

STIMULATION OF THE PRODUCTION
OF MACROLIDE ANTIBIOTICS BY
MAGNESIUM PHOSPHATE AND
RELATED INSOLUBLE MATERIALS*

Sir:

Biosynthesis of antibiotics is often regulated by carbon catabolites, nitrogen catabolites, phosphates and other metabolites^{1,2)}. Recent advances revealed the biochemical bases of the carbon catabolite regulation^{3,4)} and the phosphate regulation^{5,6)}. High production of antibiotics has generally been achieved by cultivating the producing organisms in media containing slowly utilized carbon and/or nitrogen sources or under conditions which allow a slow supply of these nutrients¹⁾. Although several papers suggested nitrogen catabolite regulation in the biosynthesis of antibiotics^{1,2)}, the importance and the methods for its relief have not been discussed in relation to antibiotic production. Recently, it was reported⁷⁾ that microbial conversion of glycine to L-serine was stimulated by magnesium phosphate (MgP), and the relevance

of the stimulation to the nitrogen catabolite regulation was postulated, based upon the fact that the ammonium ion concentration in the culture broth was depressed in the presence of MgP. In view of this observation it was of interest to examine if MgP might exert a similar effect on the production of antibiotics. The marked stimulation of leucomycin production by MgP has been reported from this laboratory⁸⁾. The present communication describes the stimulation of the production of other macrolide antibiotics, spiramycin and tylosin, in the presence of MgP and related insoluble materials.

Streptomyces kitasatoensis KA-429 (a mutant strain of the original leucomycin producer NRRL 2486), *S. ambofaciens* ATCC 23877 (a spiramycin producer), and *S. fradiae* KA-427 (a tylosin producer) were cultivated at 27°C with reciprocal shaking (240 strokes/min) in a large test tube (20 cm × 2 cm) containing 10 ml of a complex medium or chemically defined medium. The compositions of these media are given in the footnote of Table 1. Antibiotic titer was estimated by conventional paper disc method using

Table 1. Stimulation of the production of the macrolide antibiotics, leucomycin, spiramycin and tylosin, by magnesium phosphate and related materials.

Antibiotic	Addition		Basal medium No.	Antibiotic produced (μg/ml)	
	Compound	Amount (%)		No addition	Addition
Leucomycin	Mg ₃ (PO ₄) ₂ ·8H ₂ O (=MgP)	1	1	270	900
	NH ₄ MgPO ₄ ·6H ₂ O ^{a)}	1	1	85 ^{b)}	840 ^{a)}
	Mg ₃ (PO ₄) ₂ ·8H ₂ O	1	2	480	2,600
	"	1	3	<10	960
Spiramycin	Mg ₃ (PO ₄) ₂ ·8H ₂ O	3	1	<10	150
	Ca ₃ (PO ₄) ₂	1	1	<10	165
	NH ₄ MgPO ₄ ·6H ₂ O ^{a)}	1	1	<10 ^{b)}	270 ^{a)}
	Ca ₃ (PO ₄) ₂	0.5	4	200	400
	Na ₃ P ₂ W ₁₂ O ₄₃ ·18H ₂ O	1	4	200	340
Tylosin	NH ₄ MgPO ₄ ·6H ₂ O ^{a)}	1	1	86 ^{b)}	340 ^{a)}

a) NH₄MgPO₄·6H₂O was used as sole nitrogen source.

b) Ammonium lactate was used as sole nitrogen source.

c) Medium 1 contained: 3 % glycerol, 0.5 % glucose, 1 % ammonium lactate, 0.1 % MgSO₄·7H₂O, 0.01 % K₂HPO₄, 0.5 % CaCO₃ and 1 ml of trace metal solution* per liter, pH 7.2. *Trace metal solution contained (each 1 g/liter) FeSO₄·7H₂O, MnCl₂·4H₂O, ZnSO₄·7H₂O, CuSO₄·5H₂O and CoCl₂·2H₂O.

Medium 2 contained: 2 % glucose, 0.5 % peptone, 0.3 % dried yeast cells, 0.5 % meat extract, 0.5 % NaCl and 0.3 % CaCO₃, pH 7.3.

Medium 3 contained: 1 % glycerol, 1 % soybean meal, 0.3 % NaCl, pH 6.5.

Medium 4 contained: 1 % glucose, 1 % NaNO₃, 0.5 % NaCl and 0.3 % CaCO₃, pH 7.0.

* Bioconversion and biosynthesis of 16-membered macrolide antibiotics. XIX. A part of this study was presented at the 100th Annual Meeting of the Pharm. Soc. Japan, 1980 (Tokyo).

Sarcina lutea PCI 1001 as test organism (nutrient agar, 37°C, 20 hours). The antibiotic titers were assayed daily. As summarized in Table 1, the production of the three macrolide antibiotics was enhanced 2- to 100-fold in the presence of MgP or related substances. The extent of stimulation depended on the basal media, the microorganisms used and the compounds added. The major component of the antibiotics (leucomycin, A₈, spiramycin II (and III) and tylosin) remained the same in the presence or in the absence of the additions as analyzed by thin-layer chromatography described in the previous papers⁹⁻¹¹. The increase in mycelial growth was also notable especially in chemically defined media: it nearly doubled in the presence of MgP for leucomycin production; on the other hand, the increase in the antibiotic titers was always more than 2-fold, suggesting a net increase of the antibiotic formation.

In the leucomycin fermentation, the ammonium ion concentration of the supernatant of the MgP-supplemented culture was lowered in both chemically defined⁹ and complex media, whereas the sediments contained considerable amounts of ammonia. In separate experiments, high concentrations of ammonium ions were found to inhibit the leucomycin production, while pH control of the culture medium slightly increased the leucomycin titers.

From these results, it is supposed that a limited supply of ammonium ions caused by MgP led to the high production of leucomycin as well as spiramycin and tylosin. In support of this view ammonium magnesium phosphate (NH₄MgPO₄ · 6H₂O, an insoluble nitrogen source) also gave rise to an elevation of the production of these antibiotics (Table 1).

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