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The Influence of Chloramphenicol on Colicine Synthesis

Wpływ chloramfenikolu na syntezę kolicyn

In the culture of colicinogenic bacteria only few cells are capable of producing the antibiotic. It is possible to increase the number of bacteria synthesizing colicines by treatment with some chemical or physical agents. The production of colicines can be induced by UV light or X-ray irradiation, as well as by some chemical agents e.g. peroxides, mustards or mitomycin. The process of colicine induction is caused by derepression of the synthesis of the antibiotic.

In studies on the induction of colicines further progress was achieved by the method of lacunae (8).

It was found that C^{14}-thymidine was more intensively incorporated into the UV irradiated colicinogenic bacteria than into noncolicinogenic microorganisms (2). The DNA synthesis in the colicinogenic strain *Escherichia coli* was more intensive than that in the noncolicinogenic strain. About 100 copies of the colicinogenic factor were found in each induced cell. A considerable decrease in the number of colicinogenic factors was observed after treatment with mitomycin.

MATERIALS AND METHODS

Strains. In the present studies the following *E. coli* colicinogenic strains from Dr Fredericq collection were used: CA7, CA18, CA23, CA31, CA38, CA42, CA46, CA53, CA58, CA62, K235 and P14. *E. coli* C118 or ROW was employed as the indicator culture.

The induction of colicines. A modification of the Lichodied method was used in the present studies. 18 hr. culture of the colicinogenic strains was diluted with 10 volumes of warm nutrient broth and grown for 2 hr., at 37°. Next the bacteria were irradiated for various periods of time with UV light, using a 30 W Phillips lamp, at a distance of 50 cm. Immediately after the irradiation bacterial cultures were diluted with an equal volume of warm nutrient broth and incubated for 2 hr., at 37°.

The UV irradiation and postincubation were done in the dark. Afterwards, the bacteria were treated with chloroform and shaken for 10 min. After centri-

fugation at 5000 r.p.m. for 10 min. the supernant was used for titration of colicine. Various dilutions of colicine were spotted by the calibrated loop on the layer agar which contained indicator bacteria. The activity of colicines was determined after 6 hr. incubation, at 37°.

RESULTS

The induction of colicines of *E. coli* strains was tested by the Lichodied method. The concentration of colicine was estimated in nonirradiated cultures prior to UV irradiation, immediately after irradiation, and after 2 hr. incubation followed by irradiation. Strains CA7, CA18, CA23 and CA53 showed a considerable induction while strain CA31 was induced to a lesser degree. A high titre of colicines was observed after 2 hr. incubation following UV irradiation (Table 1). Further experiments were carried out on the induction of *E. coli* CA7 producing colicine V. *E. coli* CA7 culture grown for 105 min was irradiated with UV light for 30, 60, 90 and 120 seconds. A nonirradiated culture was employed as control. The results are presented in Table 2.

Table 1. The induction of colicines

Dilution of colicines / Strains	Nonirradiated			Irradiated, without incubation			Irradiated, with incubation		
	0	5	25	0	5	25	0	5	25
CA7	++	+	—	+++	++	+	CL	CLS	+++
CA18	+++	++	+	+++	+++	+	CL	CLS	+++
CA23	+	—	—	+	—	—	CL	CLS	+++
CA38	+++	++	—	+++	++	+	+++	+++	++
CA42	+	—	—	+	—	—	++	+	—
CA46	++	+	—	+++	++	—	+++	+++	—
CA53	+++	++	—	CLS	CLS	+++	CL	CL	CLS
CA62	+++	++	+	+++	++	+	+++	++	+
P14	CLS	CLS	+++	CLS	CLS	+++	CL	CLS	CLS
K235	CLS	CLS	+++	CLS	CLS	+++	CL	CL	CL

Explanation: CL — confluent lysis, CLS — semiconfluent lysis, +++, ++, + — various degrees of partial lysis

THE INFLUENCE OF CHLORAMPHENICOL (CM) ON THE SYNTHESIS OF COLICINE V

CM was added to the culture of *E. coli* CA7 after irradiation for 90 seconds. The UV irradiated bacteria were treated with CM for 2 hours. Final concentrations of CM were as follows: 0.1, 0.5, 1.0, 2.5, 5.0, 7.5,

10.0 μg/ml. The results are presented in Table 3. In highest concentrations of CM (7.5 or 10.0 μg/ml) the synthesis of colicine was completely inhibited. In low concentrations of CM (0.1 or 0.5 μg/ml) no effect of the antibiotic on the production of colicine was observed.

Table 2. The effect of UV irradiation on the induction of colicine V

Dilution of colicine	Time of irradiation in seconds				
	0	30	60	90	120
0	+	CL	CL	CL	CL
6	—	CLS	CL	CL	CL
36	—	+	CLS	CLS	CLS

For explanation see Table 1

In further studies the influence of pretreatment of *E. coli* with CM on the UV induction of colicine V was studied. The effect of pretreatment with CM depended on the concentration of CM as well as on the time of pretreatment with CM.

Table 3. The influence of CM concentration on the synthesis of the colicine induced

Dilution of colicine	Controls not irradiated without CM	Concentration of CM in μg/ml						
		0.1	0.5	1	2.5	5	7.5	10
0	CL	CL	CL	CLS	+++	+	—	—
6	CLS	CLS	CLS	+++	++	—	—	—
12	+++	+++	++	+++	++	—	—	—
CM		—	—	—	—	—	—	—

For explanation see Table 1

DISCUSSION

Only some colicinogenic strains can be induced by UV light. Some authors suggest that the inducibility of the strains depends on the type of the colicine produced. L i c h o d i e d found that the strains synthesizing colicine B or D were inducible, while those producing colicine V, I, G and H proved noninducible. J a c o b, W o l l m a n and S i m i n o v i t c h did not find any correlation between the type of colicine and inducibility.

Table 4. The influence of pretreatment of *E. coli* CA7 with CM on the induction of colicine V

Concentration of CM in μg/ml	Time of treatment	Dilution of colicine		
		0	6	12
10	120	—	—	—
	90	—	—	—
	60	—	—	—
	30	+++	+	—
7.5	120	—	—	—
	90	+	—	—
	60	+++	+	—
	30	+++	++	—
5	120	+++	+	—
	90	+++	++	—
	60	CLS	+++	+
	30	CLS	+++	+
1	120	CLS	++	+
	90	CLS	++	+
	60	CL	++	+
	30	CL	+++	+
0.1	120	CL	+++	+
	90	CL	+++	+
	60	CL	++	+
	30	CLS	+	—
Control — not washed, without CM		CL	CLS	++
Control — washed, without CM		CL	+++	++

For explanation see Table 1

Our experiments support the results of Jacob and his associates. Apart from *E. coli* CA18 and *E. coli* CA23, strains of *E. coli* CA7 (colicine V) and *E. coli* CA53 were also induced by UV light. The colicine induction is assumed to depend on some host bacteria. Amati (1) demonstrated that colicine I factor was not inducible in *Salmonella typhimurium* LT2, however, it proved inducible after it had been transferred to *E. coli* K12. Ben-Gurion observed that the addition of CM (100 μg/ml) 12 minutes prior to UV irradiation promoted the induc-

tion of colicine E2. After UV induction an increase in the colicine synthesis was observed if the antibiotic was used in a low concentration (1 or 2 μg/ml).

In the present study the synthesis of colicine was observed to be completely inhibited when UV irradiated bacteria were treated with 7.5 μg/ml of CM. However, no stimulation of the colicine V induction was observed in a low concentration of CM.

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Wpływ chloramfenikolu na syntezę kolicyn

Streszczenie

Zbadano indukcję kolicyn przy pomocy promieniowania UV. Szczepy CA7, CA18, CA23 i CA53 wykazały wysokie miano kolicyn po indukcji, podczas gdy szczep CA31 był indukowany w słabszym stopniu. Miano kolicyn było najwyższe, jeżeli naświetlane szczepy hodowano przez 2 godz. po naświetleniu. W badaniach nad indukcją kolicyny V wykazano, że proces ten zależy od dawki promieniowania. Najwyższe miano kolicyn uzyskano przy naświetlaniu bakterii przez 60 sek. lub dłużej. Chloramfenikol w stężeniu 10 lub 7,5 μg/ml hamował syntezę kolicyny V. Przy stężeniu 1 μg/ml chloramfenikolu występowało częściowe zahamowanie syntezy kolicyny, jeśli chloramfenikol był dodany natychmiast lub po 30 min. po indukcji kolicyny.

Влияние хлорамфеникола на синтез колицина

Резюме

Исследовалась индукция колицинов при помощи радиации UV. Штаммы CA7, CA18, CA23 и CA53 обнаружили высокий титр колицинов после индукции, в то время как штамм CA31 был индуци-

рован в меньшей степени. Титр колицинов был самым высоким, если облученные штаммы росли через два часа после облучения. При исследованиях индукции колицина обнаружено, что этот процесс зависит от дозы радиации. Самый высокий титр получен при времени облучения бактерий 60 сек. или дольше. Хлорамфеникол концентрации 10 или 7,5 μg/ml тормозил синтез колицина V. При концентрации хлорамфеникола, равной 1 μg/ml, появлялось частичное торможение синтеза колицина, если хлорамфеникол добавлялся сразу или спустя 30 мин. после индукции колицина.

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