On the Recovery of X-Irradiated Bacteria

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In a very recent communication [1] it has been shown that X-irradiated *E. coli* B bacteria are amenable to restoration with bacterial extract and that this phenomenon of recovery is dependent on the soluble rather than the ribosomal part of the extract. This recovery was found to be absent for irradiated bacteria treated with heated extract [1].

In an attempt to understand the mechanism of the recovery phenomenon I have studied a) the influence of various plating media on the recovery, b) the influence of prolonged heating on the sedimentation pattern of *E. coli* extract. Present communication contains the results of such study.

The bacteria used for the purpose were *E. coli* B. The preparation of bacterial sample and heating of the "Crude' extract of bacteria were done as described earlier [2]. Extract was heated at 52 °C for 1 hr. for this particular purpose. Cell-free extract was prepared in phosphate buffer from protoplasts formed by the method of LEDERBERG [3]. $Mg^{2\oplus}$ was not present in extract since the recovery of X-irradiated bacteria does not depend upon the presence of ribosomal part of extract [1]. Concentration of the extract was estimated with the help of its refractive index (determined by Abbe refractometer). The specific refractive increment of proton (1.88×10^{-3} decilitre/g) was assumed to be that for extract. Plating was done on 1. nutrient agar, 2. tris-agar and 3. salt free L-agar. These plating media were obtained by adding 1.5% agar respectively to nutrient broth, tris-glucose media [4] and to 1 *l* of growth media containing bacto-trypton 10 g, yeast extract 40 g, 50% (W/V) glucose 25 ml, 1 M $CaCl_2$ 2.5 ml. Sedimentation analysis was performed in a Spinco model E analytical ultracentrifuge with Schlieren optics. All runs were made at 50,740 rev/minute at room temperature. Sedimentation co-efficients were not calculated. The analysis was done on a purely qualitative basis.

Bacterial samples after exposure to X-rays were incubated for different periods at 37 °C in buffer containing bacterial extract at its optimal concentration (i. e., at concentration at which maximum increase in X-ray survivors, i. e., maximum recovery after a particular X-ray dose was obtained, this concentration of extract was found to be of the order of 1662.5 μg/ml, details of this was compiled in a previous paper, ref.: P. MUKHERJEE and S. B. BHATTACHARJEE, J. gen.

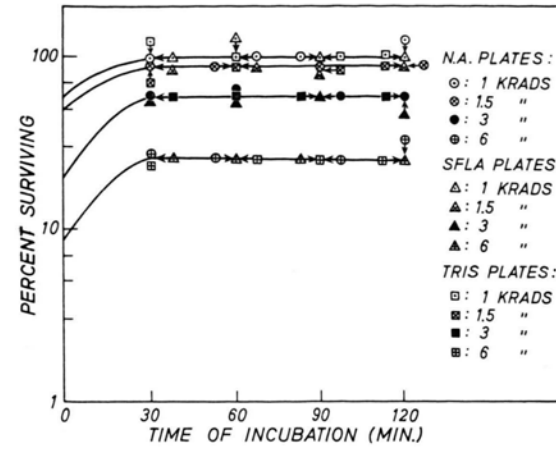

Fig. 1. Dependence of extract-promoted recovery in X-irradiated bacteria on various plating media.

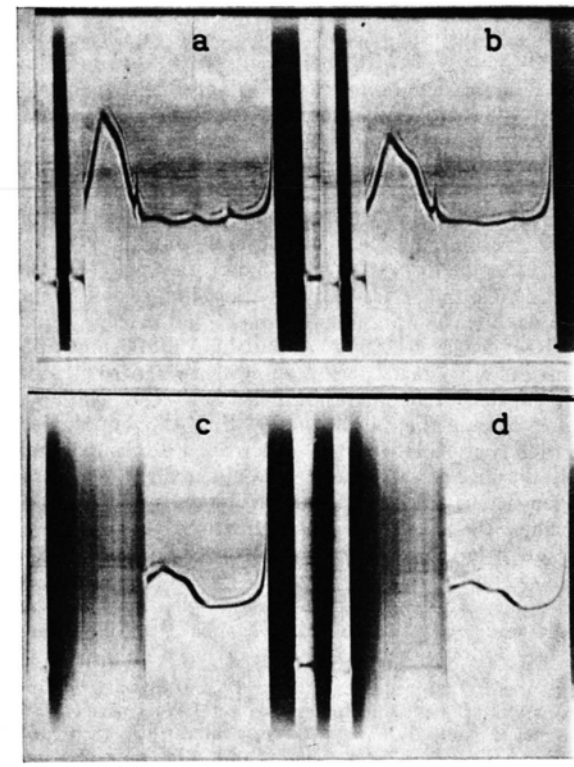

Fig. 2. Sedimentation pattern of the *E. coli* extract in buffer at a concentration of 2.2 mg/ml after centrifugation for 23 minutes (a) and 60 minutes (b) Sedimentatino pattern of extract after heating at 52 °C for 1 hr. and after centrifugation for 23 minutes (c) and 31 minutes (d).

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[1] P. MUKHERJEE and S. B. BHATTACHARJEE, J. gen. Microbiol., in press.

[2] P. MUKHERJEE and S. B. BHATTACHARJEE, J. gen. Microbiol. **60**, 233 [1970].

[3] J. LEDERBERG, Proc. nat. Acad. Sci. USA **42**, 574 [1956].

[4] O. MAALØE and P. C. HANAWALT, J. molecular Biol. **3**, 144 [1961].

Initial bacterial concentration per ml	Doses of X-ray used Krads	Plating media	Initial X-ray survivors	Final survivors after incubation in extract at minutes 30	60	90	120
$2 \cdot 10^8$	1	Trisagar	60	100	98	99	100
$2 \cdot 10^8$	1.5	Trisagar	48.5	91	88	89	90
$2 \cdot 10^8$	3	Trisagar	22	62	60	62	61
$2 \cdot 10^8$	6	Trisagar	9	28	30	25	27
$7.5 \cdot 10^8$	1	SFLA-agar	54	100	98	100	97
$7.5 \cdot 10^8$	1.5	SFLA-agar	49	85	85	86	83
$7.5 \cdot 10^8$	3	SFLA-agar	19	65	60	62	63
$7.5 \cdot 10^8$	6	SFLA-agar	6.5	30	28	29	31
$3.5 \cdot 10^9$	1	Nutrient agar	58	100	97	95	98
$3.5 \cdot 10^9$	1.5	Nutrient agar	51	90	85	88	87
$3.5 \cdot 10^9$	3	Nutrient agar	20	60	58	61	59
$3.5 \cdot 10^9$	6	Nutrient agar	8	26.8	27	26	25

Table I. Influence of plating media on the extract-promoted recovery in X-irradiated population of bacteria. Tris-glucose grown bacteria after X-irradiation were held at 37 °C in presence of bacterial extract (1662.5 μg/ml) for various periods.

Microbiol., in press). After the required period of extract-treatment, the bacterial sample was used for plating on agar plates to determine viable titre of bacteria. In this case the bacteria were not washed and freed of the extract because this would change the viable titre, rendering the maintenance of proper control impossible. However, before plating, one has to dilute the bacterial sample from $10^8 - 10^9$ cells/ml to about 10^2 cells/ml in order to get countable clones of bacteria on agar plates. In this act of dilution very little extract, if any, should be present on agar plates. This effect observed was the manifestation of a genuine recovery phenomenon and not due to limited division of bacteria. This is clarified in the previous paper (MUKHERJEE and BHATTACHARJEE, J. gen. Microbiol., in press).

Fig. 1 and Table I show the effect of various types of plating media on the extract-promoted recovery of X-irradiated *E. coli* B bacteria. It is apparent from the Fig. and the Table that the maximum level of recovery that could be attained by incubating the irradiated bacteria in presence of bacterial extract (concentration see legend Fig. 1) is similar for all the three types of agar media used for the study. This conclusion was true for all the doses of X-irradiation used in the present study.

Figs. 2 (a) and (b) give the sedimentation pattern of untreated extract of *E. coli* B. Figures show several peaks, namely, peak(s) for soluable fraction, for DNA and for ribosonal fraction of cell-extract. Figs. 2 (c) and (d) represent ultracentrifugal pattern for heated extract. Comparison of Figs. 2 (a) and (c) indicates that peaks for DNA and ribosomal fraction of cell extract have completely vanished due to heating; only that for soluable part of the extract is visible. It is also apparent that the peak for soluable fraction of cell-extract is a composite one consisting of two peaks. These two might have been reoslved at some later time. A comparison of the Figs. 2 (b) and (d) shows that the composite peak has lost some material due to heating, thereby being lowered in hight from the baseline.

The results presented above with various plating media show that after extract treatment no difference in recovery was observed on minimal and richer plates. This indicates that recovery processes due to extract-treatment or due to varying nutritional factor are overlapping.

As mentioned earlier, the analysis of the sedimentation pattern has been done on a purely qualitative basis. It would appear from the results that soluble fraction of bacterial extract is amenable to destruction by heating. We have shown that RNA is not responsible for the recovery of X-irradiated bacteria [1] so that t-RNA which is included in the soluable fraction is not the active principle. We are then left with the soluable protein fraction which when affected by heating failed to promote recovery in X-irradiated bacteria [1]. This conclusion came from the observation that the loss in biological activity of the soluble extract fraction has been reflected in the its sedimentation pattern.

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