Biosynthesis of oleandomycin by *Streptomyces antibioticus*: influence of nutritional conditions and development of resistance

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The influence of different nutritional compounds on oleandomycin biosynthesis by *Streptomyces antibioticus* was studied, resulting in the design of a chemically defined medium for production of the antibiotic. Of the variety of carbon and nitrogen compounds tested, fructose and aspartic acid (carbon and nitrogen sources, respectively) supported the highest oleandomycin titres. Addition of propionate but not acetate, both precursors of the skeleton of the macrolide lactone ring, stimulated the biosynthesis of the antibiotic. Oleandomycin biosynthesis was repressed by glucose but not by phosphate. *S. antibioticus* develops oleandomycin resistance shortly before the antibiotic begins to be synthesized, showing a triphasic pattern of resistance: spores and producing mycelium are resistant, while non-producing mycelium is sensitive.

Introduction

Antibiotics are secondary metabolites synthesized by pathways which are often connected and influenced by primary metabolism (Drew & Demain, 1977). Thus, frequently an intermediate metabolite from primary metabolism serves as precursor for the biosynthesis of the antibiotic. Therefore, the composition of the culture medium, closely connected with the metabolic capacities of the producing organism, greatly influences the biosynthesis of antibiotics. Changes in the nature and concentration of carbon and nitrogen sources, phosphorus concentration and trace elements have been reported to affect antibiotic biosynthesis in different organisms (for a review see Martin & Demain, 1980).

Oleandomycin is a macrolide antibiotic produced by *Streptomyces antibioticus*. Structurally, it contains a macrocyclic lactone ring of 14 carbon atoms with one sugar, oleandrose, and one amino sugar, desoxamine, attached to the lactone ring. Because of our interest in oleandomycin biosynthesis, we have initiated studies to establish the nutritional factors that would support growth and high oleandomycin titres by this organism. This report describes a series of experiments in the development of a defined medium for the biosynthesis of this macrolide, the influence of different factors on the regulation of oleandomycin biosynthesis and the development of oleandomycin resistance.

Methods

Organisms and culture conditions. *Streptomyces antibioticus* ATCC 11891 was routinely sporulated on GAE solid medium plates and the spores, obtained as described by Hardisson *et al.* (1978), were suspended in 20% (w/v) glycerol and stored at -20°C until used. Inocula were prepared by inoculating 50 ml YEME liquid medium in 250 ml Erlenmeyer flasks with 50 μl spore suspension (approximately 10^9 spores). After incubation at 30°C for 24 h in an orbital incubator with shaking at 250 r.p.m., the mycelia were harvested and washed by centrifugation in 50 mM-Tris/HCl buffer (pH 7.5) and then suspended to the original volume in the same buffer. Portions of this suspension were used as inocula. *Micrococcus luteus* ATCC 10240 was used as the indicator organism; it was grown overnight at 37°C in tryptone soya broth (TSB) medium (Oxoid), then diluted 1:100 in TSB containing 50% (w/v) glycerol and stored at -20°C until used.

Basal medium for nutritional studies. For studies of antibiotic production, a basal mineral-based medium was used. This contained 0.1 M-MOPS buffer, pH 7.5, 2 mM-MgSO₄, 0.5 mM-K₂HPO₄ and 1 ml trace salts stock solution (containing 4 mg ZnCl₂, 20 mg FeCl₃·6H₂O, 1 mg CuCl₂·2H₂O, 1 mg MnCl₂·4H₂O, 1 mg Na₂B₄O₇·10H₂O and 1 mg (NH₄)₆Mo₇O₂₄·4H₂O per 100 ml of distilled water) per litre. Carbon and nitrogen sources were then added at appropriate concentrations. For the experiments, 50 ml medium in a 250 ml Erlenmeyer flask containing a stainless steel spring coil was inoculated with 500 μl of a preinoculum prepared as described above and then incubated at 30°C in an orbital incubator with shaking (250 r.p.m.).

Determination of growth. Growth was determined using a method previously described (Braña *et al.*, 1985), as follows. At different times, samples (0.5 ml) were removed from the cultures and added to tubes containing 0.5 ml 2.5 M-HCl. The tubes were left to stand at room temperature for 10 min, then 3 ml distilled water was added and the

suspension was homogenized by ultrasonic treatment for 30 s. OD₆₀₀ was then determined and the values converted to dry weight of cells ml⁻¹ by means of a standard curve.

Determination of oleandomycin. At different times of incubation, samples (1 ml) were removed, centrifuged at 12000 g for 5 min in an Eppendorf minifuge and, to reduce variations among different samples, the supernatant was stored at -20 °C until all the samples from the same experiment could be assayed together; then, portions of the supernatants were tested for oleandomycin production by bioassay against *M. luteus* as follows. *M. luteus* cell suspension (150 µl) was added to 100 ml prewarmed (55 °C) TSB solid medium at half nutrient concentration containing 1% (w/v) agar. The mixture was poured onto Petri dishes and, after cooling, Whatman AA discs (8 mm in diameter) containing 5 to 20 µl of the supernatant were placed on the top of the agar. In some experiments, when the level of antibiotic production was low, Oxford cylinders (8 mm in diameter) were used containing 100 to 200 µl of supernatant. The plates were placed at 4 °C for 2 h to allow diffusion of the antibiotic and then incubated for 16 h at 37 °C. The haloes were measured and correlated with a standard curve made with commercial oleandomycin using either discs or Oxford cylinders. Each determination was run in duplicate. *M. luteus* is very sensitive to oleandomycin; it is possible to detect at least 0.5 µg oleandomycin (ml culture supernatant)⁻¹.

In some experiments, oleandomycin was also determined by high-pressure liquid chromatography (HPLC) using a reverse-phase C₁₈ column (Novapak). Samples (5 to 20 µl) were introduced and the column developed with a mobile phase consisting of acetonitrile/50 mM-phosphate buffer (pH 7.0; 50:50, v/v). The monitoring wavelength was set at 214 nm.

Uptake experiments. For studies of fructose and aspartic acid uptake, samples were centrifuged at 12000 g for 5 min at 4 °C, washed twice in 100 mM-MOPS buffer (pH 7.5) and suspended in the same buffer. Samples (0.5 ml) were then incubated at 30 °C for 5 min and the uptake experiment initiated by adding 0.1 mM (2 µCi ml⁻¹) D-[6(*n*)-³H]fructose (Amersham; sp. act. 1 Ci mmol⁻¹ = 37 GBq mmol⁻¹) or L-[2,3-³H]-aspartic acid (New England Nuclear; sp. act. 14.9 Ci mmol⁻¹ = 0.55 TBq mmol⁻¹). After incubation at 30 °C for 5 min, the uptake was terminated by adding 10 ml buffer and immediately filtering through Whatman GF/A glass-fibre filters. After washing twice with 10 ml buffer, the filters were dried and the radioactivity estimated in a toluene-based scintillation fluid.

Oleandomycin resistance. At intervals during growth, samples of the cultures were removed and washed in 50 mM-Tris/HCl buffer (pH 7.5). Then, they were briefly sonicated (5 s) to disrupt the mycelia into small fragments. Dilutions of the sonicated samples were spread over GAE plates containing either no oleandomycin, or 50 or 100 µg oleandomycin ml⁻¹. Plates were incubated at 30 °C for 48 h and then the number of colony-forming units determined. Resistance was calculated as the percentage of colonies surviving in plates containing antibiotic with respect to the control plates without oleandomycin.

Thin-layer chromatography. Antibiotic activity in the culture supernatants was also analysed by ascending thin-layer chromatography and bioautography. Samples of the supernatants were developed in silica gel F₂₅₄ (Merck) using as solvent *n*-butanol/acetic acid/water (60:20:20, by vol.). After developing, oleandomycin was detected by staining with 10% (w/v) H₂SO₄ and heating the chromatogram for 20 min at 80 °C. Parallel samples were assayed by bioautography against *M. luteus*.

Analytical methods. D-Fructose was determined by oxidation with alkaline copper (Somogyi, 1952). L-Aspartic acid was measured by HPLC using a reverse-phase C₁₈ column (Novapak); the amino acid was previously derivatized with Fluoropa as described by Hill *et al.*

(1979) and the mobile phase consisted of methanol/50 mM-sodium acetate (pH 5.9) (35:65, v/v).

Results

Nature of the antibiotic synthesized by S. antibioticus

The nature of the antibiotic synthesized by *S. antibioticus* was determined by several procedures. Samples of the culture supernatants in different complex and synthetic media were analysed by thin-layer chromatography as described in Methods. After developing, the antibiotic activity was assayed by bioautography of the plates against *M. luteus* and only one zone was detected which was inhibitory to the growth of the test organism but allowed the growth of a *Staphylococcus aureus* MLS-resistant strain (cross-resistant to macrolides, lincosamides and streptogramins B). This zone comigrated with commercial oleandomycin as demonstrated by staining of a parallel sample. In addition, the culture supernatants were analysed by HPLC and only one peak was detected having antibiotic activity which corresponded to oleandomycin. Similar results were obtained when the antibiotic synthesized in solid media was extracted from the agar and analysed as above.

Influence of carbon and nitrogen sources on oleandomycin biosynthesis

A number of carbohydrates were tested as the only carbon source for oleandomycin production in the basal mineral salts medium containing asparagine as nitrogen source (Table 1). In this, and in the remaining

Table 1. *Effect of carbohydrates on growth and oleandomycin biosynthesis*

The organism was grown in the basal mineral salts medium containing 15 mM-L-asparagine as nitrogen source and the specified carbon source at 55 mM. Data represent values obtained after 72 h incubation at 30 °C and are the mean of two determinations. No growth occurred when maltose or lactose was used as the carbon source.

Carbon source	Dry weight of mycelium (mg ml ⁻¹)	Concn of oleandomycin	
		(µg ml ⁻¹)	[µg (mg dry weight) ⁻¹]
Fructose	2.66	3.00	1.13
Galactose	0.76	2.25	2.96
Cellobiose	0.76	1.50	1.97
Xylose	0.89	1.50	1.67
Melibiose	1.14	1.90	1.67
Sucrose	1.01	1.20	1.19
Glucose	7.34	0.75	0.10
Soluble starch	7.60	1.50	0.20
Glycerol	9.50	1.20	0.13
Trehalose	9.11	0.95	0.10

Table 2. *Effect of amino acids and inorganic salts on growth and oleandomycin biosynthesis*

The organism was grown in the basal mineral salts medium containing 55 mM-fructose as carbon source and the specified nitrogen source at 15 mM. Data represent values obtained after 72 h of incubation at 30 °C and are the mean of two determinations.

Nitrogen source	Dry weight of mycelium (mg ml ⁻¹)	Concn of oleandomycin	
		(µg ml ⁻¹)	[µg (mg dry weight) ⁻¹]
Aspartic acid	1.50	4.50	3.00
NaNO ₃	1.65	4.35	2.64
Lysine	6.46	4.35	0.67
Asparagine	2.46	2.68	1.09
Proline	6.27	1.90	0.30
Urea	3.20	1.90	0.59
Alanine	3.80	1.50	0.39
NH ₄ NO ₃	3.23	0.97	0.30
Glycine	0.38	0.87	2.29
Threonine	1.28	0.65	0.51
Valine	6.83	0.60	0.09
Glutamic acid	2.47	0	0
Arginine	1.80	0	0
Histidine	1.15	0	0

experiments for the selection of a defined medium for antibiotic production, the mode of growth of the culture was noted, so as to avoid media producing too much clumping of the mycelia.

Several carbohydrates (fructose, galactose, cellobiose, xylose and melibiose) supported good levels of oleandomycin production, although to different extents. Fructose was a better carbon source than the other carbohydrates tested since it allowed production of both high oleandomycin titres and high yields of biomass. In addition, the mycelia in this carbon source had a more disperse mode of growth. In all the assays, the pH was also evaluated during growth and found to be relatively constant due to the buffering effect of MOPS.

Using fructose as carbon source, different compounds (amino acids, inorganic salts and urea) were tested as nitrogen sources for oleandomycin production (Table 2). Aspartic acid, lysine and NaNO₃ gave the highest levels of antibiotic production.

In order to select the best combination of carbon and nitrogen sources for oleandomycin biosynthesis, an experiment was designed in which four carbon sources (fructose, galactose, glucose and soluble starch) and four nitrogen sources (aspartic acid, lysine, proline and NaNO₃) were combined. The results (Table 3) showed that the combination of fructose and aspartic acid produced the highest oleandomycin titres and, consequently, these two compounds were chosen as carbon and nitrogen sources, respectively, for further studies.

To further explore the influence of the carbon to nitrogen ratio, the effects of different concentrations of fructose and aspartic acid on oleandomycin production

Table 3. *Influence of different combinations of carbon and nitrogen sources on oleandomycin biosynthesis*

The organism was grown in the basal mineral salts medium containing 55 mM-carbon source and 15 mM-nitrogen source. Data represent values obtained after 72 h of incubation at 30 °C and are the mean of two determinations.

Carbon source	Nitrogen source	Dry weight of mycelium (mg ml ⁻¹)	Concn of oleandomycin	
			(µg ml ⁻¹)	[µg (mg dry weight) ⁻¹]
Fructose	Aspartic acid	1.40	4.30	3.07
	Lysine	6.30	4.12	0.65
	Proline	6.01	1.82	0.30
	NaNO ₃	1.57	4.15	2.64
Galactose	Aspartic acid	1.20	2.08	1.73
	Lysine	3.15	3.50	1.11
	Proline	2.10	2.08	0.99
	NaNO ₃	1.80	2.02	1.12
Glucose	Aspartic acid	3.70	2.20	0.59
	Lysine	6.15	3.15	0.51
	Proline	4.15	3.10	0.75
	NaNO ₃	2.20	1.90	0.86
Soluble starch	Aspartic acid	3.50	4.02	1.15
	Lysine	4.20	5.08	1.21
	Proline	7.15	5.40	0.75
	NaNO ₃	3.15	4.08	1.30

Table 4. *Influence of different combinations of fructose and aspartic acid on oleandomycin biosynthesis*

The organism was grown in the basal mineral salts medium containing different concentrations of fructose and aspartic acid as indicated. Data represent values obtained after 72 h of incubation at 30 °C and are the mean of two determinations.

Fructose (mM)	Aspartic acid (mM)	Dry weight of mycelium (mg ml ⁻¹)	Concn of oleandomycin	
			(µg ml ⁻¹)	[µg (mg dry weight) ⁻¹]
55	5	1.20	3.77	3.14
55	10	1.35	4.20	3.11
55	15	1.42	4.58	3.22
55	20	1.48	4.25	2.87
55	30	1.45	4.10	2.83
55	60	1.47	3.52	2.39
10	15	0.83	2.27	2.74
20	15	0.95	2.68	2.82
55	15	1.37	4.38	3.20
75	15	1.54	4.36	2.83
100	15	1.90	4.23	2.23
200	15	2.97	4.19	1.41

were tested (Table 4). Optimum concentrations were 55 mM and 15 mM for fructose and aspartic acid respectively, giving a fructose/aspartic acid ratio of 3.7.

Influence of phosphate and glucose on oleandomycin biosynthesis

Oleandomycin production was not affected much by the levels of inorganic phosphate in the medium (Fig. 1a).

Fig. 1. Effect of phosphate (a) and glucose (b) on oleandomycin biosynthesis. The organism was grown in the basal mineral salts medium containing fructose (55 mM) and aspartic acid (15 mM) and supplemented with phosphate or glucose as indicated. The cultures were harvested after 72 h and oleandomycin titres and dry weight of the mycelium determined as described in Methods. ●, Growth; ▲, oleandomycin; ■, specific oleandomycin production. Each value is the mean of two determinations.

Table 5. Effect of organic acids as major carbon sources on growth and oleandomycin biosynthesis

The organism was grown in the basal mineral salts medium containing 15 mM-aspartic acid as nitrogen source and the specified organic acid at 55 mM. Data represent values obtained after 72 h of incubation at 30 °C and are the mean of two determinations.

Organic acid	Dry weight of mycelium (mg ml ⁻¹)	Concn of oleandomycin	
		(µg ml ⁻¹)	[µg (mg dry weight) ⁻¹]
Pyruvate	1.46	3.50	2.40
Citrate	0.63	1.90	3.02
Succinate	0.70	0.50	0.71
Acetate	1.65	0	0
Propionate	1.90	0	0
Butyrate	0.38	0	0

The optimum phosphate concentration was in the range 0.5 to 1.0 mM and higher levels did not produce a sharp decrease in antibiotic biosynthesis. It can, therefore, be concluded that biosynthesis of oleandomycin by *S. antibioticus* is not subject to phosphate regulation.

In contrast, the biosynthesis of oleandomycin was repressed by glucose (Fig. 1b). The addition of glucose to cultures containing fructose as the carbon source caused an increase in the rate and extent of growth but this was accompanied by a significant decrease in oleandomycin titres. Thus, antibiotic formation decreased by about 45% and 71% (oleandomycin titre and specific oleandomycin formation, respectively) in the presence of 27.5 mM-glucose and 55 mM-fructose.

Influence of organic acids on oleandomycin biosynthesis

Several organic acids were also tested as the sole carbon source for oleandomycin biosynthesis using aspartic acid as nitrogen source (Table 5). Only pyruvate, citrate and, to a lesser extent, succinate permitted oleandomycin biosynthesis. No antibiotic production was detected in the presence of acetate, propionate or butyrate.

Acetate and propionate are precursors of the skeleton of the lactone ring of oleandomycin: acetate as starter unit and propionate as extender unit. These two organic acids were independently added to the basal medium containing fructose and aspartic acid to determine whether they would stimulate oleandomycin production. The addition of propionate, but not acetate, caused an increase in the rate of growth (Fig. 2b), and produced the highest antibiotic titres. Specific oleandomycin production at the end of the process was 1.8, 1.7 and 2.1 µg oleandomycin (mg dry weight)⁻¹ in the control, in the presence of 10 mM-sodium acetate and in the presence of 10 mM-sodium propionate, respectively. Thus, the addition of 10 mM-sodium propionate to the synthetic medium increased oleandomycin biosynthesis by about 67%.

Characteristics of oleandomycin biosynthesis in the synthetic medium

The kinetic characteristics of oleandomycin biosynthesis during growth of *S. antibioticus* in the chemically defined medium are shown in Fig. 3. The stationary phase was

Fig. 2. Effect of the addition of propionate and acetate on growth (a) and oleandomycin biosynthesis (b). The organism was grown in the basal mineral salts medium containing fructose (55 mM) and aspartic acid (15 mM) and supplemented with sodium acetate or sodium propionate as indicated. At different time intervals, samples were removed and growth and oleandomycin production determined as described in Methods. ○, Control; ●, with 5 mM-sodium propionate; △, with 10 mM-sodium propionate; ▲, with 5 mM-sodium acetate; ■, with 10 mM-sodium acetate. Each value is the mean of two determinations.

Fig. 3

Fig. 3. Growth, carbon and nitrogen consumption and oleandomycin biosynthesis by *S. antibioticus*. The organism was grown in the basal mineral salts medium containing fructose (55 mM) and aspartic acid (15 mM). ○, Growth; ●, aspartic acid; △, fructose; ▲, oleandomycin; ■, specific oleandomycin production. Each value is the mean of two determinations.

Fig. 4. Fructose and aspartic acid uptake during growth of *S. antibioticus*. The organism was grown in the basal mineral salts medium with fructose (55 mM) and aspartic acid (15 mM). At different times, samples were removed and pulse-labelled with tritiated fructose (□) or aspartic acid (■) as described in Methods. Each value is the mean of two determinations.

Fig. 4

Fig. 5. Oleandomycin resistance during growth and oleandomycin biosynthesis. The organism was grown in the basal mineral salts medium containing fructose (55 mM) and aspartic acid (15 mM). At different times of incubation, samples were removed and growth (●), oleandomycin biosynthesis (▲) and oleandomycin resistance (■) were assayed as described in Methods. Each value is the mean of two determinations.

reached after 70 h of incubation and oleandomycin biosynthesis commenced at the middle of the exponential phase of growth. The nitrogen source (aspartic acid) was consumed relatively rapidly, with 50% consumption after about 60 h. In contrast, the utilization of the carbon source (fructose) was much slower: fructose disappearance was detectable only after 55 h and, at the end of the experiment, only about 22% of the sugar initially supplied had been utilized.

The uptake of fructose and aspartic acid during growth was also studied. The uptake of both substrates increased during the exponential phase, reaching maximum values after 48 h and then sharply decreasing with the stationary phase (Fig. 4). No significant differences between the uptake of these compounds was observed.

Oleandomycin resistance during growth and antibiotic biosynthesis

The basal mineral salts medium supplemented with fructose and aspartic acid was inoculated with a spore suspension and growth and oleandomycin biosynthesis were determined. To test for resistance to the antibiotic, the number of colony-forming units surviving in plates with or without oleandomycin was examined at different stages of growth (Fig. 5). Spores were highly resistant to oleandomycin, giving 100% survival when plated onto

medium containing 100 µg oleandomycin ml⁻¹. In contrast, germinating spores and young mycelia (from 15 to 30 h of incubation) showed an increased susceptibility to the antibiotic: only about 8% of the mycelia survived when challenged with 100 µg oleandomycin ml⁻¹. During this period (until 30 h of growth), oleandomycin was not detectable in the culture supernatants: it was first detected after about 40 h. From this time, the exponentially growing mycelia rapidly became resistant and after 60 h of growth no significant difference between the number of colony-forming units in plates with or without oleandomycin was observed.

The susceptibility of *S. antibioticus* to oleandomycin depending on the age of the inoculum was also tested. When spores were used as inoculum, increased oleandomycin concentrations retarded the development of the normal growth curve (Fig. 6a). This delay was also observed when the inoculum was young mycelia (about 20 h of growth; non-producing mycelia), although higher concentrations were necessary to produce similar delays than with spores (Fig. 6b). In contrast, cultures inoculated with older mycelia (about 50 h; producing mycelia) grew normally (Fig. 6c).

Discussion

Our analysis of the influence of different nutritional compounds on oleandomycin biosynthesis by *S. antibioticus* has resulted in the design of a chemically defined medium suitable for growth in a fairly disperse way and for the production of reasonable amounts of this antibiotic. It consisted of mineral salts, fructose (as carbon and energy source) and aspartic acid (as nitrogen source).

S. antibioticus grew on a variety of carbon and nitrogen sources. It is known that the biosynthesis of some antibiotics and other secondary metabolites is negatively affected when a rapidly used carbon source is utilized for growth; however, when the carbon source is slowly consumed by the producer organism, the secondary metabolite is produced effectively (Demain, 1976; Drew & Demain, 1977). This was also the situation for oleandomycin biosynthesis by *S. antibioticus*: growth with some carbon sources (i.e. glucose, glycerol, trehalose, soluble starch) led to high biomass levels, but the specific oleandomycin production was not high. Of the various carbohydrate sources tested, fructose was the most effective. Interestingly, only about 20% of this hexose was consumed at the end of the growth period. Similar results have been reported for biosynthesis of actinomycin D (Paritskaja *et al.*, 1974; Williams & Katz, 1977) and for gramicidin S (Vandamme & Demain, 1976). In *S. antibioticus*, the slow fructose utilization was

Fig. 6. Susceptibility to oleandomycin depending on the age of the inoculum. The basal mineral salts medium containing fructose (55 mM) and aspartic acid (15 mM) was inoculated with spores (a), non-producing mycelium, 20 h of incubation (b) or producing mycelium, 50 h of incubation (c) and the growth of the cultures determined in the absence of oleandomycin (○) and in the presence of $10 \mu\text{g ml}^{-1}$ (●), $25 \mu\text{g ml}^{-1}$ (△), $50 \mu\text{g ml}^{-1}$ (▲), $75 \mu\text{g ml}^{-1}$ (□) or $100 \mu\text{g ml}^{-1}$ (■) oleandomycin. Each value is the mean of two determinations.

not due to the lack of an uptake system for this sugar, since we have shown (Salas & Hardisson, 1981; this paper) that such an uptake system is present and active in this organism, although it requires some time for induction. On the other hand, it is possible that the nitrogen source (aspartic acid) would be preferentially used initially for growth (as both carbon and nitrogen source) and only when most of the amino acid has been exhausted (after about 60 h of growth) would the fructose act as the major carbon and energy source. Although a number of amino acids were favourable sources of nitrogen for oleandomycin biosynthesis, growth on one amino acid (aspartic acid) and one inorganic compound (NaNO_3) produced the highest oleandomycin titres. With these two nitrogen sources, the biomass levels were not very high, but the specific antibiotic production was the highest achieved.

Oleandomycin production by *S. antibioticus* was decreased markedly by glucose. Acidity was not responsible, since the pH of the medium containing increased glucose concentrations was similar to that of the control cultures. Therefore, our results strongly suggest some type of carbon catabolite control of oleandomycin biosynthesis in this organism. Whether the regulation is repression, inhibition or inactivation is not yet known. A

similar situation has been reported for the biosynthesis of another macrolide, erythromycin, by *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*) (Escalante *et al.*, 1982).

Neither propionate nor acetate (precursors in the biosynthesis of the lactone ring of oleandomycin) were good carbon sources for oleandomycin production. However, the addition of propionate (but not acetate) to the chemically defined medium increased the biosynthesis of the antibiotic by about 67%. This result probably reflects some metabolic difficulties in the synthesis of propionate, which could be a rate-limiting step in oleandomycin biosynthesis by this organism.

Growth and production of the antibiotic in the defined medium did not follow the typical pattern of a trophophase-idiophase fermentation. Thus, oleandomycin production began after 40 h of incubation when the organism was in the middle of the exponential phase of growth. There are many examples in the literature showing a concordance between antibiotic production and the idiophase (for a review see Weinberg, 1970). However, it is also evident in some cases that production may also parallel the growth of the producing strain (Audhya & Russell, 1975; Williams & Katz, 1977; Blanco *et al.*, 1984). In order to synthesize the antibiotic

the organism must develop a physiological change that enables it to survive during the production phase. Such a change can be constitutively expressed (Skinner & Cundliffe, 1982; Blanco *et al.*, 1984) or may appear only at or shortly before antibiotic production (Woodruff, 1966; Yoshida *et al.*, 1966; Cella & Vining, 1974; Thiara & Cundliffe, 1988). *S. antibioticus* cultures in the defined medium showed a triphasic pattern of resistance: spores and producing mycelia were resistant while non-producing mycelia were sensitive. To explain this behaviour it seems necessary to postulate the existence of a permeability barrier, probably an active efflux system which limits the access of the antibiotic to its target site. This barrier presumably does not exist in non-producing mycelium but develops just before (or simultaneously with) the onset of oleandomycin biosynthesis. Our previous studies showing the susceptibility of the ribosomes of *S. antibioticus* to oleandomycin through the growth cycle and the absence of oleandomycin-inactivating activity (Fierro *et al.*, 1987) support this proposal. Such a mechanism of resistance has not been widely reported but has been increasingly described in the recent years (Ohnuki *et al.*, 1985; Neal & Chater, 1987; Fierro *et al.*, 1988; Reynes *et al.*, 1988).

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