THE NATURE OF A COMPETENCE-INDUCING FACTOR IN BACILLUS SUBTILIS

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Competence inducing factors, which control transformation by DNA, have been demonstrated in cultures of competent cells of Streptococcus (Pakula and Walczak, 1963); Pneumococcus (Tomasz and Hotchkiss, 1964); Bacillus cereus (Felkner and Wyss, 1964) and Bacillus subtilis (Charpak and Dedonder, 1966). The pneumococcal competence factor has been isolated (Tomasz and Mosser, 1966), and found to be a polypeptide with a molecular weight of about 10^4. This report describes the isolation of a competence inducing factor from competent cells of Bacillus subtilis strain 168I . The material was partially purified by chromatography on DEAE-cellulose and appears to be polypeptide in nature.

MATERIALS AND METHODS

DNA was prepared by Marmur's method (1961) from a prototrophic strain of B. subtilis derived from the indole requiring strain $168I^-$. Cells of B. subtilis $168I^-$ were made competent by inoculation into minimal medium (Spizizen, 1958) supplemented with 20μg/ml of each of twenty amino acids, including tryptophan, and $MnSO_4$ (22.3μg/ml) (medium I). After incubation for 15 hr. at 37°, a sample of the culture was transferred into 300 ml of medium I such that the suspension had an extinction of 0.3 at 600 mμ. The culture was aerated vigorously at 37° for 4 hr. and was subsequently diluted with 1 L of medium II which differed from medium I in containing no $MnSO_4$, tryptophan at a reduced concentration of 5μg/ml and, in addition, 2,2'-dipyridyl at a concentration of 40μg/ml. The cells were incubated in medium II with slow aeration at 37° for 60 min.

The resulting competent culture was tested for frequency of transformation as follows. 5 ml. samples of the culture were incubated with DNA (5 μg/ml) for 90 min at 34° with vigorous shaking followed by incubation for 5 min. at 37° with DNase (50 μg/ml) and $MgSO_4$ (0.6 mg/ml) before plating (Levine and Strauss, 1965).

Cultures of competent cells were subjected to the following treatments:

(a) 10 ml. of cell suspension were centrifuged at 15000 rev./min. for 5 min. The cells were resuspended in 10 ml of minimal medium, centrifuged as before and resuspended in 10 ml minimal medium.

(b) 10 ml. of cell suspension were centrifuged as above, washed on the centrifuge three times with 2 ml distilled water at 2°, and resuspended in 10 ml minimal medium.

For the isolation of the competence inducing factor, the competent culture (1200 ml) was centrifuged at 15,000 x g for 5 min., washed once with cold minimal medium (80 ml) and extracted three times (50 ml each) with distilled water at 2°. The combined aqueous extracts were dialysed overnight against distilled water (4°). Samples (2 ml) were incubated for 30 min. at 37° with either trypsin (10 μg/ml) or crystalline pancreatic ribonuclease (10 μg/ml). The main part of the dialysed aqueous extract was freeze-dried, dissolved in water (3 ml) and applied to a DEAE-cellulose column, Whatman P/DE 100-C2, (46cm x 1.2cm) equilibrated with 5×10^{-3}M tris-HCl and 10^{-3}M $MgCl_2$, pH7.4. The column was eluted with a linear gradient of the above tris-$MgCl_2$ buffer (100 ml), and 5×10^{-3}M tris-HCl, 10^{-3}M $MgCl_2$ and 0.4M NaCl, pH7.4 (100 ml). The flow rate was 30 ml/hr. 3.3 ml fractions were collected and their extinctions at 260 mμ and 280 mμ measured. Selected fractions were pooled, dialysed overnight against distilled water (4°), freeze-dried and dissolved in 3 ml of minimal medium. Activities of the material from different fractions for inducing competence were measured as follows. Non-competent cells were harvested from medium I and washed twice with minimal medium and resuspended in minimal medium. Such cells were used

for measurement of frequency of transformation either without further treatment in control experiments, or after incubation at 34° for 15 min. with 1 ml of the solution under test. No transformation was observed in control experiments.

RESULTS AND DISCUSSION

From Table I, it can be seen that after being washed with minimal medium, competent cells of B. subtilis 168I⁻ suffered no significant reduction in their ability to be transformed. However, washing with distilled water reduced the number of transformants to a negligible value. Addition of the aqueous extract to the water-washed cells restored their ability to be transformed. These results indicated that a factor which is released into the aqueous extract is essential for the retention of competence in B. subtilis 168I⁻. These results are similar to those described by Parijskaya and Pukhova (1967). In the present experiments, the competence inducing activity has been partially purified and the nature of the material responsible has been investigated.

Incubation of the aqueous extract with trypsin reduced the transforming ability to a negligible value, whereas treatment with RNase was without effect. (see Table I).

TABLE I

Partial characterization of competence factor of B. subtilis 168I⁻

Transformation system	No. transformants/ ml. x 10^{-3}
Competent cells of B. subtilis 168I⁻	592
Competent cells extracted with minimal medium	575
Competent cells extracted with distilled water	0.30
Water-extracted competent cells + re-added aqueous extract	420
Water-extracted competent cells + trypsin treated aqueous extract	0.40
Water-extracted competent cells + RNase treated aqueous extract	506

Fractionation of the aqueous extract of competent cells on DEAE-cellulose is illustrated in Fig. 1A. It is seen that competence-inducing activity is confined to peak II.

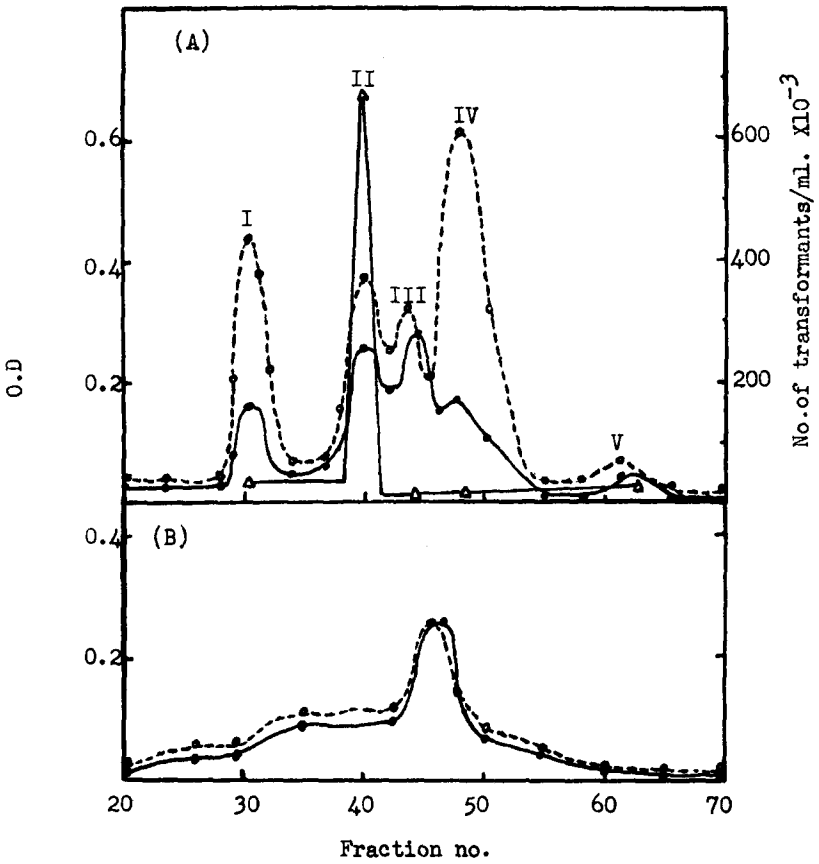

Fig. 1: Optical density of the effluents from DEAE-cellulose column of the aqueous extract of (A) competent cells of B subtilis 168 I$^-$, measured at 260 mμ o...o and 280 mμ ●—● and showing competence inducing activity of each peak Δ—Δ, and (B) non-competent cells of B.subtilis 168 I$^-$ measured at 260 o...o and 280 mμ ●—●.

As a control experiment, an aqueous extract of non-competent cells of B. subtilis 168I$^-$ was fractionated as described above. The elution profile is shown in Fig. 1B. It can be seen that considerably less ultra-violet absorbing material is released from non-competent

cells and only one absorbing peak was obtained which was not eluted at the same salt molarity as peak II from competent cells. It is suggested that the absence of this material is responsible for the inability of non-competent cells to be transformed.

Fractionation on DEAE-cellulose column of the aqueous extract of B. subtilis 168I⁻ grown in a competence medium (see text) containing chloramphenicol at a concentration 50 μg/ml is shown in Fig. 2. No fraction obtained showed competence-inducing activity.

The results presented suggest that the material responsible for inducing competence is polypeptide in nature since the activity is destroyed by treatment with trypsin but is insensitive to RNase. Nester (1964) has shown that the presence of chloramphenicol in cultures

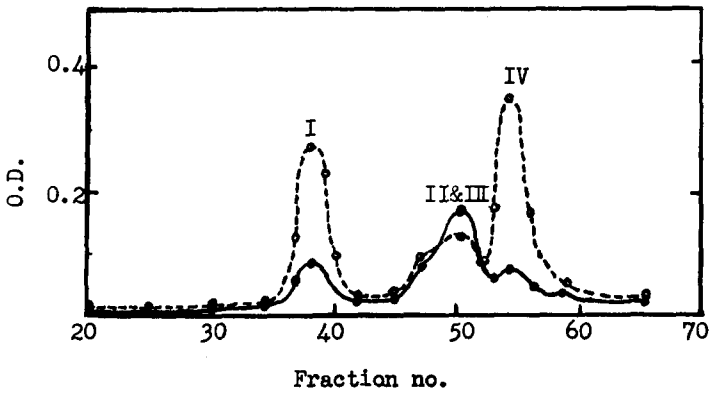

Fig. 2: Optical density at 260 mμ o...o and 280 mμ ●—● of the effluents from DEAE-cellulose column of the aqueous extract of B. subtilis 168 I⁻, grown in the competence medium in the presence of chloramphenicol.

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of B. subtilis results in loss of competence. The experiments now recorded indicate that chloramphenicol prevents the formation of the competence inducing factor, which supports the above view concerning its nature.

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