 76 and *B. megaterium* SP 14 and [sucrose, 80.0 g; NH_4 Cl, 40.0 g] for *B. megaterium* SP 86 in a 250 ml Erlenmeyer flask was inoculated with 2 ml (ca. 10^8 cells/ml) of a 24 h seed culture of the *Bacillus* strain. After 72 h incubation on a rotary shaker (160 rpm) at 30°C, lysine accumulation was assayed from the broth culture following the method described by Ekwealor and Obeta (2005). All experiments were performed in duplicate and uninoculated flasks served as control.

Effects of surfactants on lysine production by *Bacillus strains*

The effects of varying concentrations (0.01 - 1.0 µg/ml) of Tween 80, oleic acid, linoleic acid, palmitic acid and stearic acid on lysine

Table 1. Effect of antibotics on lysine accumulation by *Bacillus* strains.

Antibiotic	Concentration (µg/ml)	Lysine (mg/ml)		
		***B. megaterium* SP 86**	***B. megaterium* SP 76**	***B. megaterium* SP 14**
Erythromycin	0.01	1.97	1.41	4.34
	0.05	1.52	1.43	0.85
	0.10	1.37	1.52	0.82
	1.00	1.18	1.95	0.79
Lincomycin	0.01	1.59	1.49	1.80
	0.05	1.63	1.55	1.87
	0.10	2.10	1.48	4.14
	1.00	1.45	1.42	1.47
Chloramphenicol	0.01	1.30	1.46	0.73
	0.05	138	1.55	2.88
	0.10	1.81	1.48	0.93
	1.00	1.12	1.47	0.33
Neomycin	0.01	1.40	1.47	1.92
	0.05	1.22	1.93	3.82
	0.10	1.16	1.47	3.00
	1.00	1.10	1.43	1.70
Tetracycline	0.01	1.10	1.97	0.79
	0.05	1.37	1.52	3.46
	0.10	1.35	1.48	1.87
	1.00	1.14	1.45	0.63
		1.87x	1.59y	3.22z

x,y,z: Controls (without antibiotics).

production by *Bacillus* strains were studied. Fermentation process was as previously described.

RESULTS AND DISCUSSION

As presented in Table 1, lincomycin, neomycin and tetracycline stimulated lysine increase in only two of the strains, *B. megaterium* SP 76 and *B. megaterium* SP 14 while erythromycin enhanced lysine yield in all the strains. All the antibiotics except chloramphenicol increased lysine accumulation in *B. megaterium* SP 14. culture medium.

The effects of surfactants on lysine production by *Bacillus* strains (Table 2), showed that tween 80 and linoleic acid stimulated lysine increase in *B. megaterium* SP 76, while palmitic acid improved lysine yield in *B. megaterium* SP 86 and *B. megaterium* SP 14. Oleic acid and stearic acid did not stimulate lysine increase in any of the *Bacillus* strains (Table 2).

The reports regarding increased lysine production by bacteria, in the presence of small quantities of several kinds of antibiotics (Zaki et al., 1982; Sen and Charterjee, 1983, 1985; Israilides et al. 1989) were investigated in strains of *Bacillus megaterium*. Erythromycin, lincomycin, neomycin and tetracycline stimulated lysine increase in the *Bacillus* strains. The stimulatory effect of erythromycin and tetracycline is supported by the work of Zaki et al. (1982). They reported an increase in lysine yield by *Micrococcus glutamicus* when the antibiotics were added to the fermentation culture. In a similar work by Sen and Charterjee (1985), enhanced lysine yield by a hydrocarbon utilizing strain of *Arthrobacter* globiformis was observed when antibiotic was added to the optimal fermentation media. The exact role played by the antibiotics in lysine production is not clearly understood.

The inability of chloramphenicol to enhance lysine accumulation in the *Bacillus* strains is in line with the report of Israilides et al. (1989). These researchers believed that productivity and yields of L-lysine in bacteria were adversely affected by chloramphenicol, as a result of the great decrease in cell viability. The antibiotic, they noted, effectively arrested free cell growth, hence the decrease in lysine production.

Various kinds of surface-active agents are known to affect permeability in microorganisms (Oshima et al., 1964; Rehacek and Basappa, 1971; Smekel et al., 1982; Konicek et al., 1991); therefore, the influence of these agents on lysine accumulation by *Bacillus* strains was examined. The stimulation of lysine production in *B. me-*

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

Surfactant	Concentration (µg/ml)	L-Lysine (mg/ml)		
		***B. megaterium* SP 86**	***B. megaterium* SP 76**	***B. megaterium* SP 14**
Tween 80*	0.05	1.18	1.64	1.79
	0.10	2.05	1.47	1.63
	0.20	3.06	1.17	1.49
	0.50	1.93	1.11	1.36
Oleic acid*	0.05	2.08	1.31	2.20
	0.10	1.35	1.38	2.76
	0.20	0.89	1.41	1.97
	0.50	0.85	1.34	1.18
Linoleic acid *	0.05	0.93	1.17	3.40
	0.10	1.01	1.37	3.34
	0.20	1.06	1.67	3.10
	0.50	1.34	1.55	2.76
Palmitic acid	0.01	2.76	1.19	3.04
	0.05	3.90	1.20	3.18
	0.10	4.16	1.22	3.60
	1.00	4.02	1.40	2.38
Stearic acid	0.01	0.92	1.27	2.42
	0.05	1.86	1.30	3.22
	0.10	1.39	1.52	2.76
	1.00	1.38	1.08	2.72
		3.90m	1.53n	3.42o

*Conc., % (v/v).
m,n,o: Controls (without surfactants).

gaterium SP 76 by Tween 80 conforms to the reports of Smekel et al. (1982) and Konicek et al. (1991). While Smekel et al. (1982) observed a stimulatory effect on lysine production by *Corynebacterium glutamicum* with definite concentrations of liquid Tween 80, Konicek et al. (1991) were of the opinion that increase in lysine yield was caused by Tween 80 interfering with the cellular surface structure of the bacterium.

Oleic acid did not stimulate lysine accumulation in any of the *Bacillus* strains, but linoleic acid increased lysine yield in *B. megaterium* SP 76. The reason for the varied action of the unsaturated fatty acids on lysine production is not known.

The saturated fatty acid, palmitic acid, enhanced lysine accumulation in *B. megaterium* SP 86 and *B. megaterium* SP 14, while stearic acid had no effect on lysine yield in any of the *Bacillus* strains (Table 2).

Although Hassinen et al. (1951) reported the inactive nature of saturated fatty acids on a number of micro-organisms, Takinami et al. (1963), working on L-glutamic acid-producing bacteria, found them stimulating. They noted that C_{16} to C_{18} saturated fatty acid-treated cells, in the absence of biotin synthesized insufficient amounts of phospholipids in the cell membrane, thus, resulting in enhanced permeability toward L-glutamic acid. A similar permeability process may have been involved in the improved lysime accumulation in *B. megaterium* SP 86 and *B. megaterium* SP 14 culture medium. This experimental study has shown that antibiotics and surfactants stimulated lysine yields in *Bacillus megaterium* even though they are strain dependent.

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