LYSOGENY AND TOXINOGENY IN BACILLUS CEREUS

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Culture filtrates of many strains of Bacillus cereus contain a variety of enzymes and materials exhibiting toxic effects on laboratory animals (Slein and Logan, 1962; Molnar, 1962). The recent evidence that lysogeny and toxin production are interdependent in diphtheria bacilli (Barksdale, et al., 1961) and in some staphylocci (Blair and Carr, 1961) prompted an investigation of the possible role of lysogeny in toxinogeny of B. cereus. The following results demonstrate that a toxin-producing strain of B. cereus is lysogenic and can be induced by UV irradiation. Pronounced increases in phosphatasemia factor (Slein and Logan, 1962), lecithinase and edema-producing factor (Molnar, 1962) coincide with UV-induced lysis of the cell and release of large numbers of bacteriophage particles.

Strain 6464 of B. cereus was employed throughout. The organism was grown in the casamino acids medium as described by Thorne, Molnar and Strange (1960) except that 0.1 M phosphate buffer pH 7.6 was used and sodium bicarbonate and charcoal were omitted.

For irradiation studies, 0.1 ml of an overnight culture was added to replicate tubes containing 5 ml of fresh medium. These cultures were incubated at 37°C on a reciprocating shaker until the optical density at 650 mμ attained a value of 0.35 - 0.40 and the tube contents were pooled. Three ml of the pooled culture were added to pressed bottom Petri dishes and irradiated, with vigorous shaking, for 20 seconds at a distance of 14 inches from a GE germicidal lamp (15 watts). Two ml of the irradiated suspension were added to tubes containing 5 ml of fresh medium and the resulting cultures were reincubated at 37°C on a shaker.

At 0 time and every 30 minutes for 3 to 4 hours, the optical density of all tubes was measured and the contents of a single tube were filtered through a sintered glass filter. Such filtrates were then assayed for phospholipase, phosphatasemia factor, guinea pig edema factor, and plaque-forming units.

Phosphatasemia factor assays were kindly performed by Dr. M. W. Slein, employing the bone slice method developed by him (Slein and Logan, 1962). Phospholipase was measured by determining the optical density at 650 mμ developing after 18 hours' incubation with 5 per cent egg yolk broth. The optical densities were plotted as a function of the serial dilution of the filtrate and this line function was extrapolated to the control optical density. The graphically determined dilution at which the optical density equaled that of the control was reciprocated to yield a unitage value for phospholipase. Edema factor was assayed by intradermal injections into guinea pigs of serial dilutions prepared in gelatin-phosphate (Thorne, Molnar and Strange, 1960). Numbers of bacteriophage particles were determined by plating dilutions of filtrates on lawns of B. cereus, strain 8569, by the methods outlined by Thorne (1962). The results of a typical experiment are presented in Table I. Although there are individual variations in activities found in filtrates from separate tubes, the data clearly show that there is a close correlation between phage-mediated lysis and extracellular concentration of the various activities assayed. Unirradiated cultures fail to show any sudden release of these enzymes and/or toxin. Furthermore, heavily irradiated cultures that show no optical density increase following irradiation likewise produce no extracellular bacteriophage particles and no detectable phospholipase. Disruption by sonic oscillation in the Raytheon 10-KC oscillator of cultures possessing equal optical density (0.16 - 0.22) released no bacteriophage particles, no edema factor, and a relatively small amount of phospholipase.

Of five other strains so far tested, all appear to be lysogenic, since an ultraviolet-induced lysis can be obtained readily. No indicator strains

TABLE I. Release of various toxic and enzymatic activities during post-irradiation lysis of B. cereus strain 6464 in casamino acids medium at pH 7.6.

Post-UV	Experiment 1				Experiment 2	
time, min	O.D. 650 mμ	PL[1] titer	PFU[2] titer	PF[3] activity	O.D. 650 mμ	EF[4] titer
0	.195	< 2	6×10^4	15	.189	< 1-2
30	.255	< 2	1.9×10^3	-	.238	-
60	.325	< 2	1.4×10^6	26	.310	-
90	.380	92	-	-	.390	1-8
120	.405	300	3.9×10^6	324	.380	-
150	.399	380	-	-	.378	-
180	.361	380	1.2×10^8	845	.296	-
210	.277	380	3×10^7	-	.270	1-16
240	.223	380	1.6×10^7	583		
Sonic lysate	-	140	$< 10^2$	-	-	< 1-2

1. Units of phospholipase per ml of filtrate. 2. Number of plaque-forming units per ml of filtrate. 3. Relative activity of filtrates in releasing alkaline phosphatase from bone slices. 4. Edema-producing titer determined by intradermal injection into guinea pigs.

have been found that show plaques when exposed to filtrates of these lysed cultures, and it is currently uncertain whether this indicates defective prophages or superinfection immunity.

Strains of B. cereus produce in general the same toxins that occur in filtrates of B. anthracis cultures, although there are distinctive differences. Preliminary evidence indicates that toxin production by the Sterne strain of B. anthracis is not closely related to the lysogenic state.

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