GENETIC TRANSFORMATION OF BACILLUS SUBTILIS BY EXTRACELLULAR DNA[x]

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The occurrence of extracellular DNA in several bacterial cultures has been reported by Smithies and Gibbons (1955) and by Catlin (1956). Investigations on Micrococcus halodenitrificans using P^{32} labelled cells have shown that the DNA in culture slime was of intracellular origin (Takahashi and Gibbons, 1957). Furthermore, genetic transformation mediated by extracellular DNA has been demonstrated in Neisseria (Catlin, 1960) and in Pneumococcus (Ottolenghi and Hotchkiss, 1960).

During the course of investigations on sexual recombination in Bacillus subtilis, it was found that the two auxotrophic mutants, 168 and 170 gave rise to prototrophic colonies when they were plated together on minimal agar. It was later found that this phenomenon could be observed with several other auxotrophic mutants[xx] (Table 1).

Since the cultures were not competent for transformation at the time of plating, this phenomenon was thought to be similar to sexual recombination observed in Escherichia coli (Tatum and Lederberg, 1947). However, this possibility was excluded by the following observation: pretreatment of the mutant cultures with heterologous DNA (20 μg/ml) prepared from Xanthomonas phaseoli or addition of DNase (20 μg/ml) to the mixture inhibited the appearance of prototrophic colonies. This phenomenon therefore may be due to transformation mediated by extracellular DNA.

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[xx]The following auxotrophic mutants of B. subtilis were used: 168 (indole$^-$), 170 (tryptophan$^-$, phenylalanine$^-$), W5 (indole$^-$), W3 (leucine$^-$), C14 (cysteine$^-$), H12 (phenylalanine$^-$), N2 (serine$^-$) and S10 (glutamic acid$^-$).

TABLE 1

Development of prototrophic colonies from mixed auxotrophic cultures

Strains mixed	Prototrophs /0.1 ml	Strains mixed	Prototrophs /0.1 ml
168 + 170	17	W3 + 170	411
168 + W5	0	W3 + W5	372
168 + W3	406	W3 + N2	252
168 + C14	28	W3 + S10	337
168 + H12	31	N2 + 170	22

Cells were grown in Penassay broth (Difco) for 4 hours on a reciprocal shaker, centrifuged and resuspended in the minimal medium supplemented with 0.01% each of yeast extract and casamino acids. The cultures were diluted 10 times in the same medium, 0.5 ml of the diluted cultures were mixed and without further incubation 0.1 ml samples (about 10^7 cells) were spread on minimal agar. Prototrophic colonies were counted after 2 days. No prototrophic colonies developed when the cultures were spread separately. All incubations were carried out at 37° C.

The release of transforming DNA into culture media from *B. subtilis* cells was next investigated. Strains 168 and 170 were grown in Penassay broth (Difco) for 4 hours at 37° C on a reciprocal shaker. The cells were collected by centrifugation, resuspended in the minimal medium (Spizizen, 1958) supplemented with 0.01% each of yeast extract and casamino acids (Difco) and diluted 10 times in the same medium. The diluted cultures were further incubated with shaking. At various time intervals, cell-free culture filtrates were made by passing through Millipore filter (0.45 μ porosity). The cultures under these conditions developed a maximal competence for transformation after 90 minutes. Transforming activity in the culture filtrates, however, was not detectable even after 250 minutes.

Nevertheless, the release of transforming DNA from the cells on minimal agar could be demonstrated in the following experiment (Table 2). As shown in Table 2, the transforming activity released by 168 and W3 reached a maximal

value at 120 minutes and 150 minutes respectively, and remained almost constant thereafter. Similar results were also obtained with other auxotrophic mutants.

TABLE 2

Release of transforming activity from cells on minimal agar

Donor culture	Recipient culture	No. of prototrophs/plate: Recipient culture spread at 0 min.	90 min.	120 min.	150 min.	180 min.	210 min.
168	W3	4	50	64	32	32	35
W3	168	2	53	113	185	123	118

Donor cultures were grown in Penassay broth and diluted as described in Table 1. The diluted cultures were spread on minimal agar (0.1 ml/plate). The transforming activity released on agar plates was determined by spreading 0.1 ml samples of fully competent recipient cultures which were kept in frozen state in media containing 10% glycerol. The plates were spread with DNase (20 µg/0.1 ml) after 30 minutes to terminate the reaction.

A question that still remains to be answered is whether the cells can develop competence on minimal agar. The results of experiments designed to resolve this point clearly indicate that transformation in B. subtilis can take place on minimal agar without any special treatment (Table 3). Strains such as 168, W3, which develop their competence readily on minimal agar can, under these conditions, act effectively either as recipients or donors, while other strains such as 170 and N2 are perhaps poorly competent on minimal agar and can function effectively only as donors.

The possible occurrence in nature of transformation in Pneumococcus has been suggested (Hotchkiss, 1951). This type of transformation, without experimental intervention, now reported for B. subtilis, suggests that in specifically favorable habitats such as the root surface and the rhizosphere, transformation could be a prevalent mode of genetic exchange.

TABLE 3

Appearance of competent cells for transformation on minimal agar

Recipient culture	DNA derived from	No. of prototrophs/plate DNA spread at 0 min.	60 min.	90 min.	120 min.	150 min.
168	19 (prot.)	3	17	92	193	421
168	170	0	18	82	258	401
170	19 (prot.)	14	8	9	12	6
170	168	8	6	4	13	22
W3	19 (prot.)	1	2	7	34	48
W3	170	8	32	58	186	206
N2	19 (prot.)	1	1	1	4	8
N2	170	1	0	1	6	5

Cultures were treated as described in Table 1 and 0.1 ml samples (about 10^7 cells) were spread on minimal agar. At various time intervals the respective DNA (2 μg in 0.1 ml) solutions were spread on the agar plates and unabsorbed DNA was destroyed by spreading 0.1 ml DNase (20 μg) after 30 minutes. The numbers of prototrophs listed on the last column (150 min.) of Table 2 were almost identical to those found on plates spread with DNA at 0 time and incubated without DNase treatment, indicating that the maximal competence appeared at 150 min.

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