JOURNAL OF VIROLOGY, Mar. 1974, p. 584-589
Vol. 13, No. 3
Copyright © 1974 American Society for Microbiology
Printed in U.S.A.

Protease-Sensitive Transfection of *Bacillus subtilis* with Bacteriophage GA-1 DNA: a Probable Case of Heterologous Transfection

FRÉ ARWERT AND GERARD VENEMA

University of Groningen, Biological Centre, Department of Genetics, Haren (Gn), The Netherlands

Received for publication 2 October 1973

The host bacterium of bacteriophage GA-1, *Bacillus* sp. G1R, was compared with respect to its taxonomic relationship to *Bacillus subtilis*, *B. licheniformis*, and *B. pumilis*. The physiological-biochemical properties of *Bacillus* sp. G1R are equal to those of *B. licheniformis*, but the thermal denaturation midpoint of G1R DNA differs by 3 C and the buoyant density by 0.005 g/cm^3 from that of *B. licheniformis*. Transformation with G1R donor DNA was neither observed in *B. licheniformis* nor in *B. subtilis*-competent recipients. Bacteriophage GA-1 shows neither infectivity on *B. licheniformis* nor on *B. subtilis*. However, infection of competent *B. subtilis* cultures with phenol-extracted GA-1 DNA results in the production of infective GA-1 particles. The transfecting activity of GA-1 DNA is destroyed by treatment with proteolytic enzymes. Resistance of transfecting DNA to inactivation by trypsin develops earlier than that to inactivation by DNase. Protease-treated GA-1 DNA competes with transforming DNA to approximately the same extent as does untreated GA-1 DNA, suggesting that uptake of GA-1 DNA is not affected by protease treatment. CsCl density gradient centrifugation reveals that the density of trypsinized GA-1 DNA is 0.004 g/cm^3 greater than that of untreated DNA.

DNA of bacteriophage ϕ29, having a molecular weight of 11×10^6 (2), is the smallest phage DNA described in transfection studies of *Bacillus subtilis*. Although the host bacterium of bacteriophage ϕ29 is considered to be *B. amyloliquefaciens* (27), the phage is also infective on *B. subtilis* 168. DNAs of other *B. subtilis* phages, showing transfecting activity on competent *B. subtilis* cells, have a molecular weight that is considerably higher than that of ϕ29 DNA, e.g., SPP1, SPO2, and ϕ105 (molecular weight $\simeq 25 \times 10^6$) (5, 15, 16) and ϕ25, SPO1, 2C, SP82, and SP50 (molecular weight $\simeq 100 \times 10^6$) (25). The transfecting activity of ϕ29 DNA differs from that of the other known virulent *B. subtilis* phages in two respects: (i) transfection with ϕ29 DNA is protease sensitive (9) and (ii) transfection curves, relating the number of infective centers to the DNA concentration used, exhibit a first order concentration dependence (14, 20).

Linear dose-response curves are also observed for SPO2 DNA (20) and ϕ105 DNA (17), but these phages are known to be temperate. In addition, SPO2 transfection is sensitive to proteolytic enzymes (H. Hirokawa and T. A. Trautner, personal communication).

Originally, the aim of the present study was to extend the transfection data for *B. subtilis* with another small *B. subtilis* phage, having DNA with approximately the same molecular weight as ϕ29 DNA. When we learned that bacteriophage GA-1 is not able to infect *B. subtilis* and that its host is not *B. subtilis* as was assumed by Bradley (7), an attempt was made to obtain heterologous transfection in *B. subtilis* with the DNA of bacteriophage GA-1.

MATERIALS AND METHODS

Strains. The following bacterial strains were used: *B. subtilis* strain 1G20 (carrying the ind_{168} [= trpC2 marker]) and strain 8G5, an eightfold auxotrophic strain described by Bron and Venema (8); *B. licheniformis* strain S 859 $pur^-trp^-pep^-$ and strain S 1333 arg^-pep^- (pep refers to the gene determining glutamyl polypeptide biosynthesis); *B. pumilis* obtained from the culture collection of the Agricultural University, Wageningen, The Netherlands; *B.* sp. G1R obtained from D. E. Bradley, who classified the strain as *B. subtilis; B.* sp. G1R str^r, a streptomycin-resistant strain of *B* sp. G1R isolated after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis.

The bacteriophage strains used were: bacteriophage GA-1, obtained from D. E. Bradley; bacteriophage H1, a 5-hydroxymethyl uracil containing viru-

lent *B. subtilis* 168 phage isolated in our laboratory; bacteriophage SPP1 (15) another virulent *B. subtilis* phage.

Media. Standard media, which have been described previously, were used throughout as follows. Glucose minimal medium (21) was used for the preparation of competent *B. subtilis* cells; BLSG and TM medium (24) were used for the preparation of competent *B. licheniformis* cells. The media and methods employed by Smith et al. (19) were used for the classification tests. H-broth (0.8% Difco nutrient broth, 0.5% bactopeptone, 0.5% NaCl, 0.5% glucose, and 10^{-4} M $MnCl_2$, pH 7.2) was used for the preparation of GA-1 lysates, and TY medium (0.1% tryptone, Oxoid), 0.5% yeast extract, 1% NaCl, 10^{-4} M $MnCl_2$, and 1.5% agar, Difco) for plating of the phage.

Preparation of phage lysates. Logarithmically growing cells of *B.* sp. G1R in H-broth were infected with GA-1 at a multiplicity of infection of 1 to 3. Complete lysis occurred within 2.5 h at 37 C. Crude lysates contained approximately 10^{11} PFU/ml. Bacteriophages were collected and purified as described by Yamamoto et al. (29). H1 and SPP1 lysates were prepared in essentially the same way, using *B. subtilis* 1G20 as host.

DNA preparations. Bacterial DNAs were isolated as described previously (3). GA-1 DNA was obtained by phenol extraction as described by Hirokawa (9) for ϕ29 DNA, with the exception that sodium lauryl sulphate (0.2%) was used instead of sodium lauryl sarcosinate. Other phage DNAs were extracted by the same method.

Proteolytic enzyme treatment. Trypsin (2× crystallized, Sigma) and Pronase (B grade, Calbiochem) were dissolved in SSC (0.15 M NaCl + 0.015 M Na_3 citrate) just before use. Pronase solutions were autodigested for 3 h at 42 C to destroy possible nuclease activity. Usually 0.2 ml of DNA was mixed with 0.2 ml of enzyme solution and incubated for 30 min at 34 C. Inhibited trypsin was prepared by mixing equal amounts of trypsin with soy bean inhibitor (Sigma).

Transformation and transfection. *B. subtilis* cultures were made competent as described previously (3). *B. licheniformis* cultures were made competent as described by Thorne and Stull (24). Incubation of competent cells with transforming DNA was at 37 C for 45 min. Uptake of DNA was terminated by the addition of pancreatic DNase (50 μg/ml). For SPP1 and H1 transfection, competent *B. subtilis* cultures were incubated with transfecting DNA at 34 C for 45 min, and transfectants were plated on trypticase yeast (TY) medium in 2.5 ml of soft agar plus about 10^8 broth-grown 1G20 indicator cells. Phage GA-1 does not infect *B. subtilis*, and the host bacterium *Bacillus* sp. G1R is unable to grow in the presence of *B. subtilis*. However, we were able to demonstrate transfection of *B. subtilis* with GA-1 DNA in the following way. Competent *B. subtilis* cells were incubated with GA-1 DNA at 34 C for 60 min and transfection was terminated by addition of DNase (50 μg/ml). The transfected culture was diluted fivefold with H-broth, aerobically incubated for 3.5 h at 37 C, and plated on TY medium containing 500 μg of streptomycin per ml with *Bacillus* sp. G1R str^r as plating bacteria. The growth period in H-broth prior to plating on streptomycin-containing plates is necessary to allow GA-1 phage production in *B. subtilis*.

Buoyant density (ρ) and T_m. The methods used for buoyant density and thermal denaturation measurements (T_m) were those described by Schildkraut et al. (18) and Marmur and Doty (11), respectively.

Antisera. Anti-GA-1 phage serum (K = 80) and anti-ϕ29 phage serum (K = 790) were obtained through the kind cooperation of H. G. Seyen, Dept. of Histology, University of Groningen.

RESULTS

Classification of Bacillus spec. G1R. The observation that bacteriophage GA-1 is unable to infect *B. subtilis* 168 and the observation that the host bacterium *Bacillus* sp. G1R, classified by Bradley (7) as a strain of *Bacillus subtilis*, grows very poorly in Spizizen minimal medium, supplemented with glucose and casein hydrolysate, gave reason to doubt whether classification of *Bacillus* sp. G1R as a *B. subtilis* was justified. Since these observed differences with respect to *B. subtilis* are of minor importance for taxonomic classification we decided to adopt the morphological, cultural, and biochemical tests employed by Smith et al. (19) for the classification of *Bacillus* species. A comparison was made between *Bacillus* sp. G1R and *Bacillus subtilis* 168, *B. licheniformis* and *B. pumilis*. The results of these classification tests indicated that *Bacillus* sp. G1R is identical to *B. licheniformis*. The T_m, ρ, and percentage of guanine plus cytosine (G + C) values calculated from the T_m or ρ of the DNA of *B. subtilis*, *B. licheniformis*, and *Bacillus* sp. G1R are presented in Table 1. The values obtained for the T_m and ρ of *B. subtilis* and *B. licheniformis* DNA agree with those reported by Marmur and Doty (11), Schildkraut et al. (18), and by De Ley (19).

The transforming ability of *Bacillus* sp G1R str^r DNA was examined by use of competent *B. subtilis* 8G5 and *B. licheniformis* S 1333 and S 859 cultures. Transformation was neither observed for the auxotrophic markers tested nor for the streptomycin resistance marker. By

TABLE 1. *DNA base composition of Bacillus sp. G1R, B. subtilis, and B. licheniformis deduced from T_m and ρ*

Source of DNA	T_m (C)	G+C (%)[a]	ρ (g/cm³)	G+C (%)[b]
B. sp. G1R	85.4 ± 0.2	39.2	1.699	39.8
B. subtilis 168	87.5 ± 0.2	44.4	1.702	42.9
B. licheniformis	88.5 ± 0.2	46.8	1.704	44.9

[a] Calculated from T_m.
[b] Calculated from ρ.

using homologous DNA, about 10^6 transformants per ml were obtained for *B. subtilis*, and 2×10^4/ml for *B. licheniformis*.

On the basis of these results it is difficult to classify *Bacillus* sp. G1R as a strain of *B. subtillis*. On the basis of the criteria employed by Smith et al. (19) *Bacillus* sp. G1R should preferably be considered to be a *B. licheniformis* strain. However, the deviating T_m and ρ, and the inability of *B. spec.* G1R DNA to transform *B. licheniformis* indicate that the two organisms are genetically unrelated. Therefore, we prefer the designation *Bacillus* sp. G1R rather than a doubtful classification at the species level.

Transfection of B. subtilis with GA-1 DNA. Some properties of bacteriophage GA-1 DNA are listed in Table 2. The molecular weight of GA-1 DNA and its G+C content are close to that reported for ϕ29 DNA (1, 9), but the two phages are not related serologically, since GA-1 phage is not inactivated by anti-ϕ29 phage serum and vice versa. The production of PFU as a function of time of incubation in H-broth, after a period of 60 min of DNA uptake at 34 C, is shown in Fig. 1. As controls, we omitted from the transfection mixture (i) competent *B. subtilis* cells, (ii) GA-1 DNA, or (iii) we incubated the competent cells with DNase-treated DNA. No plaques were observed in the controls, indicating that infective GA-1 particles are produced by the infection of competent *B. subtilis* cells with GA-1 DNA.

In the presence of anti-GA-1 phage serum in the plates no plaques were formed, indicating that the plaques are not caused by induction of defective bacteriophage carried by *B. subtilis* 168 (12). Moreover, electron-microscope examination of the particles present in the plaques obtained revealed phage particles indistinguishable from GA-1. The infective centers observed during the first 2 h of incubation in H-broth (Fig. 1) were not sensitive to treatment with anti-GA-1 phage serum, indicating that they

TABLE 2. *Properties of bacteriophage GA-1 DNA*

Determination	Value
$s_{20,w}^0$	≃25
Molecular weight ($\times 10^{-6}$) from $s_{20,w}^0$	≃13.5
Length (μm) from electron micrographs (Kleinschmidt technique)[a]	6.5 ± 0.4
ρ (Trypsin-treated DNA)	1.695
G+C (deduced from ρ)	36%
T_m	84.5°C
G+C (deduced from T_m)	37%

[a] Electron micrographs were made by Sije Boonstra, Department of Biochemistry, University of Groningen.

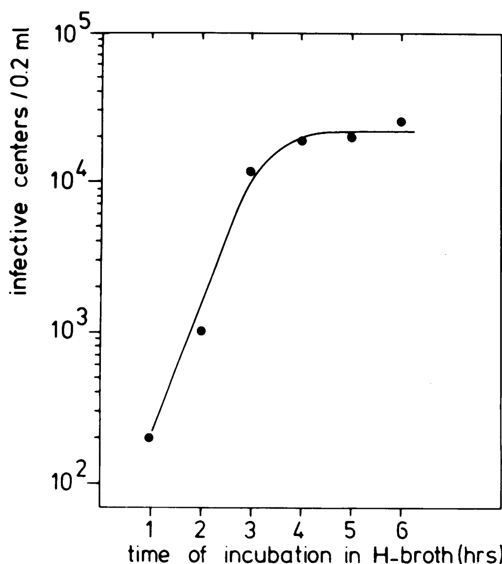

FIG. 1. *Production of infective centers after infection of a competent B. subtilis culture with GA-1 DNA. Phenol-extracted GA-1 DNA (20 µg/ml) was added to competent B. subtilis 1G20 cultures for 1 h at 34 C. Uptake of DNA was terminated with DNase (50 µg/ml), and the transfection mixture was diluted fivefold in H-broth and incubated at 37 C. Samples were withdrawn at the times indicated and plated with B. sp. G1R str^r as indicator cells.*

are due to plating of infected bacteria (i.e., *B. subtilis* cells infected with GA-1 DNA). The increase in infective centers after 2 h of incubation in H-broth is caused by the liberation of free GA-1 phage particles (± 10 to 20 phage particles per transfected cell), since from this point of time plaque formation was found to become sensitive to anti-GA-1 phage serum.

The infectivity of GA-1 DNA, previously treated with trypsin or autodigested Pronase, was assayed by transfection. As shown by Table 3, such treatment reduces the infectivity of GA-1 DNA drastically. These protease treatments caused no reduction in the infectivity of SPP1 and H1 DNA, indicating that the loss of GA-1 infectivity is not due to contaminating nuclease activity. The same conclusion can be drawn from the observation that trypsin, inhibited with soy bean inhibitor, has no effect on the transfecting activity of GA-1 DNA.

At various intervals after mixing of the competent cells with transfecting DNA, pancreatic DNase (100 µg/ml) or trypsin (200 µg/ml) was added. Figure 2 shows that the development of resistance to trypsin precedes that of resistance to DNase. These results are similar to those obtained in ϕ29 transfection by Hirokawa (9), who interpreted his results to indicate that

TABLE 3. *Effect of proteolytic enzymes on transfecting activity of GA-1 DNA*[a]

Enzymes (μg/ml)	No. of infective centers per ml
Trypsin	
0	320
2	90
20	0
200	0
Inhibited trypsin (soy bean)	
0	350
2	410
20	375
200	330
Pronase	
0	370
2	50
20	0
200	0

[a] GA-1 DNA concentration in the incubation mixture with proteolytic enzymes was 5 μg/ml; in transfection mixture 1.4 μg/ml.

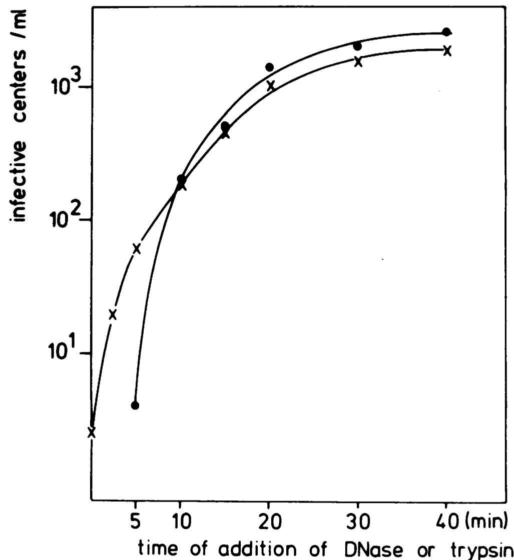

FIG. 2. *Effect of trypsin and DNase during transfection with GA-1 DNA on the number of infective centers produced. Competent B. subtilis cells (1G20) were exposed to GA-1 DNA (20 μg/ml) at 34 C. After various intervals, 0.9-ml samples were withdrawn and added to 0.1 ml of trypsin (2 mg/ml) (×) or to 0.1 ml of DNase (1 mg/ml) solutions in 0.01 M phosphate buffer (●). Fifty minutes later, 0.1 ml of trypsin was added to the DNase samples and vice versa. Samples were diluted fivefold in H-broth, incubated for 3.5 h at 37 C, and plated for infective centers with B. sp. G1R str^r as indicator cells.*

protein associated with the DNA is necessary for the uptake of φ29 DNA molecules. However, when protease-treated GA-1 DNA is used as competitive DNA in *B subtilis* transformation, the inhibitory effect on transformation is about the same as that of untreated DNA (Table 4), suggesting that uptake (including binding to competent cells) of GA-1 DNA is not affected by protease treatment.

CsCl density gradient centrifugation. Phenol-extracted GA-1 DNA was banded by preparative CsCl density gradient centrifugation. After fractionation of the gradients, the peak fractions were collected and dialyzed against SSC. After banding in CsCl density gradients, GA-1 DNA exhibits normal infectivity and protease sensitivity. This result demonstrates that the binding of protein to GA-1 DNA is stable in high concentrations CsCl and, therefore, offers the possibility to investigate the effect of protease treatment on the buoyant density of phenol-extracted GA-1 DNA. Figure 3 shows the buoyant densities of GA-1 DNA before and after trypsin treatment, relative to SP50 DNA (ρ = 1.7035, 6). Trypsinized GA-1 DNA bands at a position 0.004 g/cm³ heavier than untreated DNA. From the density difference 0.004 g/cm³, the amount of protein binding to GA-1 DNA can be estimated to be about 1% (wt/wt) of the GA-1 DNA.

DISCUSSION

The classification of the host of bacteriophage GA-1 as a strain of *B. subtilis* by Bradley (7) is difficult to reconcile with our results. On the basis of the physiological-biochemical classifi-

TABLE 4. *Competitive effect of various DNAs on transformation of B. subtilis*[a]

Competitive DNA	No. of *trpC2*⁺ transformants/ml ($\times 10^{-4}$)	Inhibition of transformation (%)
No	23	0
Untreated GA-1 DNA	5	78.2
Pronase-treated GA-1 DNA	7	69.4
Haemophilus influenzae DNA (native)	3.5	84.6
Haemophilus influenzae DNA (heat denatured)	16.5	28.5

[a] Competent 1G20 (*trpC2*) cultures were exposed to 10 μg of DNA per ml containing wild-type *B. subtilis* DNA and competing DNA in a ratio of 1:10. Transfecting activity of Pronase-treated GA-1 DNA was zero. The molecular weight of *B. subtilis* DNA and *H. influenzae* DNA was ≃2 × 10⁷.

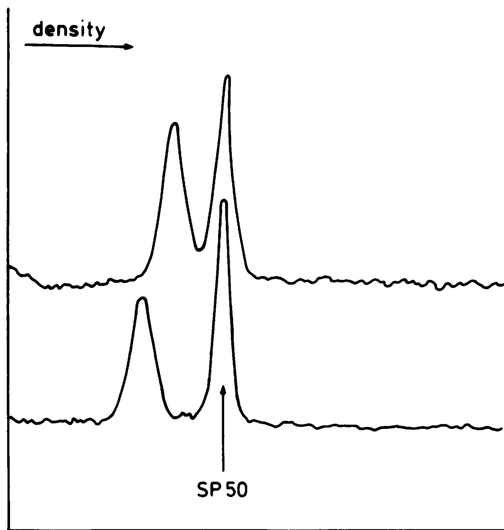

FIG. 3. Buoyant density of trypsin-treated GA-1 DNA (upper tracing) and untreated (lower tracing) DNA. GA-1 DNA (3 μg/ml) was incubated with trypsin (20 μg/ml) for 30 min at 34 C. About 0.3 μg of DNA was used for neutral CsCl density gradient centrifugation in a Spinco model E analytical ultracentrifuge. Tracings were made after 18 h at 44,000 rpm and 25 C with a photo-electric scanner. SP50 DNA was used as density marker (ρ = 1.7035).

cation tests, the bacterium should be classified as a strain of *B. licheniformis*, but because of the deviating T_m of its DNA and the lack of genetic homology with *B. subtilis* as well as with *B. licheniformis* we are inclined to assume that *Bacillus* sp. G1R is a species of *Bacillus* neither closely related to *B. subtilis* nor to *B. licheniformis*.

Attempts to transfect *B. subtilis* with DNA obtained from non-*B. subtilis* phages have all been unsuccessful (λ, T1, T4: Trautner, personal communication; T7, Mu-1: Arwert, unpublished data), with a possible exception reported by Bayreuther and Romig (4) who used polyoma virus DNA. This paper shows that bacteriophage GA-1, which in view of the properties of its host is considered to be a non-*B. subtilis* phage, is produced after infection of competent *B. subtilis* cells with GA-1 DNA. The observation that a bacteriophage genome is fully expressed in the cytoplasm of different bacterial species is not unique. It is known that several *Bacillus* phages can be propagated on different *Bacillus* species (23, 30). By using a different system, Wais and Goldberg (26) have demonstrated that urea-treated T4 phage can infect and grow in spheroplasts of several genera of bacteria which are usually resistant to T4.

Similar to transfection with ϕ29 DNA, transfection with GA-1 DNA is abolished by treatment of the DNA with proteolytic enzymes. Electron microscopy of ϕ29 DNA has revealed that circular molecules are converted to linear molecules by protease treatment (9, 13). From these results, and from the observation that the S value of single-stranded trypsinized ϕ29 DNA was essentially the same as that of untreated single-stranded DNA, Hirokawa (9) has argued that the protein associated with ϕ29 DNA is likely to be bound to the ends of the ϕ29 DNA molecules. Additional enzymatic evidence has suggested that ϕ29 DNA has short single-stranded ends to which the protein is bound (9).

The development of trypsin and DNase resistance in transfection with GA-1 DNA is very much similar to that of ϕ29 DNA observed by Hirokawa (9). Hirokawa concluded from his observations that the association of protein with ϕ29 DNA is prerequisite to uptake of the DNA by the competent cells. If this were true, one would expect to find that untreated bacteriophage DNA competes more strongly with transforming DNA than protease-treated bacteriophage DNA. This is not observed for GA-1 DNA. Therefore, other possibilities should be considered, namely, that the protein bound to GA-1 DNA either plays an essential role in the intracellular development of bacteriophage or protects the GA-1 chromosome from being degraded by cellular nucleolytic activity. If GA-1 DNA resembles the structure of ϕ29 DNA in that the association with protein is located at the ends of the GA-1 DNA molecules, resistance to protease activity will precede that to DNase activity, since DNA enters competent *B. subtilis* cells in a linear way, starting from one end of the DNA molecule (22, 28).

As a consequence of trypsin treatment the buoyant density of GA-1 DNA shifts to a heavier position. The distribution profile of untreated GA-1 DNA in CsCl density gradients is symmetrical and equal to that of trypsin-treated DNA. We interpret these results to indicate that a constant amount of protein is associated with a GA-1 DNA molecule. The amount of protein binding to GA-1 DNA can be estimated from the density shift to be about 13 $\times 10^4$ daltons. If there is one protein molecule bound to each end of the GA-1 DNA molecule, the molecular weight of a protein molecule binding to one end of GA-1 DNA would amount to about 65,000, a value which is the same order of magnitude as determined for the protein associated with ϕ29 DNA by Ortin et al. (13).

ACKNOWLEDGMENTS

We thank W. J. Feenstra for a critical discussion, and Liesbeth Steendam and Hans Koster for valuable technical assistance.

This investigation was carried out under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.) and with financial aid of the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

LITERATURE CITED

1. Anderson, D. L., D. D. Hickman, and B. E. Reilly. 1966. Structure of *Bacillus subtilis* bacteriophage ϕ29 and the length of ϕ29 deoxyribonucleic acid. J. Bacteriol. **91**:2081-2089.
2. Anderson, D. L., and E. T. Mosharrafa. 1968. Physical and biological properties of phage ϕ29 deoxyribonucleic acid. J. Virol. **2**:1185-1190.
3. Arwert, F., and G. Venema. 1973. Transformation in *Bacillus subtilis*. Fate of newly introduced transforming DNA. Mol. Gen. Genet. **123**:185-198.
4. Bayreuther, K. E., and W. R. Romig. 1964. Polyoma virus: production in *Bacillus subtilis*. Science **146**:778-779.
5. Birdsell, D. C., G. M. Hathaway, and L. Rutberg. 1969. Characterization of temperate *Bacillus* bacteriophage ϕ105. J. Virol. **4**:264-270.
6. Biswal, N., A. K. Kleinschmidt, H. Ch. Spatz, and T. A. Trautner. 1967. Physical properties of the DNA of bacteriophage SP50. Mol. Gen. Genet. **100**:39-55.
7. Bradley, D. E. 1965. The isolation and morphology of some new bacteriophages specific for *Bacillus* and *Acetobacter* species. J. Gen. Microbiol. **41**:233-241.
8. Bron, S., and G. Venema. 1972. Ultraviolet inactivation and excision repair in *Bacillus subtilis*. I. Construction and characterization of a transformable eightfold auxotrophic strain and two ultraviolet-sensitive derivatives. Mutat. Res. **15**:1-10.
9. Hirokawa, H. 1972. Transfecting deoxyribonucleic acid of *Bacillus* bacteriophage ϕ29 that is protease sensitive. Proc. Nat. Acad. Sci. U.S.A. **69**:1555-1559.
10. Ley, J. de. 1970. Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. J. Bacteriol. **101**:738-754.
11. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109-118.
12. Okamoto, K., J. A. Mudd, J. Magnan, W. M. Huang, T. V. Subbaiah, and J. Marmur. 1968. Properties of the defective phage of *Bacillus subtilis*. J. Mol. Biol. **34**:413-428.
13. Ortin, J., E. Vinuela, M. Salas, and C. Vasquez. 1971. DNA-protein complex in circular DNA from phage ϕ29. Nature N. Biol. **234**:275-277.
14. Reilly, B. E., and J. Spizizen. 1965. Bacteriophage deoxyribonucleate infection of competent *Bacillus subtilis*. J. Bacteriol. **89**:782-790.
15. Riva, S., M. Polsinelli, and A. Falaschi. 1968. A new phage of *Bacillus subtilis* with infectious DNA having separable strands. J. Mol. Biol. **35**:347-356.
16. Romig, W. R. 1968. Infectivity of *Bacillus subtilis* bacteriophage deoxyribonucleic acids extracted from mature particles and from lysogenic hosts. Bacteriol. Rev. **32**:349-357.
17. Rutberg, L., J. A. Hoch, and J. Spizizen. 1969. Mechanism of transfection with deoxyribonucleic acid from the temperate *Bacillus* bacteriophage ϕ105. J. Virol. **4**:50-57.
18. Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. **4**:430-443.
19. Smith, N. R., R. E. Gordon, and F. E. Clark. 1952. Aerobic sporeforming bacteria. U. S. Dept. Agr. Monogr. no. 16.
20. Spatz, H. Ch., and T. A. Trautner. 1971. The role of recombination in transfection of *B. subtilis*. Mol. Gen. Genet. **113**:174-190.
21. Spizizen, J. 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. Proc. Nat. Acad. Sci. U.S.A. **44**:1072-1078.
22. Strauss, N. 1965. Configuration of transforming deoxyribonucleic acid during entry into *Bacillus subtilis*. J. Bacteriol. **89**:288-293.
23. Taylor, M. J., and C. B. Thorne. 1963. Transduction of *Bacillus licheniformis* and *Bacillus subtilis* by each of two phages. J. Bacteriol. **86**:452-461.
24. Thorne, C. B., and H. B. Stull. 1966. Factors affecting transformation of *Bacillus licheniformis*. J. Bacteriol. **91**:1012-1020.
25. Truffaut, N., B. Revert, and M. O. Soulie. 1970. Etude comparatives des DNA phages 2C, SP8, SP82, ϕe, SP01 et SP50. J. Biochem. **15**:391-400.
26. Wais, A. C., and E. B. Goldberg. 1969. Growth and transformation of phage T4 in *Escherichia coli* B/4, *Salmonella*, *Aerobacter*, *Proteus* and *Serratia*. Virology **39**:153-161.
27. Welker, N. E., and L. L. Campbell. 1967. Unrelatedness of *Bacillus amyloliquefaciens* and *Bacillus subtilis*. J. Bacteriol. **94**:1124-1130.
28. Williams, G. L., and D. M. Green. 1972. Early extracellular events in infection of competent *Bacillus subtilis* by DNA of bacteriophage SP82G. Proc. Nat. Acad. Sci. U.S.A. **69**:1545-1549.
29. Yamamoto, K. R., B. M. Alberts, R. Benzinger, L. Lawhorne, and G. Treiber. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology **40**:734-744.
30. Yelton, D. B., and C. B. Thorne. 1970. Transduction in *Bacillus cereus* by each of two bacteriophages. J. Bacteriol. **102**:573-579.