PROTECTION OF LYOPHILIZED *E. COLI* FROM OXYGEN BY COLICIN E1 TREATMENT

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1. Introduction

Lyophilized bacteria are susceptible to oxygen upon exposure to air [1–4]. The dried bacteria, exposed to air lose their ability to form colonies on nutrient media, and "die" in an exponential fashion. We found that upon reconstitution, these "dead" bacteria were inducible for beta-galactosidase and could incorporate [^{3}H] TdR into their DNA, but were not able to initiate a new cycle of DNA synthesis [5].

Colicin E1 adsorbed to membranal receptor of a sensitive *E. coli* [6] is known to "kill" these bacteria by blocking the initiation of DNA synthesis.

We assumed, that both colicin and oxygen exert their lethal effect through the same membranal structure, connected with the initiation of DNA synthesis. We then predicted, that colicin-treated bacteria after freeze-drying should become less sensitive to oxygen; in other words, colicin E1 should protect freeze-dried bacteria from the toxic effect of oxygen.

2. Materials and methods

2.1. *Bacteria*

a) A colicinogenic strain of *E. coli* Col. El. b) A thymine requiring strain of *E. coli* B, sensitive to colicin El.

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2.2. *Media*

a) L-broth containing per 1 liter of water: 10 g bacto trypton (Difco); 5 g yeast extract; 5 g NaCl; 1 g glucose. b) Solid medium, containing per 1 liter: 20 g bacto tryptose; 5 g NaCl; 5 g yeast extract; 2% bacto agar.

Preparation of colicin E1: Colicin E1 was prepared from Col E1 after induction by mitomycin C (1 μg/ml). The cells were centrifuged at 9,000 rpm for 20 min, and washed twice in TM buffer (10^{-2} M Tris, Mg^{2+} 10^{-4} M; [11]). The cells were frozen and thawed the next day, and were broken in the "Yeda Press" at 2,000 PSI, centrifuged in the Beckman Spinco ultracentrifuge at 120,000 *g* for 30 min. The supernatant was dialysed 3 times against TM buffer. The colicin preparation had a titer of 10^{-5}–10^{-6} as determined by the plaques method [12].

2.3. *Decay curves of colicin treated E. coli B T$^-$*

Colicin E1 was added to a culture of bacteria in the stationary phase, at a final titer of 10^{-2}; after 30 min at 37° the cells were harvested, washed 3 times in water and freeze-dried in ampules. At this stage, control cultures which were not treated with colicin contained 9×10^8 per ml viable bacteria, colicin treated bacteria had only 2×10^4 viable per ml. For experiments described below, these respective viabilities were taken as 100%. The ampules were broken open, and the dried bacteria were exposed to air at 28° and at relative humidity of 45% for various periods.

Following various treatments, pellets of bacteria were fixed in 2.5% glutaraldehyde, postfixed in 2%

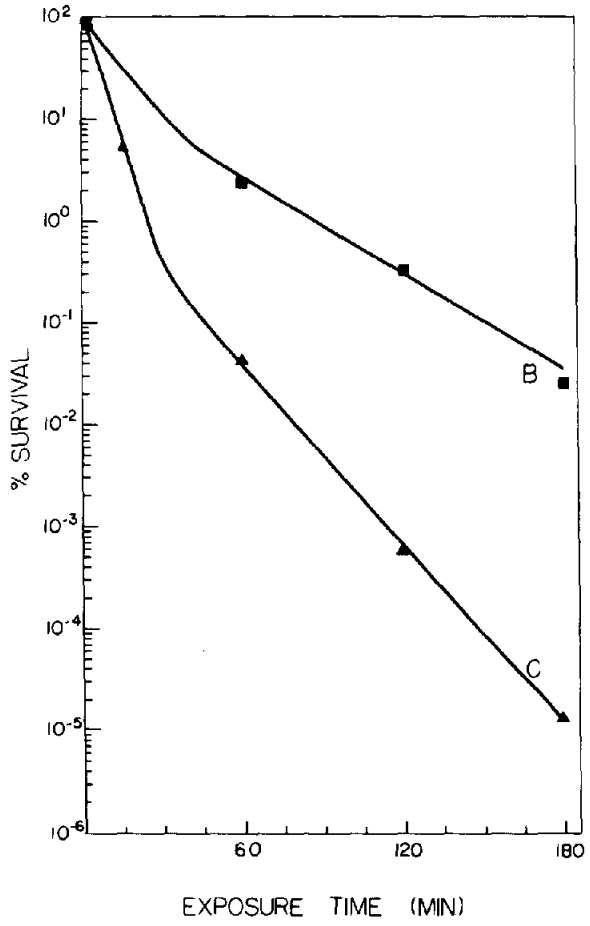

Fig. 1. Decay curves of lyophilized *E. coli* B T⁻ treated with colicin, upon exposure to air. B: *E. coli* B treated with colicin. 100% = 9×10^8 viable cells/ml; C: control, non treated cells. 100% = 2×10^4 viable cells/ml.

osmium tetroxide and embedded in epon. Preparations were sectioned, with Omu-2 Reichart ultramicrotome, stained with uranyl acetate and lead citrate, and observed in a JEM 100 B electron microscope.

3. Results and discussion

Decay curves of control bacteria treated with colicin, were studied. Fig. 1 depicts the results of such an experiment. Decay curves constants during the first and second hours of exposure were determined for the control bacteria (C), and for bacteria treated by the colicin (B). The decay constants of the controls were 3.56 and 1.80 during the 1st and 2nd hour, respectively. For the colicin treated bacteria they were 2.00 and 0.97. The colicin treatment thus reduced the decay constants of oxygen treated bacteria by a factor of two.

The results obtained in different experiments were reproducible and in all cases the adsorption of colicin E1 to bacteria before lyophilization reduced their decay curves constants during the first hour from 3.70 ± 0.25 to 1.80 ± 0.23, and during the second hour of exposure – from 2.08 ± 0.35 to 0.85 ± 0.20. These results indicate that colicin E1 probably protected from oxygen a sensitive site in the cells. This protective effect of colicin was specific. The specificity of protection by colicin is supported by the following data:

i) The protein concentration in the lyophilization medium did not exceed 0.1 μg/ml, which is too low

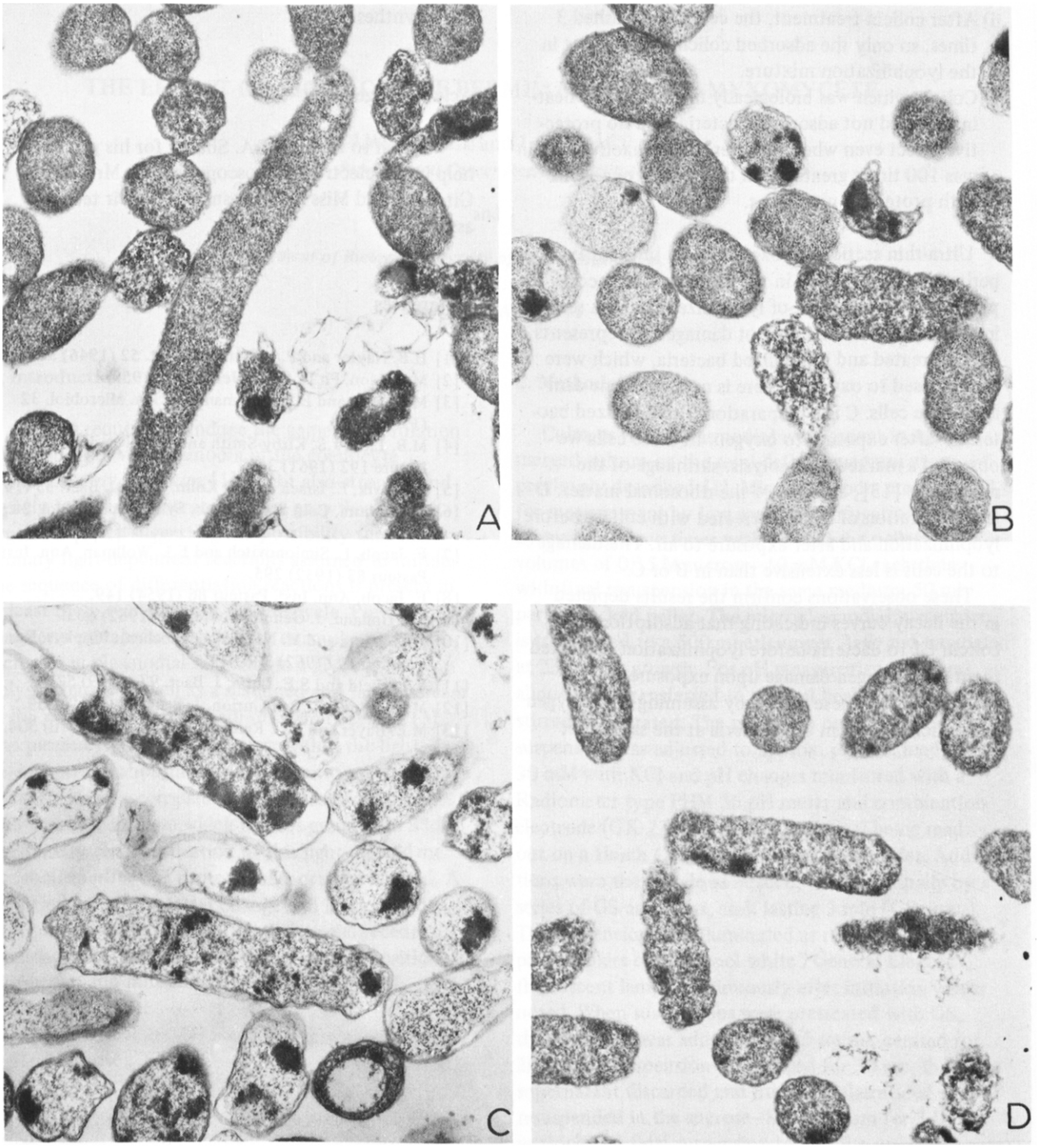

Plate 1. Ultra-thin sections of lyophilized *E. coli* B T⁻. A: Control of lyophilized bacteria; B: colicin treated and lyophilized bacteria; C: bacteria lyophilized and exposed to oxygen; D: bacteria, treated with colicin before lyophilization, and after exposure to oxygen. Magnification: × 18,000.

for non-specific protection.

ii) After colicin treatment, the cells were washed 3 times, so only the adsorbed colicin was present in the lyophilization mixture.

iii) Colicin which was biologically inactivated by heating and did not adsorb to bacteria, had no protective effect even when the titer of the inactive colicin was 100 times greater than that of native colicin with protective properties.

Ultra-thin sections of bacteria used in these experiments were studied in the electron microscope. In plate 1 – A is a control of lyophilized bacteria sealed in vacuo. The bacteria are not damaged. B represents colicin-treated and lyophilized bacteria, which were not exposed to oxygen. There is no significant damage to the cells. C is a preparation of lyophilized bacteria – after exposure to oxygen. In these cells we observed a marked plasmolysis, shrinkage of the membrane [13], and loss of the ribosomal matter. D is a preparation of bacteria treated with colicin before lyophilization and after exposure to air. The damage to the cells is less extensive than in B or C.

These observations confirm the results depicted in the decay curves indicating that adsorption of colicin E1 to bacteria before lyophilization protected them from oxygen damage upon exposure to air.

We interpret these results by assuming that oxygen and colicin E1 act in the bacteria at the same site, which is probably connected with the initiation of DNA synthesis.

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References

[1] H.B. Naylor and P.A. Smith, J. Bact. 52 (1946) 565.
[2] M.B. Lion, Ph.D. thesis, Jerusalem (1959).
[3] M.B. Lion and E.D. Bergmann, J. Gen. Microbiol. 32 (1963) 321.
[4] M.B. Lion, J.S. Kirby-Smith and M.L. Randolph, Nature 192 (1961) 34.
[5] O. Novik, E. Israeli and A. Kohn, J. Appl. Bact. 35 (1972) 1.
[6] M. Nomura, Cold Spring Harb. Symp. Quant. Biol. 28 (1963) 315.
[7] F. Jacob, L. Simionovitch and E.L. Wollman, Ann. Inst. Pasteur 83 (1952) 295.
[8] F. Jacob, Ann. Inst. Pasteur 86 (1954) 149.
[9] I.B. Holland, J. Gen. Microbiol. 29 (1962) 603.
[10] M. Nomura and M. Nakamura, Biochem. Biophys. Res. Commun. 7 (1962) 306.
[11] K.L. Field and S.E. Luria, J. Bact. 97 (1969) 57.
[12] M. Elgat and R. Ben-Gurion, J. Bact. 38 (1969) 359.
[13] M.E. Bayer and C.C. Remsen, J. Bact. 101 (1970) 304.