BIOTECHNOLOGY PROCESSES

Scale-up and Mixing

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Scale-up and Mixing

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Dispersal of Insoluble Fatty Acid Precursors in Stirred Reactors as a Mechanism to Control Antibiotic Factor Distribution

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Biosynthesis of factors in the A-21978C antibiotic complex was controlled by addition of appropriate fatty acid precursors. Toxicity associated with higher fatty acids was avoided by continuous addition of the fatty acid at a rate nearly equal to the uptake by the producing organism. Inability of the producing organism to assimilate a solid, insoluble long-chain fatty acid was overcome by dissolution of the acid in another substrate.

Substance A21978C is a complex of antibiotics produced by Streptomyces roseosporus, having a common cyclic polypeptide nucleus and different fatty acid side chains (1) (2) (Figure 1). Separation of the major factors C1, C2, and C3 revealed differences in both in vitro antibiotic activity and toxicology for the different naturally occurring compounds. Deacylation of the alkanoyl side chain, followed by reacylation with a series of fatty acids indicated the n-decanoyl substitution at position R, resulted in the best therapeutic potential (3) (4) (5).

The natural occurrence of the n-decanoyl factor, designated LY146032, was too low to permit isolation in sufficient quantity directly from fermentation broth (Table 1).

TABLE 1
Distribution of Naturally Occurring Factors in A21978C Fermentation

A21978C Factor	Concentration µg/ml	% of Total A21978C Complex
C1	77	27.3
C2	113	40.1
C3	72	25.5
C5	trace	-
LY146032	trace	-
	282	

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In an attempt to produce LY146032 by fermentation, the addition of decanoic acid during the antibiotic production phase of the fermentation was proposed. Initial efforts in shaken cultures were unsuccessful due to either the toxicity or insolubility of the fatty acid. In this paper we will describe efforts to direct the biosynthesis of A21978C factors in continuously stirred reactors operating in a fed-batch mode.

MATERIALS AND METHODS

A mutant strain of Streptomyces roseosporus NRRL 11379 was used to inoculate 50 ml of vegetative medium of the following composition: Trypticase soy broth (Baltimore Biological Laboratories, Baltimore, Maryland), 30 mg/ml; potato dextrin, 25 mg/ml. The inoculated medium was incubated for 48 hours at 30°C in a 250 ml Erlenmeyer flask on a shaker rotating through an arc of two inches in diameter at 250 RPM. One-half ml of the mature vegetative culture was dispensed into multiple containers and stored in the vapor phase of liquid nitrogen. One ml of the stored culture was used to inoculate 800 ml of the vegetative medium described above. The inoculated vegetative medium was incubated in a 2000 ml Erlenmeyer flask at 32°C for 120 hours on a shaker

rotating through an arc of two inches in diameter at 250 RPM. The entire contents of the two flasks (approximately 1400 ml after incubation) were used to inoculate 1900 liters of a secondary vegetative stage having the following composition (mg/ml): soybean flour, 5.0; yeast extract (Difco Laboratories, Detroit, Michigan), 5.0; calcium gluconate, 10.0; KCl, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2; $FeSO_4 \cdot 7H_2O$, 0.004; Sag 471 antifoam (Union Carbide, Danbury, Connecticut). The potassium, magnesium, and ferrous salts were prepared separately as follows: 7.6 g $FeSO_4 \cdot 7H_2O$ was dissolved in 76 ml of concentrated HCl. 380 g of $MgSO_4 \cdot 7H_2O$ and 380 g of KCl and deionized water were added to bring the total volume to 3800 ml. The inoculated medium was incubated 24 hours in a stainless steel vessel at 30°C. The vessel was aerated at 0.85 v/v/m and stirred with conventional agitators.

The mature secondary seed (8.33% v/v) was used to inoculate a production medium of the following composition (mg/ml): soybean flour, 22.0; $Fe(NH_4)_2SO_4 \cdot 6H_2O$, 0.66; glucose monohydrate, 8.25; Sag 471, 0.22; potato dextrin, 33.0; and molasses (blackstrap), 2.75.

Two types of stirred reactors were used. The smaller vessel, operated at 120 liters, was agitated with two conventional flat Rushton type impellers at relatively high power input. The larger vessel, operated at 4550 liters, was equipped with impellers having curved paddles and was operated at relatively low power input. Air flow in both reactors was supplied at 0.5 v/v/m by large open tubes which were estimated to contribute very little to the overall mixing. Respiration rates were estimated by difference in inlet and exhaust gas concentration via a Perkin-Elmer mass spectrometer. Distribution of A21978C factors was estimated by high performance liquid chromatography as described previously (2).

Examination of the batch fermentation medium suggested that the growth limiting nutrient was carbon in the form of carbohydrate. It was then hypothesized that in a fed-batch operation a moderately toxic substrate, such as decanoic acid, could be fed continuously to the fermentation if the metabolic consumption rate exceeded the addition rate.

Delivery of decanoic acid to the culture presented a problem. With a melting point of 34°C the compound is a solid at the fermentation temperature of 30°C, and the compound has very low solubility in water. In order to avoid the obvious problems arising in supplying a limiting nutrient as a solid phase, the substrate was dispensed to the stirred reactor as a five percent solution dissolved in a fifty percent ethanol/water mixture. There was an immediate response in oxygen uptake to the onset of the decanoic acid feed, as illustrated in Figure 2. Also, a significant improvement in LY146032 concentration was immediately realized (Table 2).

TABLE 2
Distribution of A21978C Factors with Decanoic Acid Feed[a]

A21978C Factor	Concentration µg/ml	% of Total A21978C Complex
C1	72	19.8
C2	109	29.9
C3	42	11.5
C5	19	5.2
LY146032	122	33.5
	364	

(a) N-decanoic acid/ethanol/water 1:2:2 fed 50 ml per hour to 120 L operating volume.

Material balances suggested that only a small portion of the decanoic acid that was fed could be accounted for by incorporation into the product. Thus, most of the fatty acid was apparently catabolized, presumably by the beta-oxidation pathway. In an attempt to increase the amount of decanoic acid available for the incorporation, the concentration of fatty acid in the

TABLE 3
Distribution of A21978C Factors with Increased Decanoic Acid Feed[a]

A21978C Factor	Concentration µg/ml	% of Total A21978C Complex
C1	131	10.4
C2	189	15.0
C3	107	8.5
C5	52	4.1
LY146032	784	62.1
	1263	

(a) N-decanoic acid/ethanol/water 1:2:2 fed 50 ml per hour to 120 L operating volume.

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