# THE EFFECT OF NALIDIXIC ACID, RIFAMPICIN AND CHLORAMPHENICOL ON THE MITOMYCIN C - INDUCED SYNTHESIS OF A BACTERIOCIN OF SERRATIA MARCESCENS.

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SUMMARY

Nalidixic acid inhibits the mitomycin C-induced production of the bacteriocin marcescin B in S. marcescens HY indicating that DNA replication is an essential step for its production. Using nalidixic acid, rifampicin and chloramphenicol the temporal sequence of macromolecular synthesis following mitomycin C induction was determined. Marcescin B is effectively produced if (i) DNA synthesis is allowed during the first 30 min, (ii) RNA synthesis can proceed up to about 60 min and (iii) protein synthesis continues for about 3 hours.

## INTRODUCTION

Bacteriocins of various species of bacteria are coded by plasmids (1). In a bacteriocinogenic culture there is little spontaneous bacteriocin production which can be induced by mitomycin C (2). In earlier investigations of the colicinogenic factors E1 and E2 (3, 4) it was suggested that induction raises the number of plasmid copies per cell and thereby increases the bacteriocin production by a 'gene-dosage-effect'. Recent studies (5, 6) in which the plasmid DNA content of colicinogenic cells was determined after induction did not support this hypothesis. On the other hand in an R-factor containing strain of Escherichia coli it was found that the degree of resistance to antibiotics was correlated with the number of R-factor copies per cell (7). A similar observation was made in mutants of Serratia marcescens HY with increased

spontaneous bacteriocin production. Such mutants contained higher amounts of plasmid DNA than the wild type strain (8).

The present communication describes the molecular events in S. marcescens HY following mitomycin C induction of marcescin B.

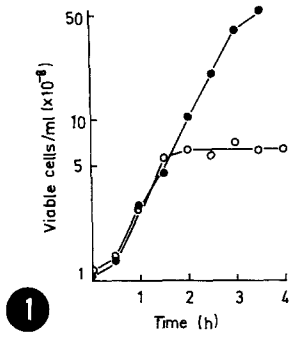
## MATERIALS AND METHODS

Bacterial strain: Serratia marcescens HY, W 831/3. Growth conditions, media, mitomycin C induction, and bacteriocin tests were as described elsewhere (9).
Measurement of the synthesis of DNA, RNA and protein: Cultures of W 831/3 in a shaking water bath (30°C) were grown to a cell titre of $2 \times 10^8$/ml and then diluted 1 : 4 in fresh YPM. ($^3$H)thymidine, ($^3$H)uridine or ($^3$H)leucine was added as appropriate to a final concentration of 0.1 μCi/ml, and the incubation was continued. Samples of 0.5 ml were withdrawn, added to 0.5 ml of ice-cold trichloroacetic acid (TCA)*, mixed rapidly, and cooled with ice for 30 min. The precipitates were collected on glass filters (Whatman GF/C), and washed succesively with 5 ml cold 5 % TCA and 5 ml cold 1 % acetic acid. Filters were dried in glass tubes for 15 min at 100°C. The radioactivity content of the filters was determined after addition of 5 ml scintillant (0.5 % PPO and 0.022 % POPOP in toluene) in a liquid scintillation counter.
More information is given in the legends to the figures.

## RESULTS

Mitomycin C induction of logarithmically growing S. marcescens HY stopped growth but did not kill the cells (Fig. 1). This shows that the concentration of mitomycin C used (1 μg/ml) is bacteriostatic but not lethal for S. marcescens. Marcescin B production under these conditions yields about 400 AU/ml after 5 h (9). Since the viable cell count is constant after induction, this might indicate that marcescin B production is not a lethal event if one does not assume that only a minor fraction of cells was induced. When

*Footnote: Abbreviations used are NA (Nalidixic acid), CMP (Chloramphenicol), RIF (Rifampicin), TCA (Trichloroacetic acid).



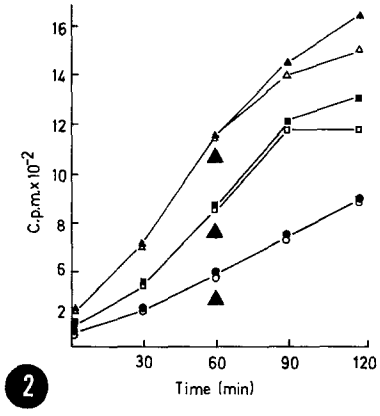




Figure 1
Growth of S. marcescens after induction. Two 10ml cultures in YPM were grown at 30°C in a shaking water bath. After 90min one culture was induced with mitomycin C (1µg/ml). The viable cell titre was determined by plating on NB agar. Control (●). Induced culture (O).

Figure 2
Synthesis of macromolecules after induction. The incorporation of ($^3$H)uridine (▲,△), ($^3$H)thymidine (■,□), and ($^3$H)leucine (●,O) was measured as described in the Methods. Mitomycin C (1µg/ml) was added at 60min (open symbols). Control (solid symbols).

the synthesis of macromolecules after induction was measured (Fig. 2) it was observed that DNA synthesis continued at a normal rate and finally stopped after about 30 min. Synthesis of protein was unimpaired whereas RNA synthesis was slightly reduced. We wanted to know whether the DNA synthesis observed after mitomycin C induction is of importance for the production of marcescin B.

As shown in Fig. 3 NA immediately blocks DNA synthesis early and late after induction. The production of marcescin B was inhibited when NA was added during a period of 30 min after induction (Table 1) which coincides with the period of unimpaired DNA synthesis observed after addition of mitomycin C (Fig. 2). This is taken as evidence for an essential role of DNA synthesis for the induced production of marcescin B.

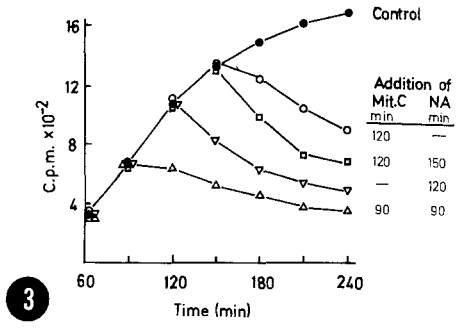
Table 1

| t (min after induction) | Marcescin B production (AU/ml) of mitomycin C-induced cultures after addition of NA | RIF | CMP |
|---|---|---|---|
| 0 | 50 | 50 | 10 |
| 10 | 50 | - | - |
| 20 | 75 | - | - |
| 30 | 150 | 75 | 10 |
| 60 | 450 | 350 | 10 |
| 90 | - | 450 | 50 |
| 120 | - | 500 | 100 |
| 180 | - | - | 450 |

Influence of NA, RIF, and CMP on the production of marcescin B. Cultures of S. marcescens (10ml) were grown in YPM at 30°C. At $5 \times 10^8$ cells/ml (t=0) the cultures were induced with mitomycin C (1µg/ml) and NA (10µg/ml), RIF (100µg/ml), and CMP (100µg/ml), respectively, were added at the times indicated. Cultures were further incubated for 4h. Cultures were then sonicated with a Branson Sonifier (2min at 90W) and centrifuged for 10 min at 43300 xg. For the titration of marcescin B in the supernatant, aliquots (0.01ml) of serial dilutions were placed on agar plates, seeded with E.coli W4110. The reciprocal of the greatest dilution which inhibited growth of the indicator lawn was defined as the number of arbitrary units per ml (AU/ml). Addition of NA, RIF, and CMP alone did not raise marcescin B production above the background level of about 50AU/ml, whereas mitomycin C-induction yielded about 500AU/ml.

Since marcescin B is a protein molecule, synthesis of RNA and protein are necessary for its production (9). The sequence and the length of time required for these processes was studied by means of specific inhibitors.

RNA synthesis was inhibited by RIF (11). In S. marcescens a concentration of 100 µg/ml is sufficient to stop RNA synthesis immediately but allows the synthesis of DNA and protein for at least 1 h at a reduced rate (unpubl. results).



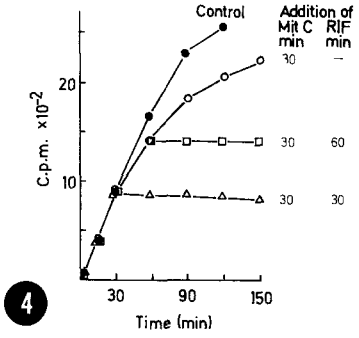




Figure 3
Effect of NA on DNA synthesis during induction. DNA synthesis was measured by incorporation of ($^3$H)thymidine into acid insoluble material as described in the Methods. Concentrations of mitomycin C were 1μg/ml and of NA 10μg/ml, resp..

Figure 4
Effect of RIF on RNA synthesis during induction. RNA synthesis was measured by incorporation of ($^3$H)uridine into acid insoluble material as described in the Methods. Mitomycin C concentrations used were 1μg/ml and of RIF 100μg/ml, resp..

Even after mitomycin C induction RIF inhibited RNA synthesis (Fig. 4) but reduced the amount of marcescin B produced only when it was added to the cells during the first 60 min after induction (Table 1).

CMP (12) at a concentration of 100 μg/ml blocked protein synthesis in S. marcescens completely, even after induction (Fig. 5). However, marcescin B production was only inhibited up to 2 h after induction (Table 1).

## DISCUSSION

After induction of marcescin B by mitomycin C, DNA synthesis in S. marcescens HY continued for about 30 min and has been shown to be an essential step for the production of marcescin B. This synthesis might be a result of repair and/or continued replication of chromosomal DNA or might be

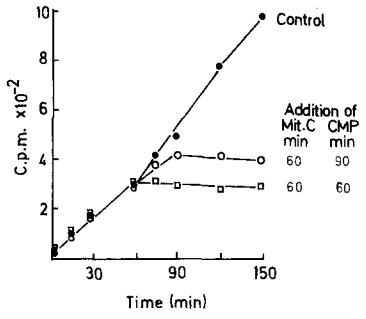



Figure 5
Effect of CMP on protein synthesis during induction. Protein synthesis was measured by incorporation of ($^3$H)leucine into acid insoluble material as described in the Methods. Concentrations of mitomycin C were 1µg/ml and of CMP 100µg/ml, resp..

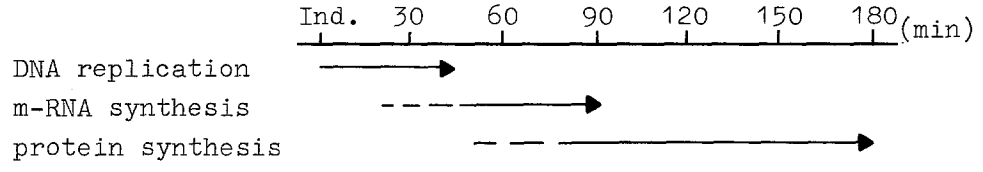
caused by the start of plasmid DNA replication. We favour the latter interpretation because preliminary experiments indicated that the content of supercoiled plasmid DNA in induced cells of S. marcescens was increased 2-3 fold as compared to the uninduced control. Therefore, according to the 'gene-dosage-hypothesis', inhibition of plasmid DNA replication should result in a decrease of marcescin B production. This, in fact, was found.

Recently it has been reported that NA also reduces the rate of S13 m-RNA synthesis (13). Inhibition of RNA synthesis by NA does not seem to be the reason for the reduction of marcescin B production since RNA synthesis in S. marcescens is only slightly effected by NA. Furthermore, NA and RIF should then inhibit marcescin B production during the same period after induction, but this is not the case.

Addition of RIF reduced drastically marcescin B production within about 60 min after mitomycin C induction. It has been shown that RIF interferes with the DNA replication

of Col-factor E1 by inhibition of RNA-primer synthesis (14). The extended RIF sensitive phase of marcescin B production as compared to the shorter NA sensitive phase is consistent with the interpretation that RIF inhibits m-RNA synthesis under our conditions (Table 1). However, our experiments do not exclude the possibility that RIF also inhibits DNA synthesis at an early stage after induction.

In the experiments presented above the approximate temporal sequence of DNA, RNA and protein synthesis essential for mitomycin C-induced production of marcescin B has been determined and is summarized in the following scheme:




Similar results have been obtained with E. coli W3110 containing Col-factors E1-K30 or D-CA23 (unpubl. results).

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