Induction of Bacteriocins (Phage Tails) of *Proteus rettgeri* with Trimethoprim

Trimethoprim (TMP), 2,4-diamino-5 (3,4,5-trimethoxybenzyl)-pyrimidine, is a potent inhibitor of dihydrofolate reductases, causing deprivation of cellular tetrahydrofolate[1,2]. This drug inhibits deoxyribonucleic acid (DNA) and protein synthesis more extensively than ribonucleic acid synthesis in susceptible strains of *Escherichia coli*[3], a process that is reversed neither by the addition of thymidine alone[3] nor by the addition of purines and amino acids[4]. Very recently, TMP was shown to eliminate a resistance factor (R factor 1818) from a strain of *E. coli*; the 'curing' was interpreted as a result of the induction of thymineless conditions by TMP[5]. It is known that thymine deficiency promotes prophage induction in lysogenic bacteria[6,7].

We wished to examine the suitability of TMP for the induction of 2 bacteriocinogenic (b^+) strains of *Proteus rettgeri*[8], which elaborate phage tails following induction with mitomycin C (MC) (manuscript in preparation). For this purpose, the b^+ strains of P. *rettgeri* No. III and VI were exposed to 10, 5, 2.5, 1.25, and 0.63 µg/ml of TMP (TMP was a gift of Hoffmann-La Roche, Grenzach), 1 µg/ml of MC (Sigma Chemical Co., St. Louis USA), or no drug (control) in tryptic soy broth (TSB, Difco) and in SCASB, a defined medium[9]. The technique employed of induction at 32 °C was the same as described previously[8]; *P. rettgeri* strain LF 9i served as the indicator strain to determine the number of lethal units of bacteriocin/0.05 ml cell lysate. The minimal inhibitory concentrations of TMP against the two b^+ strains as well as against our control strain *E. coli* ATCC 25922 were determined utilizing a previously published microtiter procedure[10]; the cell inocula were adjusted to yield approximately 7.5×10^5 and 7.5×10^3 colony-forming units/ml at 0 time, respectively. Standardized disk susceptibility tests[11] were carried out with 25 µg TMP-sulfamethoxazole (TMP-

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Table I. Induction of b^+ *P. rettgeri* with trimethoprim

P. rettgeri b^+ strain No.	Induced with	Titer (against *P. rettgeri* LF 9i) SCASB	TSB
	Trimethoprim (µg/ml)		
III	10	0[a]	0
	5	0	20
	2.5	10	80
	1.25	20	160
	0.63	20	160
	0 (control)	10	0
	Mitomycin (µg/ml)		
	1	320	160
	Trimethoprim (µg/ml)		
VI	10	0	10
	5	0	40
	2.5	20	160
	1.25	40	160
	0.63	40	320
	0 (control)	0	0
	Mitomycin (µg/ml)		
	1	320	320

[a] Lethal units/0.05 ml.

Table II. Minimal inhibitory concentrations (MIC) of trimethoprim against b^+ *P. rettgeri* strains and control strain of *E. coli*

Strain	MIC (µg/ml)	
	7.5×10^5 cfu/ml at 0 min	7.5×10^3 cfu/ml at 0 min
P. rettgeri III	50	3.2
P. rettgeri VI	50	3.2
E. coli ATCC 25922	3.2	3.2

SMZ) disks (Nährböden und Chemie GmbH, Wesel) and Mueller-Hinton agar (MHA, Difco).

It was found that TMP, at a concentration over the range of 0.63 to 2.5 µg/ml, induced synthesis of *P. rettgeri* bacteriocins at titers comparable to those obtained with 1 µg/ml of MC (Table I); however, in SCASB, the defined medium, TMP yielded far lower titers of bacteriocins. Both b^+ *P. rettgeri* strains proved resistant against TMP (Table II); no detectable inhibition zones were obtained with TMP-SMZ disks on MHA; the control strain of *E. coli* yielded inhibition zones that measured 29 mm in diameter. It may be added that TMP was found to induce elaboration of group A bacteriocins (phage tails) of subgroups I and II of *Serratia marcescens*[12] as well, though at lower titers as compared with our two b^+ strains of *P. rettgeri* (unpublished results).

At the moment, we have no explanation for the seemingly paradoxical finding, namely that TMP at subinhibitory concentrations is an efficient inducer of defective prophage (phage tail bacteriocins) in b^+ strains of *P. rettgeri*, that are resistant to this drug, as determined with conventional antimicrobial susceptibility tests. Most likely, the 2 strains of *P. rettgeri* examined are not completely, but rather 'relatively', resistant against TMP, thus allowing for subtle interactions between the drug and the cells' metabolism, with, among other(s), resultant production of phage tails.

Zusammenfassung. Zwei bacteriocinogene (Phagenschwänze) Stämme von *Proteus rettgeri* konnten mit subinhibitorischen Konzentrationen von Trimethoprim (0.63 bis 2.5 µg/ml) induziert werden.

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Antagonism Between Plant Growth Regulators in Pollen Tube Elongation of *Calotropis procera*

Abscisic acid is an inhibitory hormone found to be involved in control of growth regulation together with other naturally occurring growth promoters[1]. Studies with synthetic abscisic acid have shown that applications of the chemical caused inhibition of germination, cessation of extension growth, leaf senescence and formation of resting buds in woody species[2]. But contrary to these observations, recently abscisic acid has been reported to induce pollen tube elongation in *Calotropis procera*[3]. The possible role of abscisic acid in the regulation of pollen tube growth led us to study its interaction with growth-promoting hormones when applied in combination with the abscisic acid in culture medium exogenously.

Isolated pollinia, dissected out from fully opened flowers of *C. procera* were incubated in hanging drop cultures consisting of basal medium, 0.3 *M* sucrose (analar) with or without abscisic acid and other growth hormones (IAA, GA_3 and kinetin) separately and in combination with abscisic acid. The pollinia were allowed to germinate under the conditions of light received from two 40-watt fluorescent lamps hanging at a distance of 1 m. Each hanging drop culture contained 3 pollinia, and 3 replicates for each were run. The mean length of pollen tubes were measured after 24 h of incubation. In order to avoid genetical discrepancies, pollinia were obtained from flowers of one and the same plant.

Growth of pollen tube in relation to different plant growth regulators

Treatment	Length of pollen tubes (µm)
Control	660 ± 140
ABA (1.0)	1110 ± 120
IAA (1.0)	909 ± 75
IAA (0.1)	872 ± 120
GA_3 (10.0)	1180 ± 180
GA_3 (1.0)	1050 ± 135
Kinetin (5.0)	740 ± 130
ABA + IAA (1 + 1)	360 ± 40
ABA + IAA (1 + 0.1)	285 ± 60
ABA + GA_3 (1 + 10)	393 + 150
ABA + GA_3 (1 + 1)	480 ± 150
ABA + Kinetin (1 + 5)	440 ± 30

The number in parenthesis indicates concentration of the chemical in ppm.

It is evident from the results presented in the Table that all the growth hormones tested promoted the pollen tube elongation. The maximum promotion of pollen tube elongation was observed in basal medium containing 10 ppm GA_3. The growth hormones when applied alone caused increase in tube length, but in combination with abscisic acid they acted antagonistically and greatly inhibited the elongation of the tube. It may be postulated that some sort of physiological antagonism exists between abscisin and other growth hormones studied as regards their role in pollen tube elongation of *C. procera*. Such antagonism between abscisin and GA_3 or abscisin and kinetin has been reported earlier in relation to plant growth[1].

Zusammenfassung. Während Abscisinsäure allein das Pollenschlauchwachstum in *Calotropis procera* stimuliert, hebt sie in binärer Kombination mit Auxin, Gibberellinsäure (GA_3) und Kinetin deren fördernde Wirkung teilweise auf.

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