FATE OF TRANSFORMING DNA IN BACILLUS SUBTILIS STRAIN SENSITIVE TO METHYL METHANESULFONATE

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SUMMARY: From Bacillus subtilis strain 168 (Trp^-, Leu^-), which had been treated with nitrosoguanidine, a mutant showing the properties of the recombination-defficient strain (rec^-) was isolated according to its sensitivity to methyl methanesulfonate (MMS, 0.004 M). By using neutral and alkaline CsCl density gradients for the fractionation of lysates of the transformed cultures evidence was obtained showing that the decreased transformability of the rec^- strain is caused by its inability to bind covalently the part of transforming ^{32}P-DNA (labeled by bromouracil) with the recipient chromosome (labeled by C^3H_3-thymidine).

During the past five years, many Bacillus subtilis mutants sensitive to ulraviolet radiation (uvr^-) and alkylating agents (MMS-s and similar) have been isolated and characterized and relationship between this sensitivity and the repair mechanism and process of genetic recombination has been demonstrated (1-3). Certain strains have been found to show considerably decreased transformability which has been ascribed - on the basis of comparison with transduction whose resulta are independent of the same process of DNA uptake by the host cell - to the defficiency of the mutants in recombination (4-7).

Even the recombination process, however, may not be entirely the same for both types of transfer of genetic properties (7). This study provides direct evidence that the decreased transformability of the rec^- mutant is brought about by a disturbance in the recombination mechanism during which the received part of the transforming DNA has to be covalently bound to the host chromosome.

METHODS

As parent strain for the isolation of the MMS-s mutant served *B. subtilis* 168 (Trp^-, Leu^-, MMS-r and rec^+, respectively), a wild type with respect to its sensitivity to the alkylating agent. From approximately 18,000 colonies after the treatment with nitrosoguanidine (30/ug/ml, 20 min, 37 C with aeration), tested by the replica-plating on agar plates (Spizizen minimal medium (8), 1% of casamino acids , tryptophan 20/ug/ml, 0.004M-MMS, and 1.5% of agar), 7 sensitive colonies were isolated whose transformability varied between 0.5 and 12% of values observed with the parent strain. In other experiments a strain with transformability decreased to 0.8% and marked *B. subtilis* 168/1a (Trp^-, Leu^-, MMS-s and rec^-, resp.) was used.

Transforming DNA was isolated from *B. subtilis* SMYW (prototrophic strain) and *B. subtilis* 10 (Thy^-), respectively. In the latter case the culture growing in a L.P. medium (9) was labeled by ^{32}P and, after thymine had been washed off, also by bromouracil as density marker (100/ug/ml for the period of two generations, again in the presence of $Na_2H^{32}PO_4$, 1.5/uC/ml, spec. activity 48 C/mg phosphorus). The isolation was effected in both cases by the modified (10) method of Marmur (11).

The transformation experiments were carried out with both strains as described by Šrogl (12), the final concentration of transforming DNA being 5 /ug/ml. The processing of the culture after the transformation, discontinued by DNase (5 /ug/ml) and by washing, was carried out in the medium and also according to Hanawalt (13), as described briefly in the legend to Fig. 1. The same holds true for the preparation of

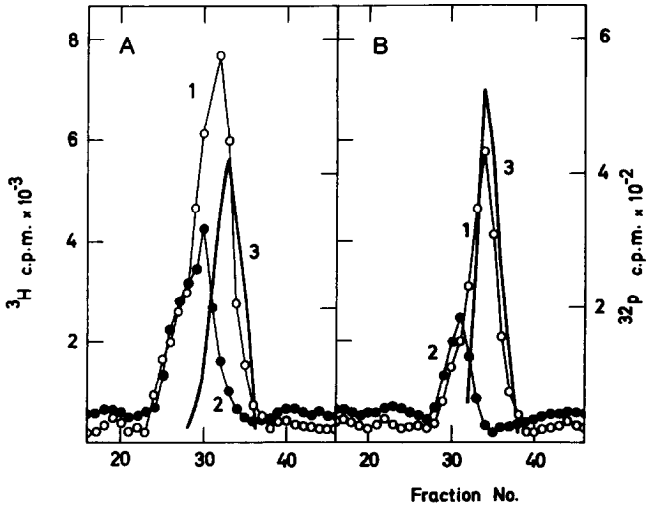

Fig. 1 Neutral Buoyant CsCl Density Gradient of Lysates of B. subtilis rec$^+$ and rec$^-$ Cultures. The culture (60 ml), labeled with (^{3}H-methyl)-thymidine, which had been transformed with BUdR-^{32}P-DNA (5 /ug/ml; 20.4 S, spec. activity 3,190 cpm per /ug) and treated with pancreatic DNase (5 /ug/ml) was lyzed and dialyzed according to Hanawalt (13). The lysate at pH 8.0 was adjusted to a density of 1.72 g/cm^3 by the addition of solid CsCl and centrifuged 72 hours at 36,000 rpm and 21 C in Spinco SW 39 rotor (4.5 ml of sample and 0.5 ml paraffin oil). To each fraction (8 drops; thin needle of MSE Piercer), carrier DNA was added (15 /ug in 0.8 ml) and 1 ml of 10% CCl_3COOH at 0 C. After filtration through a mambrane nitrocellulose filter Synpor 6 (0.4 /u) the ^{3}H- and ^{32}P-radioactivity was measured with 5 ml of toluene scintillation solution in Packard Tricarb apparatus.
A - 168 rec$^+$ culture, B - 168/1a rec$^-$ culture; 1 - ^{3}H (— o —), 2 - ^{32}P (— • —), 3 - position of ^{3}H-DNA (———) from control untransformed cultures treated by the same method.

the density gradient and for the centrifugation. The fixation of transforming ^{32}P-DNA in the cell was measured in terms of the radioactivity of the culture treated with DNase and washed three-times on a membrane filter (7).

RESULTS AND DISCUSSION

The MMS-s mutant isolated according to its inability to grow on agar plates containing 0.004M-MMS was found to be also very sensitive to ultraviolet light (the same effect was brought about by a dose ten-times lower than required for the parent strain). The change in transformability of the mutated strain is shown in Table I. To demonstrate that the transformation was not affected by bromouracil present in transforming DNA, also the results obtained with unlabeled DNA isolated from prototrophic strain are given in the Table. There is no substantial difference in the frequency of transformation of different markers with both donor DNAs; only the transfer of MMS resistance is less frequent with BUdR-^{32}P-DNA. The extent of the resistance, however, was not investigated with both donor strains. It is therefore obvious that the decreased transformability of the rec$^-$ strain is caused neither by the specific effect of BUdR-DNA nor by a change in the competence of the culture decisive for the uptake of exogeneous DNA, as indicated by the radioactivity value of ^{32}P-DNA which had been bound irreversibly to the competent cells during the incubation. However, not even in this respect is the situation with various rec$^-$ mutants as yet described clear (6,7).

The next step in the process of transformation is the synapsis of DNA with the homologous region of the recipient chromosome which represents the start to integration. Fig. 1 shows that in both transformed cultures can be found in the

Table I. Transformation of Bacillus subtilis rec^+ and rec^- strains

Recipient Strain	Transforming DNA (donor)	Uptake of DNA (cpm/ml)	Marker Transformed	Transformants (per ml) [a]	Total Number of Cells (per ml) [a]	Frequency of Transformants	% of Parent Values
168 rec^+	BUdR-^{32}P-DNA (Thy^- strain)	319	Trp	1.64×10^5	7.43×10^7	2.21×10^{-3}	100
			Leu	1.49×10^5	7.43×10^7	2.0×10^{-3}	100
168/1a rec^-		332	Trp	1.05×10^3	5.58×10^7	1.88×10^{-5}	0.9
			Leu	9.02×10^2	5.58×10^7	1.62×10^{-5}	0.8
			MMS-r	4.01×10^2	5.58×10^7	7.29×10^{-6}	-
168 rec^+	SMYW (prototrophic strain)	-	Trp	3.36×10^5	1.31×10^8	2.56×10^{-3}	100
			Leu	2.71×10^5	1.31×10^8	2.07×10^{-3}	100
168/1a rec^-		-	Trp	3.10×10^3	1.29×10^8	2.38×10^{-5}	0.9
			Leu	1.82×10^3	1.29×10^8	1.40×10^{-5}	0.7
			MMS-r	8.03×10^3	1.29×10^8	6.18×10^{-5}	-

[a] The values are averages from six competent cultures growing in parallel and transformed 120 min at final concentration of transforming DNA 5 μg/ml .

neutral CsCl gradient (after lysozyme treatment and dialysis of the obtained lysate) a DNA fraction which contains both the activity of the ^{3}H-labeled recipient genome and also of ^{32}P-labeled BUdR-DNA used for transformation. The impaired symmetry of the DNA peak resulting from the presence of bromouracil is especially apparent with 168 rec^{+} strain where also a shift of the peaks of ^{32}P- and ^{3}H-DNA from the transformed culture (— o —) with respect to the position of the ^{3}H-DNA peak from the control culture (———) can be observed. Hence, the chromosomes of both strains possess the ability to take up a homologous part of transforming DNA; the quantitative differences which may exist are difficult to explain. The results of a similar fractionation in the alkaline CsCl density gradient shown in Fig. 2 clearly indicate that the essential

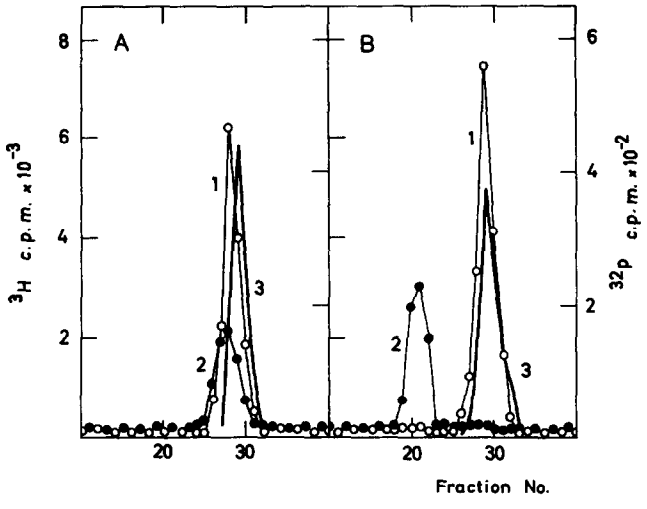

Fig. 2 Alkaline Buoyant CsCl Density Gradient of Lysates of B. subtilis rec^{+} and rec^{-} Cultures. 100 ml of each culture after the transformation was processed as shown in Fig. 1. The obtained lysates before being adjusted to a density of 1.76 g/cm^{3}, were made alkaline with KOH (final concentration 0.1 N). The centrifugation and subsequent treatment of samples as well as their designation in the Figure are the same as in Fig. 1.

difference between the two cultures exists only in the recombination step which is responsible for covalent binding of the homologous part of transforming DNA to the recipient chromosome. While with the 168 rec^+ strain remains the picture essentially the same and ^{32}P-DNA stays integrated even with single-stranded polynucleotide chains after alkaline denaturation, ^{32}P-radioactivity is released under these conditions from the 168/1a rec^- strain and a new peak is formed, in the region of higher density.

The results given above show that the difference in the transformation process of rec^+ and rec^- strains pertains only to its last step, i.e. to recombination which permits the normal expression of the corrected gene during replication and transcription. In spite of the fact that this study deals merely with a comparison of the integration of exogenous DNA in two strains differing in recombination, the obtained results correspond to the present concept of the fate of transforming DNA in a competent B. subtilis culture (14-17).

Other studies on this rec^- strain will be reported in detail in the Collection of Czechoslovak Chemical Communications.

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REFERENCES

1. Reiter, H., and Strauss, B., J.Mol.Biol., 14, 179 (1965).
2. Mahler, I., Biochem.Biophys.Res.Commun., 21, 384 (1965).

3. Okubo, S., and Romig, W.R., J.Mol.Biol., 14, 130 (1965).
4. Searashi, T., and Strauss, B., Biochem.Biophys.Res.Commun., 20, 680 (1965).
5. Okubo, S., and Romig, R.W., J.Mol.Biol., 15, 440 (1966).
6. Hoch, J.A., Barat, M., and Anagnastopoulos, C., J.Bacteriol., 93, 1925 (1967).
7. Dubnau, D., Davidoff-Abelson, R., and Smith, J., J.Mol.Biol., 45, 155 (1969).
8. Spizizen, J., Proc.Natl.Acad.Sci.U.S., 44, 1072 (1958).
9. Goodman, H.M., Abelson, J.N., Landy, A., Zadražil, S., and Smith, J.D., Europ.J.Biochem., 13, 461 (1970).
10. Zadražil, S., and Mach, O., in Reid, E., (Ed.), Zonal Centrifugation, University of Surrey, Guildford,1970.
11. Marmur, J., J.Mol.Biol., 3, 208 (1961).
12. Šrogl, M., Dissertation, Czech. Acad. Sci., Prague, 1965.
13. Hanawalt, P.C., and Ray, D.S., Proc.Natl.Acad.Sci.U.S., 52, 125 (1964).
14. Bodmer, W.F., and Ganesan, A.T., Genetics, 50, 717 (1964).
15. Bodmer, W.F., J.Mol.Biol., 14, 534 (1965).
16. Venema, G., Pritchard, R.H., and Venema-Schröder, T., J. Bacteriol., 89, 1250 (1965).
17. Harris, W.J., and Barr, G.C., J.Mol.Biol., 39, 245 (1969).