Resistance to Inhibitors of RNA Polymerase in Actinomycetes Which Produce Them

By M. GLORIA BLANCO, CARLOS HARDISSON* AND JOSE A. SALAS

Departamento de Microbiología, Universidad de Oviedo, Oviedo, Spain

(Received 22 May 1984; revised 28 June 1984)

Resistance to the endogenous antibiotic was studied in three actinomycetes that produce inhibitors of RNA polymerase. The three producers, *Nocardia mediterranei* (rifamycin producer), *Streptomyces spectabilis* (streptovaricin producer) and *Streptomyces lydicus* (streptolydigin producer), were each highly resistant to the antibiotic they produce ($MIC > 200 \mu g\ ml^{-1}$) and *in vivo* RNA synthesis was also resistant. However, cross-resistance to the other RNA polymerase inhibitors was not found. Resistance to these antibiotics was due to target site modification, since the RNA polymerase enzymes of the three producing organisms were highly resistant *in vitro* to the corresponding antibiotic, and no antibiotic-inactivating enzymes were detected. A mutant was isolated from *S. spectabilis* which was sensitive to streptovaricin (its own product) and also showed an increased sensitivity to rifamycin and streptolydigin. This mutant had RNA polymerase which was extremely sensitive to the three antibiotics.

INTRODUCTION

Antibiotic-producing organisms must protect themselves from their own products. In many cases this problem does not really occur, since there is no target site for the antibiotic inside the producing cell; such antibiotics are called xenotoxic (Vining, 1979). In contrast, prokaryotic organisms synthesizing autotoxic antibiotics have to develop specific mechanisms to overcome the toxic effects of their products. Several ways to achieve this have been described. These include modification of the target site (Cundliffe, 1978; Thompson & Cundliffe, 1980; Cundliffe & Thompson, 1981; Piendl & Bock, 1982; Thompson *et al.*, 1982; Skinner & Cundliffe, 1982), inactivation of intracellular antibiotic (Benveniste & Davies, 1973; Skinner & Cundliffe, 1980; Hotta *et al.*, 1981; Perez-González *et al.*, 1983) and exclusion of the antibiotic from the cytoplasm (Malik & Vining, 1972).

There are several antibiotics produced by actinomycetes that inhibit bacterial DNA-dependent RNA polymerase. These include the rifamycins, streptovaricins and streptolydigin. Rifamycin (RIF) and streptovaricin (STV), produced by *Nocardia mediterranei* and *Streptomyces spectabilis* respectively, seem to act by inhibition of the initiation of transcription (Sippel & Hartman, 1968; Wehrli *et al.*, 1968), although it has been reported that RIF, at high concentrations, may also inhibit elongation (Kessler & Hartmann, 1977). On the other hand, streptolydigin (SLG), produced by *Streptomyces lydicus*, interferes with RNA chain elongation rather than initiation (Schleif, 1969; Siddhikol *et al.*, 1969; Cassani *et al.*, 1971; Helm *et al.*, 1972), and may lead to premature termination (von Meyenburg *et al.*, 1978).

There has been only one report in the literature concerning resistance to these antibiotics in the producing organisms (Watanabe & Tanaka, 1976). In that report, RNA polymerase of *Streptomyces* (now *Nocardia*) *mediterranei* (RIF producer) was shown to be resistant to the produced antibiotic when compared with that of *Streptomyces griseus* (non-producing organism) and

Abbreviations: RIF, rifamycin; SLG, streptolydigin; STV, streptovaricin.

Escherichia coli. Here, we have extended these investigations to the producers of STV and SLG, and we have also looked for the possible existence of cross-resistance to these three inhibitors at the level of RNA polymerase.

METHODS

Bacterial strains and culture conditions. *Nocardia mediterranei* ATCC 13685 was kindly provided by Professor D. A. Hopwood, Department of Genetics, John Innes Institute, Norwich, UK. *Streptomyces spectabilis* UC-2472 and *Streptomyces lydicus* UC-2056 were obtained from Dr J. E. Grady, Upjohn Co., Kalamazoo, Mich., USA. These organisms were grown in YEME liquid medium containing the following components (l^{-1}): glucose, 10 g; yeast extract, 3 g; malt extract, 3 g; peptone, 5 g. Flasks containing 100 ml YEME liquid medium were inoculated with spore suspensions and incubated for 24 h at 35 °C and then 10 ml of such cultures were used as inocula for 500 ml cultures. After growth at 35 °C for 24 h in an orbital incubator (Gallenkamp) with shaking, the mycelia were harvested by centrifugation (12000 g for 15 min), washed twice in buffer A (50 mM-Tris/HCl pH 8.4 at 4 °C, 10 mM-MgCl₂, 7 mM-2-mercaptoethanol, 1 mM-EDTA) and stored at -20 °C until use. *Micrococcus luteus* ATCC 10240 and *Bacillus megaterium* ATCC 11562 were grown overnight at 35 °C in Müller-Hinton broth (Difco).

Determination of the minimal inhibitory concentration (MIC). The organisms were inoculated into YEME liquid medium and incubated overnight at 35 °C. YEME solid medium plates containing different antibiotic concentrations were then inoculated using a multipoint inoculator (Steer replicator) and growth on the plates was monitored after 2 d incubation at 35 °C. Because *Streptomyces* spp. tend to form clumps in YEME liquid medium, the mycelia were crushed prior to replicating.

Test for antibiotic production. At different times of the growth cycle, samples were centrifuged in Corex tubes (12000 g for 15 min) and the amount of antibiotic in the supernatant was determined by bioassay. To reduce variations, the supernatant solutions were stored at -20 °C until all the samples from the experiment could be assayed together. For the assay, plates of Müller-Hinton medium (15 ml) containing 2% (w/v) agar were inoculated with 0.1 ml of an overnight culture of *M. luteus* (for STV and RIF production) and of *B. megaterium* (for SLG production). The inoculum was spread over the surface of the medium by means of glass beads (2-3 mm diameter). Metal Oxford cylinders containing 50-200 µl of the supernatant were then placed on the top of the medium and the antibiotic allowed to diffuse into the medium for 2 h at 4 °C. Incubation was then carried out at 35 °C for 16 h and the diameters of the haloes were measured and compared with those obtained with known concentrations of STV, RIF and SLG in plates run in parallel.

Isolation of mutants. Mutagenesis of spores was carried out by UV-light treatment. Spore suspensions (5 ml in 20%, w/v, glycerol; OD₅₈₀ = 0.3) were exposed for 1 min to UV irradiation in an uncovered glass Petri dish. This time was previously found to produce about 95% loss of spore viability. Adequate dilutions were spread over YEME solid medium plates and incubated for 3-4 d at 35 °C until the surviving spores formed colonies and sporulated. These were then replica-plated onto YEME solid medium plates containing 100 µg antibiotic ml⁻¹, and colonies unable to grow on these plates were selected as possible sensitive mutants. Such colonies were tested again for antibiotic sensitivity after several rounds of colony isolation on solid medium plates without antibiotic. The MICs of these individual colonies were then determined as described above.

Measurements of *in vivo* RNA synthesis. At different stages of the growth cycle, samples (30 ml) were withdrawn, centrifuged and resuspended in a minimal synthetic medium previously described (Hardisson *et al.*, 1980). After preincubation of the cells for 5 min with different concentrations of the antibiotics, the samples were pulse-labelled with [³H]uridine (1 µCi ml⁻¹, specific activity 30 Ci mmol⁻¹) for 5 min and the reaction stopped by the addition of 1.0 ml ice-cold 10% (w/v) trichloroacetic acid (TCA). The tubes were placed in an ice-cold water bath for 30 min before the contents were filtered and washed with 20 ml ice-cold 5% TCA. The filters were dried and counted in a Beckman LS 100 C liquid scintillation spectrophotometer using a toluene-based scintillation fluid. Corrections were made in all cases for the corresponding blanks.

Partial purification of RNA polymerase. The whole process was carried out at 4 °C. About 10 g wet weight of mycelium was suspended at 0.2 g ml⁻¹ in buffer A containing 1 mM-phenylmethylsulphonyl fluoride (PMSF) and 1 mM-o-phenanthroline to inhibit proteolytic enzymes. The suspension was then submitted to brief sonication (four 1 min bursts with intermittent cooling) in an 150 W ultrasonic disintegrator. This produced disaggregation and partial breakage of the mycelia and was important in order to get maximal yields of the enzyme. Lysozyme (1 mg ml⁻¹) was added and the mixture incubated overnight. The extract was treated with polyethyleneglycol (PEG) 6000 and dextran T-500, essentially as described by Babinet (1967), but with minor modifications. A sample of 50 ml extract was mixed with 23.5 ml 30% (w/v) PEG 6000 and 8.6 ml 20% (w/v) dextran T-500, both dissolved in water. After stirring for 30 min, the mixture was centrifuged at 30000 g for 20 min and the upper phase discarded. To the lower phase (dextran phase), 18 ml 30% PEG, 43 ml buffer A and 20 g NaCl were added and the mixture was again stirred for 30 min. After centrifugation at 30000 g for 20 min, the upper phase (PEG phase) was recovered and dialysed against 21 buffer A which was changed several times. This dialysed extract was submitted to two successive ammonium sulphate precipitations (35% and 70% saturation) and most of the RNA polymerase

activity was recovered in the 70% precipitation. This was dissolved in 4 ml buffer B (25 mM-Tris/HCl, pH 8.4 at 4 °C, 1 mM-EDTA, 7 mM-mercaptoethanol) containing 15% (v/v) glycerol, 1 mM-PMSF, 1 mM-*o*-phenanthroline and 25 mM-KCl and again dialysed against the same buffer. The material obtained was applied to a DEAE-cellulose column (10 × 2 cm), previously equilibrated with buffer A plus 25 mM-KCl at a flow rate of 30 ml h⁻¹ and washed with 75 ml buffer B. Buffer B (50 ml) containing KCl (25–400 mM linear concentration gradient) was applied at a flow rate of 50 ml h⁻¹. Fractions (2 ml) were collected and those containing RNA polymerase activity pooled, precipitated with 70% saturated ammonium sulphate and suspended in 2 ml buffer B containing 50% glycerol and 100 mM-KCl. These fractions were kept at -20 °C until use.

RNA polymerase assays. The assay mixture for determination of RNA polymerase activity contained, in a total volume of 100 µl, the following components: 50 mM-Tris/HCl pH 7.9 at 25 °C, 10 mM-MgCl₂, 10 mM-MnCl₂, 200 mM-KCl, 0.1 mM-dithiothreitol, 0.1 mM-EDTA, 1 mM-K₂HPO₄ pH 7.5, 0.1 mM-GTP, 0.1 mM-CTP, 0.1 mM-UTP, 0.05 mM-[2,8,5-³H]ATP (0.2 µCi nmol⁻¹), 0.05 µg bovine serum albumin, 1 µg poly(dA-dT) and 5–50 µl extract. The reaction was stopped after 15 min at 37 °C by the addition of 1 ml 5% TCA containing 10 mM-sodium pyrophosphate. After 30 min at 4 °C, the samples were filtered through Whatman GF/C glass fibre discs (2.5 cm diameter) and washed with 12 ml 2% TCA plus 10 mM-sodium pyrophosphate. The radioactivity in the dried filters was estimated in a Beckman LS 100 C liquid scintillation spectrophotometer. Under these incubation conditions, an incorporation of 40 c.p.m. represented approximately 1 pmol [³H]AMP incorporated into RNA.

Dry weight determinations. Samples were centrifuged, washed twice with double-distilled water and dried at 100 °C for 24 h in small glass vials before weighing.

Chemicals and media. Streptovaricin (U-7750, complex) and streptolydigin (U-5481, free acid) were the kind gift of Dr J. E. Grady. Rifamycin SV was from Sigma. The three antibiotics were dissolved in 50% (v/v) ethanol at 4 mg ml⁻¹. Poly(dA-dT), adenosine 5'-triphosphate, guanosine 5'-triphosphate, cytidine 5'-triphosphate, uridine 5'-triphosphate, malt extract, 2-mercaptoethanol, EDTA, PEG 6000, dextran T-500, lysozyme, DEAE-cellulose (DE-52), peptone, dithiothreitol, phenylmethylsulphonyl fluoride, *o*-phenanthroline and yeast extract were from Sigma. Ammonium sulphate (enzyme grade) was from Merck. [5-³H]Uridine (specific activity 30 Ci mmol⁻¹; 1.11 TBq mmol⁻¹) was from Amersham and [2,8,5-³H]adenosine 5'-triphosphate (specific activity 46 Ci mmol⁻¹; 1.70 TBq mmol⁻¹) was from New England Nuclear.

RESULTS

Growth, antibiotic production and in vivo resistance

When inoculated as spore suspensions into YEME liquid medium, *N. mediterranei*, *S. spectabilis* and *S. lydicus* reached the stationary phase of growth after approximately 60 h incubation at 35 °C. Production of STV and RIF by *S. spectabilis* and *N. mediterranei*, respectively, started very early in the growth cycle (after about 10 h incubation), while that of SLG by *S. lydicus* occurred after a lag phase of about 38 h (Fig. 1). The three organisms did not produce very high antibiotic concentrations under our culture conditions but, as will be seen later, the levels of resistance were high. In conventional agar diffusion assays, each of the three organisms was resistant to its own antibiotic product, but cross-resistance to the other antibiotics was not found. Calculations of the MICs (Table 1) confirmed these observations. After UV-light treatment, a mutant of *S. spectabilis* was isolated which was sensitive to STV (MIC 25 µg ml⁻¹) and also showed increased sensitivity to RIF and SLG (MIC 5 µg ml⁻¹ for both). This mutant was also examined for its ability to produce STV and was found to produce 12 times less STV than the wild-type strain (0.03 µg ml⁻¹ and 0.36 µg ml⁻¹ at 100 h incubation, respectively).

Having established that the three producers were each resistant to their product, the effect of the three antibiotics on RNA synthesis by whole cells was assayed by measuring the incorporation of [³H]uridine into TCA-insoluble material. The tendency of *Streptomyces* to grow as clumps was reduced in these experiments by using a medium consisting of YEME and 34% sucrose, which promoted diffuse growth (Chater, 1974). Pulse-labelling experiments for each of the three producers showed that *in vivo* RNA synthesis was not affected by the endogenous antibiotic (Fig. 2). However, cross-resistance to the other antibiotics was found only for *N. mediterranei* at concentrations lower than 25 µg STV ml⁻¹. Higher concentrations of STV (50 µg ml⁻¹) caused inhibition (data not shown). Significantly, there was no difference in the response to the antibiotics when cells in different stages of the growth cycle were used, showing that resistance was a permanent property of the cell and was not acquired solely during the phase of antibiotic production.

Fig. 1. Growth and antibiotic production by cultures of (a) *N. mediterranei* (rifamycin production), (b) *S. spectabilis* (streptovaricin production), and (c) *S. lydicus* (streptolydigin production). ○, Growth; ●, antibiotic production. Each value is the average of three determinations.

Table 1. MICs of antibiotic-producer and test organisms

Organism	MIC of antibiotic (μg ml ⁻¹)		
	Rifamycin	Streptovaricin	Streptolydigin
<i>N. mediterranei</i>	> 200	75	25
<i>S. lydicus</i>	25	25	> 200
<i>S. spectabilis</i>	25	> 200	50
<i>S. spectabilis</i> STV ¹	5	10	5
<i>M. luteus</i>	1	1	75
<i>B. megaterium</i>	5	25	25

Fig. 2. Effect of rifamycin (●), streptovaricin (○), and streptolydigin (Δ) on RNA synthesis by whole cells of (a) *N. mediterranei*, (b) *S. spectabilis*, and (c) *S. lydicus*. Cell suspensions (0.5 ml) were incubated with the antibiotic for 5 min before initiating the assay by the addition of [³H]uridine (1 μCi ml⁻¹). After 5 min incubation at 35 °C, the reaction was stopped by adding 1 ml ice-cold 10% TCA. The samples were then processed as described in Methods.

Table 2. Requirements for RNA polymerase activity of *Streptomyces spectabilis*

Similar results were found using enzyme fractions of the other two producers (data not shown).

Conditions	Enzyme activity*
Complete†	275
Minus DNA	25
Minus $MnCl_2$	2
Minus $MgCl_2$	29
Minus enzyme	18
Minus UTP, CTP and GTP	12
Plus RNAase ($5 \mu g\ ml^{-1}$)	12

* Expressed as pmol [3H]AMP incorporated into cold 5% TCA-insoluble material in 30 min at 35 °C. All the values are the average of duplicate assays and were in a range $\pm 10\%$.

† See Methods for composition of complete assay mixture.

RNA polymerase resistance

The results described above support the view that RNA synthesis is resistant to the produced antibiotic, but it was possible that the antibiotic was unable to penetrate the cell wall and the cytoplasmic membrane of the producer organism. In order to clarify this point, we tested the sensitivity of the target site of these antibiotics. To do this, we partially purified the RNA polymerase of the three organisms. It was essential to obtain RNA polymerase fractions which were dependent on the addition of exogenous DNA template for the transcription process, since RIF and STV only inhibit the initiation of transcription (Sippel & Hartmann, 1968; Wehrli *et al.*, 1968) and anomalous results might have been obtained if DNA from *Streptomyces* or *Nocardia* were not totally eliminated. This would happen if RNA polymerase could finish transcription of those contaminating DNA fragments in which this process was initiated prior to the addition of antibiotic. Preliminary experiments with crude enzyme preparations from sensitive non-producing *Streptomyces* species and very sensitive organisms such as *B. megaterium* and *M. luteus* confirmed this. Contaminating endogenous DNA was removed by partial purification of the enzyme (see Methods) and, in particular, by the use of fractions eluted from DEAE-cellulose columns at KCl concentrations between 0.2 and 0.3 M. These fractions obtained from the producing organism were able to transcribe a wide range of DNA templates (including chromosomal, plasmid and phage DNAs) and also showed high efficiency with a synthetic template, poly(dA-dT) (data not shown). The activities of the three enzymes were dependent on the presence of DNA template, nucleoside triphosphates and both magnesium and manganese ions. As expected, the transcripts were sensitive to RNAase treatment (Table 2). The enzyme was stable for at least 1 month at $-20\text{ }^{\circ}\text{C}$ in the presence of 50% glycerol and 1 mM-dithiothreitol.

The sensitivity of these partially purified enzyme preparations to the three antibiotics was tested. The RNA polymerase of each of the three producers was resistant to the produced antibiotic which, in the case of *S. lydicus* and *S. spectabilis*, even stimulated transcription (Figs 3 and 4). Moreover, in agreement with the results cited above relating to the inhibition of RNA synthesis *in vivo*, the RNA polymerase of each producer was not cross-resistant to the other antibiotics. Only in the case of *N. mediterranei* did the RNA polymerase show a partial resistance to STV. This corresponded with an elevated MIC and the effects upon RNA synthesis *in vivo* described above. The response of the STV-sensitive mutant of *S. spectabilis* to the three antibiotics was also tested. This mutant was highly sensitive to the three antibiotics (STV, SLG and RIF at $0.1\text{ }\mu g\ ml^{-1}$ caused about 98%, 71% and 85% inhibition, respectively; Fig. 4b).

Since the RNA polymerase used was only partially purified, and in order to rule out the possible presence of antibiotic-inactivating enzymes in such preparations, several additional experiments were done. Each individual antibiotic was incubated at 35 °C for 2 h with S100 (supernatant following centrifugation at 100000 g for 4 h) obtained from each producer. This was done in the presence of ATP and acetyl-coenzyme A. Then the residual antibiotic activity of the three antibiotics was tested by two methods: (i) bioassay against *M. luteus* (for STV and RIF) and against *B. megaterium* (for SLG), and (ii) inhibition of RNA polymerase activity of the STV-sensitive mutant. No inactivation of the antibiotics was detected by either method.

Fig. 3. Effect of rifamycin (●), streptovaricin (○) and streptolydigin (△) on *in vitro* RNA polymerase activity of (a) *N. mediterranei* and (b) *S. lydicus*. The enzyme fractions were incubated for 5 min with antibiotic before initiating the reaction. After 15 min at 37 °C, the reaction was stopped with 1 ml ice-cold TCA containing 10 mM-sodium pyrophosphate and the radioactivity incorporated into TCA-insoluble material determined after processing of the samples, as described in Methods.

Fig. 4. Effect of rifamycin (●), streptovaricin (○) and streptolydigin (△) on *in vitro* RNA polymerase activity of wild-type (a) and a streptovaricin-sensitive mutant (b) of *S. spectabilis*. Conditions were the same as those described for Fig. 4.

DISCUSSION

The study of antibiotic-resistance mechanisms in actinomycetes has become a very attractive field of research, particularly since the suggestion that the resistance determinants found in clinical isolates could have an origin in the producing organisms (Walker & Walker, 1970; Benveniste & Davies, 1973). Evidence supporting this hypothesis has appeared in recent reports showing sequence homology between antibiotic-resistance genes from producing bacteria and from transposon-located genes (Herbert *et al.*, 1983; Thompson & Gray, 1983). However, most of the work on these resistance mechanisms has been done with antibiotics acting on protein synthesis, and only a few reports have been published on inhibitors of RNA polymerase. Mutants resistant to inhibitors of RNA polymerase have been isolated from non-producing organisms and have been shown to have RNA polymerase resistant to the drug (Schleif, 1969; Yura & Igarashi, 1968; Iwakura *et al.*, 1973; Sonenshein *et al.*, 1974; Halling *et al.*, 1978). This

was also the case for RIF resistance in *N. mediterranei* (Watanabe & Tanaka, 1976). Most of these resistant mutants showed cross-resistance between RIF and STV (Yura & Igarashi, 1968) but cross-resistance between these two antibiotics and SLG was not always found (Iwakura *et al.*, 1973; Sonenshein *et al.*, 1974). We report here that the actinomycetes producing RIF, STV and SLG are resistant to their own antibiotics due to target site modification. Resistance seems to be constitutively expressed and was observed throughout the cell cycle, independent of the period of antibiotic production. In addition, we could separate resistance to RIF and STV completely in *S. spectabilis* (STV producer) and partially in *N. mediterranei* (RIF producer), suggesting that the modifications of RNA polymerase responsible for resistance in these organisms are different. Additional support for the view that resistance is associated with the alteration of RNA polymerase comes from a mutant of *S. spectabilis* selected as STV-sensitive and which also has RNA polymerase sensitive to STV. In this strain, the mutation for STV sensitivity increased the affinity of the RNA polymerase for both STV and RIF. This suggests that, although the resistance mechanisms in the respective producers may not be exactly the same, there must be a relation between them. This mutant showed another feature. The acquisition of the STV-sensitive phenotype was accompanied by a great decrease in antibiotic production. The importance of this is clear if one considers the necessity for an antibiotic-sensitive organism to be a non-producer (or low-level producer) in order to avoid 'suicide'. It is possible that this mutant is the result of a unique lesion in structural gene of the RNA polymerase yielding an antibiotic-sensitive product which, in some way, regulates the expression of the genes for antibiotic synthesis. Alternatively, this mutant could be the result of a double mutation (one in a structural gene for RNA polymerase and another in an antibiotic production gene), but the statistical probability of this occurring is very low.

Another interesting point arising from our study was the fact that antibiotics produced by *S. lydicus* (SLG) and *S. spectabilis* (STV) not only failed to inhibit the RNA polymerase of the producer but even enhanced its activity. This phenomenon was observed previously in a streptolydigin-resistant mutant of *Bacillus subtilis* (Halling *et al.*, 1978). It is possible that the interaction of the antibiotic with the modified subunit of the enzyme induced some conformational changes in the multimeric protein structure making the binding of RNA polymerase to the DNA template more effective. It is interesting in this context that RIF-dependent mutants have been reported in *E. coli* (Dabbs, 1979).

Resistance to inhibitors of RNA polymerase in non-producer organisms has been reported to be associated with the β subunit (Rabussay & Zillig, 1969; Boyd *et al.*, 1974; Linn *et al.*, 1975; Halling *et al.*, 1977, 1978; Boothroyd *et al.*, 1983). In some cases, the modification alters the electrophoretic mobility of the subunit (Linn *et al.*, 1975) and it has recently been shown that rifampicin resistance mutations in *E. coli* were due to a change in only one amino acid in the β subunit sequence (Ovchinnikov *et al.*, 1983). Work in progress in our laboratory is focused on the elucidation of the modification in the RNA polymerase subunit responsible for resistance in the producer organisms.

We wish to thank Dr Eric Cundliffe for encouraging us in the realization of this project and for critical reading of the manuscript, and Dr F. Parra for helpful suggestions in the RNA polymerase purification. We also thank Professor D. A. Hopwood for the kind gift of *Nocardia mediterranei* and Upjohn Co. for the kind gift of streptovaricin, streptolydigin and both producer organisms.

M. G. B. was the recipient of a predoctoral fellowship of the Ministry of Education and Science, Spain. This work was supported in part by a grant of the Comisión Asesora para el desarrollo de la Investigación Científico-Técnica, Spain.

REFERENCES

- BABINET, C. (1967). A new method for the purification of RNA polymerase. *Biochemical and Biophysical Research Communications* **26**, 639-644.
- BENVENISTE, R. & DAVIES, J. (1973). Aminoglycoside-antibiotic inactivating enzymes in actinomycetes similar to those present in clinical isolates of antibiotic-resistant bacteria. *Proceedings of the National Academy of Sciences of the United States of America* **70**, 2276-2280.
- BOOTHROYD, C. M., MALET, R. M., NENE, V. & GLASS, R. E. (1983). Genetic studies on the β subunit of *Escherichia coli* RNA polymerase. III. Analysis of low level rifampicin-resistant mutants. *Molecular and General Genetics* **190**, 523-526.

- BOYD, D. H., ZILLIG, W. & SCAIFE, F. J. G. (1974). Reference mutations for the β subunit of RNA polymerase. *Molecular and General Genetics* **130**, 315-320.
- CASSANI, G., BURGESS, R. R., GOODMAN, H. M. & GOLD, L. (1971). Inhibition of RNA polymerase by streptolydigin. *Nature New Biology* **230**, 197-200.
- CHATER, K. F. (1974). Rifampicin-resistant mutants of *Streptomyces coelicolor* A3(2). *Journal of General Microbiology* **80**, 277-290.
- CUNDLIFFE, E. (1978). Mechanism of resistance to thiostrepton in the producing-organism *Streptomyces azureus*. *Nature, London* **272**, 792-795.
- CUNDLIFFE, E. & THOMPSON, J. (1981). The mode of action of nosiheptide (multihomycin) and the mechanism of resistance in the producing organism. *Journal of General Microbiology* **126**, 185-192.
- DABBS, E. R. (1979). New tool for studying interactions of components of ribonucleic acid polymerase: rifampin-dependent mutants. *Journal of Bacteriology* **139**, 1072-1074.
- HALLING, S. M., BURTIS, K. C. & DOI, R. H. (1977). Reconstitution studies show that rifampicin resistance is determined by the largest polypeptide of *Bacillus subtilis* RNA polymerase. *Journal of Biological Chemistry* **252**, 9024-9031.
- HALLING, S. M., BURTIS, K. C. & DOI, R. H. (1978). β subunit of bacterial RNA polymerase is responsible for streptolydigin resistance in *Bacillus subtilis*. *Nature, London* **272**, 837-839.
- HARDISSON, C., SALAS, J. A., GUJARRO, J. A. & SUÁREZ, J. E. (1980). Macromolecular synthesis during the germination of *Streptomyces* spores in a chemically defined medium. *FEMS Microbiology Letters* **7**, 233-235.
- HELM, K. VON DER. & KRAKOW, J. S. (1972). Inhibition of RNA polymerase by streptolydigin. *Nature New Biology* **235**, 82-83.
- HERBERT, C. J., GILES, I. G. & AKHTAR, M. (1983). The sequence of an antibiotic resistance gene from an antibiotic-producing bacterium: homologies with transposon genes. *FEBS Letters* **160**, 67-71.
- HOTTA, K., YAMAMOTO, H., OKAMI, Y. & UMEZAWA, H. (1981). Resistance mechanisms of kanamycin-, neomycin-, and streptomycin-producing streptomycetes to aminoglycoside antibiotics. *Journal of Antibiotics* **34**, 1175-1182.
- IWAKURA, Y., ISHIHAMA, A. & YURA, T. (1973). RNA polymerase mutants of *Escherichia coli*. II. Streptolydigin resistance and its relation to rifampicin resistance. *Molecular and General Genetics* **121**, 181-196.
- KESSLER, C. & HARTMANN, G. R. (1977). The two effects of rifampicin on the RNA polymerase reaction. *Biochemical and Biophysical Research Communications* **74**, 50-56.
- LINN, T., LOSICK, R. & SONENSHEIN, A. L. (1975). Rifampicin resistance mutation of *Bacillus subtilis* altering the electrophoretic mobility of the β subunit of ribonucleic acid polymerase. *Journal of Bacteriology* **122**, 1387-1390.
- MALIK, V. S. & VINING, L. C. (1972). Chloramphenicol resistance in a chloramphenicol-producing *Streptomyces*. *Canadian Journal of Microbiology* **18**, 583-590.
- MEYENBURG, K. VON, NIELSEN, L. D., JOHNSEN, K., MOLI, S., SVENNINGSEN, B. & MIOZZARI, G. (1978). Reevaluation of the mode of action of streptolydigin in *Escherichia coli*: induction of transcription termination in vivo. *Antimicrobial Agents and Chemotherapy* **13**, 234-243.
- OVCHEVNIKOV, Y. A., MONASTYRSKAYA, G. S., GURIER, S. O., KALININA, N. F., SVERDLOW, E. D., GRAGEROV, A. I., BASS, I. A., KIVER, I. F., MOISEYEVA, E. P., IGUMNOV, V. N., MINDLIN, S. Z., NIKIFOROV, V. G. & KHEVIN, R. B. (1983). RNA polymerase rifampicin resistance mutations in *Escherichia coli*: sequence changes and dominance. *Molecular and General Genetics* **190**, 344-348.
- PÉREZ-GONZÁLEZ, J. A., VARA, J. & JIMÉNEZ, A. (1983). Acetylation of puromycin by *Streptomyces alboniger* the producing organism. *Biochemical and Biophysical Research Communications* **113**, 772-777.
- PIENDL, W. & BOCK, A. (1982). Ribosomal resistance in the gentamicin producer organism *Micromonospora purpurea*. *Antimicrobial Agents and Chemotherapy* **22**, 231-236.
- RABUSSAY, D. & ZILLIG, W. (1969). A rifampicin resistant RNA polymerase from *Escherichia coli* altered in the β subunit. *FEBS Letters* **5**, 104-106.
- SCHLEIF, R. (1969). Isolation and characterization of a streptolydigin resistant RNA polymerase. *Nature New Biology* **223**, 1068-1069.
- SIDDHIKOL, C., ERNSTOETZER, J. W. & WEISBLUM, B. (1969). Mode of action of streptolydigin. *Journal of Bacteriology* **99**, 151-155.
- SIPPEL, A. & HARTMANN, G. R. (1968). Mode of action of rifampicin on the RNA polymerase reaction. *Biochimica et biophysica acta* **157**, 218-219.
- SKINNER, R. H. & CUNDLIFFE, E. (1980). Resistance to the antibiotics viomycin and capreomycin in the *Streptomyces* species which produce them. *Journal of General Microbiology* **120**, 95-104.
- SKINNER, R. H. & CUNDLIFFE, E. (1982). Dimethylation of adenine and the resistance of *Streptomyces erythreus* to erythromycin. *Journal of General Microbiology* **128**, 2411-2416.
- SONENSHEIN, A. L., CAMI, B., BREVET, J. & COTE, R. (1974). Isolation and characterization of rifampicin-resistant and streptolydigin-resistant mutants of *Bacillus subtilis* with altered sporulation properties. *Journal of Bacteriology* **120**, 253-265.
- THOMPSON, J. & CUNDLIFFE, E. (1980). Resistance to thiostrepton, siomycin and sporangiomycin in actinomycetes that produce them. *Journal of Bacteriology* **142**, 455-461.
- THOMPSON, C. J. & GRAY, J. (1983). Nucleotide sequence of a streptomycete aminoglycoside phosphotransferase gene and its relationship to phosphotransferases encoded by resistance plasmids. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 5190-5194.
- THOMPSON, J., CUNDLIFFE, E. & STARK, M. (1982). On the mode of action of berninamycin and the mechanism of resistance in the producing organism, *Streptomyces bernensis*. *Journal of General Microbiology* **128**, 875-884.
- VINING, L. C. (1979). Antibiotic tolerance in producer organisms. *Advances in Applied Microbiology* **25**, 147-168.
- WALKER, M. S. & WALKER, J. B. (1970). Streptomycin biosynthesis and metabolism. *Journal of Biological Chemistry* **245**, 6683-6689.

- WATANABE, S. & TANAKA, K. (1976). The effect of rifampicin on *in vitro* RNA synthesis of *Streptomyces mediterranei*. *Biochemical and Biophysical Research Communications* **72**, 522-529.
- WEHRLI, W., KNUSEL, F., SCHMID, K. & STAEHELIN, M. (1968). Interaction of rifamycin with bacterial RNA polymerases. *Proceedings of the National Academy of Sciences of the United States of America* **61**, 667-673.
- YURA, T. & IGARASHI, K. (1968). RNA polymerase mutants of *Escherichia coli*. I. Mutants resistant to streptovaricin. *Proceedings of the National Academy of Sciences of the United States of America* **61**, 1313-1319.