JOURNAL OF BACTERIOLOGY, Apr. 1978, p. 338-344
0021-9193/78/0134-0338$02.00/0

Vol. 134, No. 1
Printed in U.S.A.

Biochemical and Genetic Properties of Site-Specific Restriction Endonucleases in *Bacillus globigii*

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Received for publication 21 November 1977

Bacillus globigii contains two site-specific endonucleases, *Bgl*I and *Bgl*II. A rapid technique for selection of mutants deficient in each of these enzymes was developed using sensitivity to infection by bacteriophage SP50 as an indication of the levels of enzyme. Mutants defective in *Bgl*I, *Bgl*II, and both *Bgl*I and *Bgl*II retained the wild-type modification phenotype. Genetic and biochemical studies have established that these enzymes are involved in restriction in vivo. Simplified purification procedures for *Bgl*I and *Bgl*II using these mutants are described.

The study of restriction and modification of bacteriophage has led to a deeper understanding of many important biological processes. Specifically, biochemical studies have resulted in the discovery and isolation of type I and type II site-specific endonucleases. The type II nucleases, which require only Mg^{2+} as a cofactor, were essential for the development of techniques for gene cloning and for rapid sequencing of DNA. In *Escherichia coli*, genetic studies of restriction and modification preceded the biochemical identification of the enzymes involved (19). However, in *Haemophilus influenzae* the site-specific endonuclease *Hin*dII was isolated and the recognition sequence was determined several years before genetic studies confirmed that this enzyme was responsible for restriction in vivo (10, 14). For many other bacterial strains, the isolation of a site-specific endonuclease(s) has been reported, but evidence for restriction in vivo due to this enzyme(s) was not provided. The genus *Bacillus* is typical in this regard. Although 31 different strains of bacilli have been shown to contain site-specific endonucleases (13), only in the cases of *Bsu*I (16) and *Bst* 1503 (3; N. E. Welker, personal communication) has the presence of these enzymes been correlated with restriction activity in vivo.

Investigations in our laboratory resulted in the discovery of two type II site-specific endonucleases in *Bacillus globigii* (18). The persistent contamination of *Bgl*I by *Bgl*II rendered purification difficult. Accordingly, techniques were devised to select mutants that were deficient in either *Bgl*I, *Bgl*II, or both *Bgl*I and II. These strains not only greatly facilitated the purification of the enzymes but also demonstrated that *Bgl*I and *Bgl*II were responsible for restriction in vivo.

MATERIALS AND METHODS

Media and buffers. Supplemented L broth contained, per liter: tryptone (Difco), 10 g; yeast extract, 5 g; and NaCl, 5 g; neutralized to pH 7.0 with 1 N NaOH. After autoclaving (121°C for 15 min), $MgCl_2$ was added to a final concentration of 2 mM and glucose was added to 0.1% (wt/vol).

Supplemented BHI contained 32 g of brain heart infusion (BHI; Difco) per liter. After autoclaving, $MgCl_2$ was added to a final concentration of 2 mM and glucose was added to 0.1%.

SLBH medium (H. Boyer, personal communication) contained, per liter: tryptone, 11 g; yeast extract, 22.5 g; glycerol, 4 ml; 1 M K_2HPO_4, 51 ml; and 1 M KH_2PO_4, 15.6 ml. Salts were autoclaved and added separately.

Semisolid-plus-glucose agar was used in the selection of rifampin-resistant (Rfm^r) transformants. This overlay medium contained, per liter: tryptone, 10 g; NaCl, 8 g; agar, 6 g. After autoclaving, 12 ml of sterile 50% (wt/vol) glucose was added.

When solid media were required, agar was added to a final concentration of 1% before autoclaving.

TMM buffer contained 6 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.4), 6 mM $MgCl_2$, and 6 mM 2-mercaptoethanol.

S buffer contained 50 mM potassium phosphate (pH 7.0), 10 mM 2-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid, and 1 mM sodium azide.

C buffer consisted of S buffer containing 10% glycerol.

HTP buffer contained 25 mM potassium phosphate (pH 7.0), 10 mM 2-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid, and 1 mM sodium azide.

D buffer contained 20 mM potassium phosphate (pH 7.0), 10 mM 2-mercaptoethanol, 200 mM NaCl, 1 mM ethylenediaminetetraacetic acid, and 50% glycerol.

Bacterial strains, viruses, and DNA. The bacterial strains used in this study are listed in Table 1. Bacteriophages SP50 and β22 and the host strain *B. subtilis* BR151 (*lys-3 metB10 trpC2*) were obtained from B. Reilly. *B. subtilis* strain RUB300 (*lys-3 hisB2*

TABLE 1. *Strains of B. globigii*

Designation	Description	Source[a]
SB512	Wild-type *B. globigii*; produces *BglI* and *BglII*	J. Lederberg
RUB550	Rifampin resistant (Rfm^r); produces *BglI* and *BglII*	Spontaneous mutant of SB512
RUB562	Rfm^r; produces only *BglII*	EMS treatment of RUB550
RUB561	Rfm^r; produces only *BglI*	EMS treatment of RUB550
RUB560	Rfm^r; produces neither *BglI* nor *BglII*	EMS treatment of RUB561

[a] EMS, Ethyl methane sulfonate.

metB10) was constructed in this laboratory.

Simian virus 40 and adenovirus type 2 DNA were gifts from R. J. Roberts. Lambda DNA was prepared as described (18).

Genetic procedures. DNA was isolated from bacteriophage by the methods of Foldes and Trautner (6) and Yehle and Doi (21) for SP50 and β22, respectively. Bacterial DNA was isolated as described by Yasbin et al. (20). Cells were grown to competence as described by Boylan et al. (2) and incubated with either bacterial DNA (19) or bacteriophage DNA (6) as described previously. The efficiency of plating of bacteriophage SP50 was determined essentially as described by Yasbin et al. (20), using *B. subtilis* BR151 or mutants of *B. globigii* as hosts and assaying the bacteriophage in a semisolid agar overlay on tryptose blood agar base (20). Transfection was assayed in the same medium (19).

Isolation of mutants deficient in restriction. Spores of *B. globigii* RUB550 were treated with 0.5 M ethyl methane sulfonate (Eastman-Kodak Co.) according to the method of Ito and Spizizen (9). Standard methods of selecting "nibbled" colonies were not successful. Therefore, survivors (0.1% of the total population) were streaked onto BHI plates, incubated for 24 h at 37°C, and subsequently replicated onto BHI spread with 10^6 plaque-forming units of SP50 grown on *B. subtilis* BR151 (see Fig. 1). Of 1,200 colonies tested, 60 showed increased lysis on medium containing bacteriophage SP50. The increased lysis assay is qualitative and yields many false positives. Generally, the replicated streak of wild-type clones showed little or no lysis (less than two plaques per streak), whereas presumptive mutants had increased lysis (five plaques to confluent lysis of the streak). The clones (21/60) that had an increased efficiency of plating by standard techniques were assayed for nuclease activity. Only those clones that had qualitative alterations in enzyme activity were studied further.

Determination of endonuclease activity. Two general procedures were used for analysis of enzyme activity. Endonucleases that had been partially purified by column chromatography were assayed by incubating 2 μl of the column fraction with 2 μg of DNA in a total volume of 50 μl of 2× TMM buffer. After 10 min of incubation at 37°C, bromophenol blue, sucrose, and ethylenediaminetetraacetic acid were added, and the sample was subjected to electrophoresis on a 1% agarose gel (5). DNA from bacteriophage λ and ϕ3T was used to assay the activity of *BglI* and *BglII*, respectively.

To determine whether mutant strains synthesized endonucleases, a rapid, crude analysis was devised. Strains or clones were cultured in 100 ml of supplemented L broth to the middle of the exponential phase of growth. The cells were concentrated by centrifugation at 10,000 rpm at 4°C for 10 min in a Sorvall GSA rotor. After suspension in 1.25 ml of C buffer, cells were disrupted by sonic treatment three times for 10 s (red probe, Bronwill Biosonic III) and centrifuged for 30 min at 4°C in a Sorvall SP table-top centrifuge. A 4-μl volume of the crude supernatant was added to 1 μg of plasmid pCD1 DNA in a total volume of 50 μl of 2× TMM buffer. After 10 min of incubation at 37°C, the mixture was subjected to electrophoresis as described above. pCD1 is a chimeric plasmid consisting of pMB9 with the *thyP3* region of *B. subtilis* bacteriophage ϕ3T genome inserted at the *Eco*RI site (5). This plasmid contains four sites for *BglI* and two sites for *BglII*. Digestion of pCD1 with *BglI*, *BglII*, or *BglI* and *BglII* gives easily distinguishable patterns upon gel electrophoresis.

Purification of *BglI*. (i) Crude extract. The purification of *BglI* and *BglII* was based on the purification procedure of *BamHI* communicated to us by H. Boyer. Strain RUB561 was grown in SLBH medium at 37°C with rapid aeration to late log phase in a New Brunswick MF114 fermentor. A 50-g sample of cells was obtained from 12 liters of culture. All subsequent steps were performed at 4°C. The cells were collected by centrifugation, washed with 1 liter of 1 M KCl, and suspended in 100 ml of S buffer. Lysozyme (Worthington Biochemicals) was added (final concentration, 100 μg/ml), and the mixture was incubated for 15 min on ice. Acid-washed glass beads (10 g; 0.1-mm diameter) were added, and the cells were disrupted by sonic treatment five times for 1 min with the large probe of a Bronwill Biosonic III Sonifier. The mixture was centrifuged at 10,000 rpm for 10 min in a Sorvall SS34 rotor. The supernatant was centrifuged for 2 h at 33,000 rpm in a Beckman type 35 rotor to remove debris.

(ii) Phosphocellulose column chromatography. The crude extract was applied to a phosphocellulose column (2.5 by 40 cm bed) that had been equilibrated with S buffer. A flow rate of 1.5 ml/min was maintained by applying pressure with a peristaltic pump. The column was then washed with 250 ml of S buffer and eluted with a 1-liter, 0 to 1.0 M KCl linear gradient. Fractions of 10 ml were collected and subsequently assayed. Over 95% of the nucleic acid and protein were voided from the column. The fractions giving complete digestion of DNA eluted at approximately 0.4 M KCl. These fractions, which constituted approximately 50 ml, were pooled.

(iii) Hydroxylapatite chromatography. The pooled fractions from phosphocellulose chromatography were mixed with an equal volume of a solution containing 10 mM 2-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid, and 1 mM sodium azide. This mixture was applied to a hydroxylapatite column (HTP, Bio-Rad; 1.2 by 9.0 cm bed) that had been equilibrated with HTP buffer. The column was eluted with a 200-ml gradient of HTP buffer in which potas-

sium phosphate ranged from 25 to 250 mM, and fractions (4 ml) were assayed. *BglI* elutes at 150 to 170 mM potassium phosphate. The fractions giving complete digestion were pooled (12 ml) and dialyzed to equilibrium against buffer D. This preparation was stored at −20°C and used without further purification.

***BglII* purification. (i) Crude extract.** This procedure was the same as for *BglI* except that the bacterial strain was RUB562 and the S buffer contained 0.1 M KCl.

(ii) Phosphocellulose column chromatography. The procedure was the same as for *BglI* except that the column was eluted with an 0.1 to 0.8 M linear gradient of KCl. *BglII* elutes in a broad peak from 0.3 to 0.5 M KCl.

(iii) Hydroxylapatite column chromatography. The fractions from the phosphocellulose column giving complete digestion were pooled (100 ml) and dialyzed twice for 4 h against 6 liters of HTP buffer. The pooled fractions were then applied to a hydroxylapatite column and eluted as for *BglI*. *BglII* elutes between 75 and 85 mM potassium phosphate. Fractions giving complete digestion were pooled (12 ml) and dialyzed to equilibrium against D buffer. This enzyme was stored at −20°C and used without further purification.

RESULTS

Isolation of mutants. Endonuclease-deficient mutants were obtained according to the selection scheme illustrated in Fig. 1. Of 21 mutants that showed increased sensitivity to phage infection, 5 produced only *BglI*. One of these was designated RUB561. Two produced only *BglII*. One of these was designated RUB562. A second round of mutagenesis using spores of RUB561 yielded a strain that had lost both restriction enzymes. This strain was designated RUB560. The remaining 13 mutants were capable of making both *BglI* and *BglII* and were not characterized further.

Endonuclease from these mutants was isolated according to procedures described in Materials and Methods. A yield of approximately 15,000 U of *BglI* without detectable *BglII* was obtained from 50 g of the strain RUB561. Similar amounts of RUB562 yielded 15,000 U of *BglII* with no detectable *BglI*. We have adopted the convention that 1 U is the amount of enzyme required for limit digestion of 1 μg of lambda DNA after 30 min at 37°C, although other DNAs may display slightly different kinetics. Figure 2 shows an ethidium bromide agarose gel electrophoresis pattern of DNA treated with the purified enzymes. DNA from simian virus 40, adenovirus-2, and lambda are standards demonstrating the purity of these preparations.

Restriction and modification of bacteriophage. Bacteriophage SP50 was propagated on the three mutant strains RUB560, RUB561, and RUB562, and also on wild-type *B. globigii* (RUB550) and *B. subtilis* (BR151). Bacteriophage from each host were used to determine the efficiency of plating on the other four strains as shown in Table 2. These results demonstrate that both *BglI* and *BglII* function as restriction

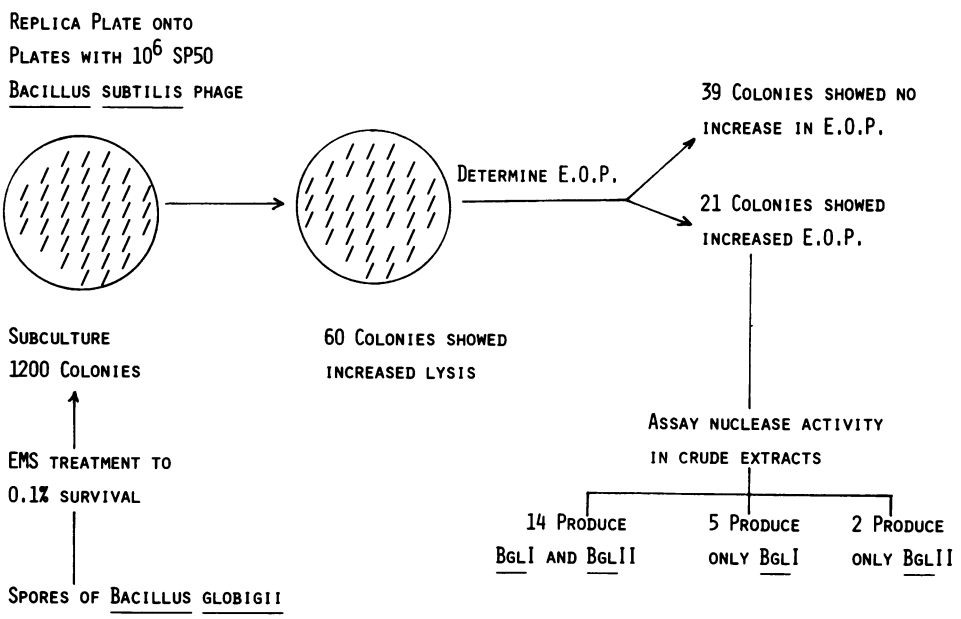

FIG. 1. *Scheme for the selection of restriction-deficient mutants of B. globigii. EMS, Ethyl methane sulfonate; E.O.P., efficiency of plating.*

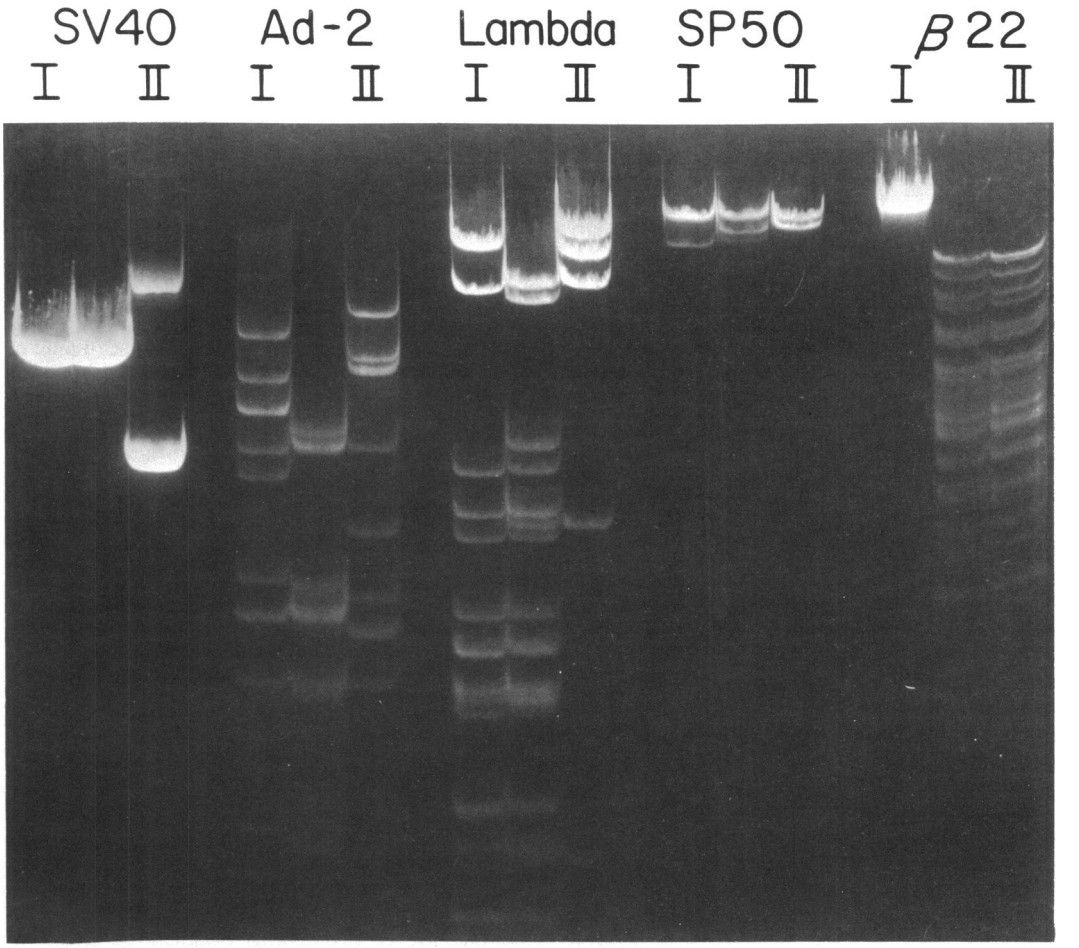

FIG. 2. *Agarose gel electrophoresis of DNA cleaved with BglI and BglII. A 2-μg sample of DNA was used in each gel slot. The DNAs were digested with 3 U of BglI, BglII, or both enzymes for 1 h at 37°C and fractionated by gel electrophoresis. The center slots in each group of three are the combined digests with BglI and BglII.*

enzymes in vivo. An analysis of the efficiency of plating of unmodified bacteriophage on the mutant strains RUB560 (R_I^- M_I^+, R_{II}^- M_{II}^+), RUB561 (R_I^+ M_I^+, R_{II}^- M_{II}^+), and RUB562 (R_I^- M_I^+, R_{II}^+ M_{II}^+) indicates that the *BglI* enzyme accounts for a 70-fold restriction of SP50 · BR151 and that *BglII* accounts for a 7-fold restriction of the bacteriophage.

These three mutant strains of *B. globigii* retained the modification characteristics of the wild-type strain. For example, bacteriophage that had been propagated on the restrictionless mutant (strain RUB560) formed plaques with equal efficiency on the strain having both enzymes (RUB550), the strain with only *BglI* (RUB561), and the strain with only *BglII* (RUB562; see Table 2).

To complement these genetic studies, we have also performed biochemical tests. Linear DNA from SP50 · *B. subtilis* BR151 was cleaved only once by the *BglI* enzyme and once by the *BglII* enzyme at different sites (see Fig. 2). DNA from bacteriophage SP50 propagated on any of the *B. globigii* strains, including the restrictionless mutant strain RUB560, was not cleaved by either *BglI* or *BglII* (data not shown). These findings correlate well with the genetic data, indicating that the mutant strains retain the site-specific modification of the wild-type strain for both enzymes.

Another bacteriophage of *B. subtilis*, β22, was found to form plaques on two strains of *B. globigii*, RUB560 and RUB561. Unfortunately, β22 gives variable, nonreproducible results in plaque assays (8), so the efficiency of plating could not be determined. However, these qualitative results were supported by the nuclease digestion patterns of β22 · *B. subtilis* BR151 DNA (see

TABLE 2. *Efficiency of plating on strains of B. globigii and B. subtilis*

Bacteriophage[a]	Host and relevant restriction-modification phenotype[b]				
	151	550 (R_I^+ M_I^+) (R_{II}^+ M_{II}^+)	560 (R_I^- M_I^+) (R_{II}^- M_{II}^+)	561 (R_I^+ M_I^+) (R_{II}^- M_{II}^+)	562 (R_I^- M_I^+) (R_{II}^+ M_{II}^+)
SP50·BR151	1.0	0.01	2.8	0.04	0.42
SP50·RUB550	0.77	1.0	0.9	1.0	1.1
SP50·RUB560	0.61	1.0	1.0	1.0	1.1
SP50·RUB561	0.67	0.9	1.0	1.0	1.1
SP50·RUB562	0.58	0.61	0.94	0.70	1.0

[a] Bacteriophage SP50 propagated on host BR151 is designated SP50·BR151.

[b] R_I^+ designates the restriction activity associated with *BglI*; M_I^+ designates the corresponding modifying activity. The same convention (R_{II} M_{II}) is used for *BglII* activity.

TABLE 3. *Transfection[a] with SP50·BR151 DNA treated in vitro with BglI and BglII*

Donor DNA	No. of plaques
Uncleaved DNA	2,000
DNA cleaved with *BglI*	0
DNA cleaved with *BglII*	0
DNA cleaved with *BglI* and *BglII*	0
DNA cleaved with *BglI* mixed with an equal amount of DNA cleaved with *BglII*	44

[a] Performed according to Foldes and Trautner (6). Each transfection used a total of 3 μg of DNA in a volume of 0.5 ml of competent cells of *B. subtilis* BR151. After 25 min of incubation at 30°C, 1.5 ml of semisolid medium was added to each sample, and the entire mixture was transferred rapidly to a petri dish containing 20 ml of solidified tryptose blood agar base.

Fig. 2). This DNA was cleaved more than 50 times by digestion with *BglII*, but was not cleaved at all by digestion with *BglI*.

Transfection with SP50 DNA. DNA from bacteriophage SP50 is highly efficient in transfection of *B. subtilis*, but SP50 DNA that has been fragmented by hydrodynamic shear is not biologically active (7). It was of interest to determine the effect of nuclease treatment on the infectivity of SP50 DNA, since this DNA is cleaved once by *BglI* or *BglII*. When competent cells of *B. subtilis* BR151 were mixed with SP50·BR151 DNA that was cleaved with *BglI*, *BglII*, or both enzymes, no plaques were observed. However, when a mixture of *BglI*-cleaved DNA and *BglII*-cleaved DNA was used, plaques were formed, as shown in Table 3.

Transfection studies with a number of different bacteriophage demonstrated that physically intact molecules of DNA are required for infectivity. For bacteriophage SP50 this was not so. A mixture containing *BglI*-cleaved SP50 DNA and *BglII*-cleaved SP50 DNA could infect competent cells, albeit with a 50-fold reduction in plaques compared to the same amount of intact DNA. Evidently, the overlapping fragments contain a region of homology, allowing them to be joined by the host cell recombination systems. This is not surprising in view of earlier work demonstrating that *rec* strains of *B. subtilis* show a greatly reduced efficiency of transfection with uncleaved SP50 DNA, and that three molecules of SP50 DNA are required in the process of transfection (15).

Transformation with modified and unmodified chromosomal DNA. Transformation, unlike transfection, has been shown to be unaffected in vivo by restriction endonucleases in the one case where it has been examined (16). However, the transforming activity of DNA is reduced by treatment with site-specific endonucleases (18). Because *B. globigii* is closely related to *B. subtilis*, it is possible to transform *B. subtilis* with DNA isolated from this heterologous source (4). Although the efficiency of transformation was low with DNA from *B. globigii*, it was possible to markedly increase the efficiency of transformation by the foreign gene simply by integrating it into the genome of *B. subtilis*. The foreign DNA, surrounded by a neighborhood of homologous DNA, is called the intergenotic DNA. It is possible to compare the efficiency of transformation of homologous (*B. subtilis*), heterologous (*B. globigii*), and intergenotic (*B. subtilis* transformed by *B. globigii* DNA) DNA in *B. subtilis* and to study the effect of nucleases on this transformation process. As indicated in Table 4, transformation to rifampin resistance by homologous DNA was quite efficient, whereas heterologous DNA transformed poorly. Intergenotic DNA was markedly more efficient than heterologous DNA even though the *rfm* gene was derived from the foreign source. Only DNA from *B. globigii* was resistant to both endonucleases, suggesting that it was modified. The foreign gene was subject to restriction in the intergenotic configuration because the host cell, *B. subtilis*, lacks the enzymes necessary for modification. Until a competent strain of *B. globigii* can be found, it is not possible to determine whether restriction of DNA occurs in vivo in this system.

DISCUSSION

B. globigii produces two type II restriction enzymes, *BglI* and *BglII* (18). *BglII* recognizes

TABLE 4. *Transformation with DNA treated in vitro with BglI and BglII*

Source of DNA	Class of donor	Enzyme treatment	Rfm^r transformants per ml	Relative frequency[a] of Rfm^r transformation	Percent inhibition by endonuclease
B. subtilis	Homologous	None	1.68×10^6	1.00	NA[b]
		BglI	4.51×10^5	0.27	73
		BglII	1.05×10^6	0.63	37
B. subtilis transformed by *B. globigii* DNA	Intergenotic	None	6.1×10^5	0.36	NA
		BglI	5.0×10^3	0.003	99
		BglII	3.82×10^4	0.02	94
B. globigii	Heterologous	None	1.20×10^5	0.07	NA
		BglI	1.25×10^5	0.07	0
		BglII	1.24×10^5	0.07	0

[a] Relative frequency of transformation is based on comparing the frequency of transformation with that obtained with homologous DNA. DNA (10 μl; approximately 3 μg) was treated with either *BglI* or *BglII* (after phosphocellulose column chromatography) for 45 min at 37°C in TMM buffer. Competent cells (*B. subtilis* RUB300, 0.5 ml) were added, and DNA uptake was allowed to proceed for 30 min. Transformation mixtures were then plated on tryptose blood agar base using a 2-ml semisolid-plus-glucose overlay agar. After 2 h at 37°C, recipients were challenged with 25 μg of rifampin in 2 ml of semisolid-plus-glucose overlay.

[b] NA, Not applicable.

the sequence A↓GATCT, leaving a 5′ tetranucleotide extension, GATC (12; S. Zain and R. J. Roberts, personal communication). However, the sequence recognized by *BglI* is not known. In 1976, B. Reilly (personal communication) found that *B. subtilis* bacteriophage SP50 would form plaques on a lawn of *B. globigii* and, in addition, SP50 propagated on *B. subtilis* was restricted when plated on *B. globigii*. These observations formed the rationale for the isolation of restriction-deficient mutants. Strains that lack one or both of the site-specific nucleases associated with *B. globigii* should be more sensitive to bacteriophage infection. By screening mutagenized clones of *B. globigii* for sensitivity to SP50 · *B. subtilis* strain BR151, we have isolated nuclease-deficient mutants. In all the single-step mutants, restriction of infectivity by bacteriophage has been reduced but not eliminated (the R$^\pm$ phenotype), while the full modification characteristics of the wild-type strain are maintained (the M$^+$ phenotype). Mutagenic treatment of one of these R$^\pm$ M$^+$ strains resulted in the isolation of an R$^-$ M$^+$ mutant. The results permit two conclusions: (i) *BglI* and *BglII* are restriction enzymes; and (ii) *BglI* and *BglII* activities are encoded by separate genes. In addition, although only a few mutants have been isolated, we may tentatively conclude that (i) the restriction enzymes do not share a common subunit necessary for activity, (ii) the modification enzyme(s) of *B. globigii* is encoded by genes distinct from the genes encoding the restriction endonucleases, and (iii) the enzymes responsible for modification do not share a gene product that is necessary for their activity in common with the restriction enzymes.

Mutants that are defective in restriction have been isolated from a number of other bacterial restriction-modification systems. Our studies have revealed that restriction and modification in *B. globigii* have features in common with these systems. For example, 44 different restriction-defective mutants have been isolated in the RI and RII host specificity systems of *E. coli*. All these mutants had the R$^-$ M$^+$ phenotype (22). Different results are obtained with the *E. coli* host specificities K, B, P1, IS, and H. Mutants selected solely on the basis of restriction deficiency are divided equally into two classes. One class shows the R$^-$ M$^+$ phenotype, while the other shows the R$^-$ M$^-$ phenotype (19). These data, along with other work, indicate that the latter modification and restriction enzymes share a protein subunit. The *H. influenzae* Ra host specificity system also show results analogous to the *B. globigii* system. Single-step restriction mutants of *H. influenzae* Ra show a R$^\pm$ M$^+$ phenotype. R$^-$ M$^+$ mutants are obtained only after a second round of mutagenesis (11). As in *B. globigii*, these data are interpreted as evidence for two separate restriction systems that do not share a gene product necessary for activity.

Aside from their usefulness as selection tools for restriction mutants, these studies revealed unexpected and anomolous facts concerning the bacteriophages SP50 and β22. Both of these virulent phages have genomes consisting of double-stranded DNA of molecular weight exceed-

ing 100×10^6 with no detectable levels of methylated bases (1, 21). SP50 DNA has a molecular weight of 100 Mdal, yet it is cleaved only once by either *BglI* or *BglII*. Based on the assumption of random nucleotide distribution, one would expect 30 cleavage sites for *BglII* and even more sites for the *BglI* enzyme. β22 DNA is cleaved more than 50 times by *BglII*, but not cleaved at all by *BglI*. While these data are consistent with our genetic studies, the phenomenon is puzzling. We do not know whether the lack of cleavage is caused by a deficiency in sites for the enzymes or by protection by methylation of the DNA or by protein associated with the DNA.

Many strains of bacteria produce two site-specific nucleases. Often the two enzymes possess similar molecular weights and ionic properties, making their separation difficult. Whereas impure preparations may be suitable for physical mapping of nuclease sites, the techniques of gene cloning and DNA sequence analysis require highly purified enzymes, free of extraneous nucleases. Removal of one enzyme activity by mutation may be a generally applicable way to solve this problem, provided two conditions can be met: (i) there exists a related strain that lacks the modification characteristics of the strain of interest; and (ii) a phage can be found that will propagate on both of the strains.

ACKNOWLEDGMENTS

We thank B. Reilly for the identification of bacteriophage that form plaques on *B. globigii*, and S. Hattman for advice and discussion.

This work was aided by grant V27 from the American Cancer Society. C.H.D. is a predoctoral fellow in the Department of Radiation Biology and Biophysics.

LITERATURE CITED

1. **Biswal, N., A. K. Kleinschmidt, H. C. Spatz, and T. A. Trautner.** 1970. Physical properties of the DNA of bacteriophage SP50. Mol. Gen. Genet. **100**:39–55.
2. **Boylan, R. J., N. H. Mendelson, D. Brooks, and F. E. Young.** 1972. Regulation of the bacterial cell wall: analysis of a mutant of *Bacillus subtilis* defective in biosynthesis of teichoic acid. J. Bacteriol. **110**:281–290.
3. **Catterall, J. F., and N. E. Welker.** 1977. Isolation and properties of a thermostable restriction endonuclease (Endo R · *Bst*1503). J. Bacteriol. **129**:1110–1120.
4. **Chilton, M. D., and B. J. McCarthy.** 1969. Genetic and base sequence homologies in bacilli. Genetics **62**:697–710.
5. **Duncan, C. H., G. A. Wilson, and F. E. Young.** 1977. Transformation of *Bacillus subtilis* and *Escherichia coli* by a hybrid plasmid pCD1. Gene **1**:153–167.
6. **Foldes, J., and T. A. Trautner.** 1964. Infectious DNA from a newly isolated *B. subtilis* phage. Z. Vererbungsl. **95**:57–65.
7. **Havender, W. R., and T. A. Trautner.** 1972. Genetic and transfection studies with *B. subtilis* phage SP50. III. Biological effects of DNA cleavage and the physical basis of the map. Mol. Gen. Genet. **116**:51–67.
8. **Hemphill, H. E., and H. R. Whiteley.** 1970. Nucleic acid synthesis in *Bacillus subtilis* infected with bacteriophage β22. J. Virol. **6**:381–392.
9. **Ito, J., and J. Spizizen.** 1971. Increased rate of asporogenous mutations following treatment of *Bacillus subtilis* spores with ethyl methane sulfonate. Mutat. Res. **13**:93–96.
10. **Kelly, T. J., and H. O. Smith.** 1970. A restriction enzyme from *Haemophilus influenzae*. II. Base sequence of the recognition site. J. Mol. Biol. **51**:393–409.
11. **Piekarowicz, A., and S. W. Glover.** 1972. Host specificity of DNA in *Haemophilus influenzae:* the two restriction and modification systems in strain Ra. Mol. Gen. Genet. **116**:11–25.
12. **Pirrotta, V.** 1976. Two restriction endonucleases from *Bacillus globigii*. Nucleic Acid Res. **3**:1747–1760.
13. **Roberts, R. J.** 1977. Restriction endonucleases, p. 757–768. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements: plasmids and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Smith, H. O., and K. W. Wilcox.** 1970. A restriction enzyme from *Haemophilus influenzae*. I. Purification and general properties. J. Mol. Biol. **51**:379–392.
15. **Spatz, H. C., and T. A. Trautner.** 1971. The role of recombination in transfection of *B. subtilis*. Mol. Gen. Genet. **113**:174–190.
16. **Trautner, T. A., B. Pawlek, S. Bron, and C. Anagnostopoulos.** 1974. Restriction and modification in *Bacillus subtilis:* biologic aspects. Mol. Gen. Genet. **131**:181–191.
17. **Wilson, G. A., and F. E. Young.** 1972. Intergenotic transformation of the *Bacillus subtilis* genospecies. J. Bacteriol. **111**:705–716.
18. **Wilson, G. A., and F. E. Young.** 1976. Restriction and modification in the *Bacillus* genospecies, p. 305–357. *In* D. Schlessinger (ed.), Microbiology—1976. American Society for Microbiology, Washington, D.C.
19. **Wood, W. G.** 1966. Host specificity of DNA produced by *Escherichia coli:* bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. **16**:118–133.
20. **Yasbin, R. E., G. A. Wilson, and F. E. Young.** 1975. Transformation and transfection in lysogenic strains of *Bacillus subtilis*: evidence for selective induction of prophage in competent cells. J. Bacteriol. **121**:296–304.
21. **Yehle, C. O., and R. H. Doi.** 1967. Differential expression of bacteriophage genomes in vegetative and sporulating cells of *Bacillus subtilis*. J. Virol. **1**:935–947.
22. **Yoshimori, R., D. Roulland-Dussoix, and H. W. Boyer.** 1972. R factor-controlled restriction and modification of deoxyribonucleic acid: restriction mutants. J. Bacteriol. **112**:1275–1279.