Journal of Bacteriology, Oct. 1976, p. 473-476
Copyright © 1976 American Society for Microbiology

Vol. 128, No. 1
printed in U.S.A.

NOTES

Site-Specific Deoxyribonucleases in *Bacillus subtilis* and Other *Bacillus* Strains

TAKEHIKO SHIBATA,* SHUKUKO IKAWA, CHOLUNG KIM, AND TADAHIKO ANDO
Laboratory of Microbiology, The Institute of Physical and Chemical Research, Wako-shi, Saitama 351, Japan

Received for publication 21 July 1976

We systematically studied site-specific deoxyribonucleases in *Bacillus* strains and detected deoxyribonuclease activities in 20 of 62 strains tested.

Recently, site-specific deoxyribonucleases (DNases) (endonucleases) that cleave deoxyribonucleic acid (DNA) strands at unique sites were found in various kinds of microorganisms. (5, 9; R. J. Roberts, personal communication); many of them are of the so-called type II restriction endonuclease (1). In *Bacillus* species, only three strains were reported to possess site-specific DNases; *B. subtilis* strain R (Endo.R.*Bsu*R) (2, 3) and *B. amyloliquefaciens* (*B. subtilis*) strain H (Endo.R. *Bam*HI) (12) and strain N (Endo.R.*Bam*NI and Endo.R.*Bam*Nx) (8; T. Shibata and T. Ando, Biochim. Biophys Acta, in press). Therefore, we systematically studied the distribution of site-specific DNases in *Bacillus* strains.

Cell extracts were prepared as follows. Cells harvested from 150 ml of a culture grown overnight at 30°C in Penassay broth (Difco) or from 150 ml of an early stationary-phase culture grown in CI medium (8) at 37°C were suspended in 1.5 ml of 20 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (pH 7.5) containing 2 mM $MgCl_2$, 0.1 mM ethylenediaminetetraacetate (EDTA), and 2 mM 2-mercaptoethanol. The cells were treated with lysozyme (0.3 mg/ml) for 30 min at 37°C and then sonically oscillated (ultrasonic disruptor UR200P, Tomy Seiko Co., Tokyo, Japan) three to six times at 20 kHz for 15 s in an ice-water bath. Cell debris was removed by centrifugation at 80,000 × *g* for 60 min at 3°C (fraction I), and a streptomycin sulfate supernatant fraction (fraction II) was prepared (8). Since site-specific DNases cleave DNA into fragments characteristic of each enzyme, their activities and specificities were determined by examining the electrophoretic patterns of treated DNA (6). A 0.3-μg sample of each DNA was treated with a cell extract (fraction I or fraction II, 0.1 to 0.7 mg of protein) for 50 min at 37°C in 90-μl reaction mixtures containing 50 mM Tris-hydrochloride buffer (pH 7.5), 5 mM $MgCl_2$, 0.2 mM EDTA, and 5 mM 2-mercaptoethanol. The treated DNAs were extracted with phenol, precipitated by the addition of cold ethanol, and dissolved in 25 μl of 50 mM Tris-hydrochloride buffer (pH 7.5) containing 0.2 mM EDTA, 8% sucrose, and 0.01% bromophenol blue. Then, 10-μl samples were subjected to electrophoresis on a 0.7% agarose gel slab, with E buffer in the presence of 0.5 μg of ethidium bromide per ml (6), at 110 V for 1.5 to 2 h at room temperature.

We tested 34 strains of *B. subtilis*, including almost all of those available from the culture collection of Institute of Applied Microbiology (University of Tokyo): 2 strains of *B. amyloliquefaciens*, 5 strains of *B. cereus*, 4 strains of *B. licheniformis*, 7 strains of *B. megaterium*, 2 strains of *B. polymyxa*, 7 strains of *B. pumilus*, and 1 strain of *B. sphaericus*. Table 1 shows the strains from which site-specific DNase activities were detected; Fig. 1 shows the electrophoretic patterns of various treated DNAs.

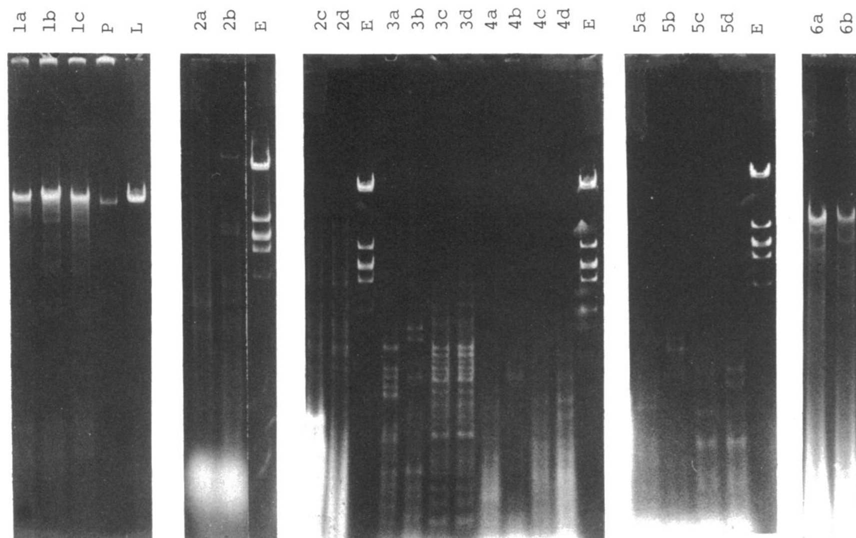
It is interesting that the site-specific DNase of *B. subtilis* Marburg 168 is apparently not a restriction enzyme because this enzyme was as active on DNA from phage ϕ105C·168 (grown on this strain) as on DNA from phage ϕ105C·N (grown on *B. amyloliquefaciens* N), which is restricted by *B. subtilis* 168 in vivo (7) (Fig. 1: 1b and 1c).

The DNases from *B. amyloliquefaciens* strains F and K and from *B. subtilis* IAM 1259 have the same specificity as that of Endo.R.*Bam*HI or Endo.R.*Bam*NI because; (i) the electrophoretic patterns of the treated phage λ DNA were identical, (ii) they were inactive on phage ϕ105C·168 DNA (12; Shibata and Ando, in press), and (iii) they were inactive on phage λ DNA carrying a *Bam*HI-type modification (Fig. 1: 9a, 9b, 10, 11a through 11c, 12a,

TABLE 1. *Bacillus strains from which site-specific DNases were detected*

Strain	DNase[a]	Origin[b] reference	Note	No. in Fig. 1
B. subtilis				
Marburg 168 (GSY 1026)	Endo.*BsuM*	Hoch et al. (4)		1
ATCC 6633	Endo.R.*Bsu*6633	ATCC		2
IAM 1076	Endo.R.*Bsu*1076	IAM		7
IAM 1114	Endo.R.*Bsu*1114	IAM		8
IAM 1145	Endo.R.*Bsu*1145	IAM	ATCC 14593	6
IAM 1192	Endo.R.*Bsu*1192	IAM		5
IAM 1193	Endo.R.*Bsu*1193	IAM		3
IAM 1231	Endo.R.*Bsu*1231	IAM		4
IAM 1247	Endo.R.*Bsu*1247	IAM		16
IAM 1259	Endo.R.*Bsu*1259I	IAM		12
B. amyloliquefaciens				
F	Endo.R.*Bam*FI	Welker and Campbell (11); stock of T. Kaneko (this institute)	ATCC 23350	9
K	Endo.R.*Bam*KI	Welker and Campbell (11); stock of T. Kaneko (this institute)		11
B. cereus				
ATCC 14579	Endo.R.*Bce*14579	ATCC		13
Rf sm st	Endo.R.*Bce*R			14
IAM 1229	Endo.R.*Bce*1229	IAM		17
Bacillus sp.				
170	Endo.R.*Bce*170	Sunaga et al. (10)	*B. cereus*	15
B. megaterium				
899	Endo.R.*Bme*899	Institute Pasteur, Paris		20
B205-3	Endo.R.*Bme*205	Stock of T. Kaneko (this institute)		18
B. pumilus				
AHU 1387	Endo.R.*Bpu*1387	AHU; stock of Y. Sasaki		19
B. sphaericus				
IAM 1286	Endo.R.*Bsp*1286	IAM		21

[a] According to the nomenclature of Smith and Nathans (9).
[b] Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; IAM, Culture Collection of Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; AHU, Culture Collection of the Faculty of Agriculture, Hokkaido University, Sapporo, Japan.



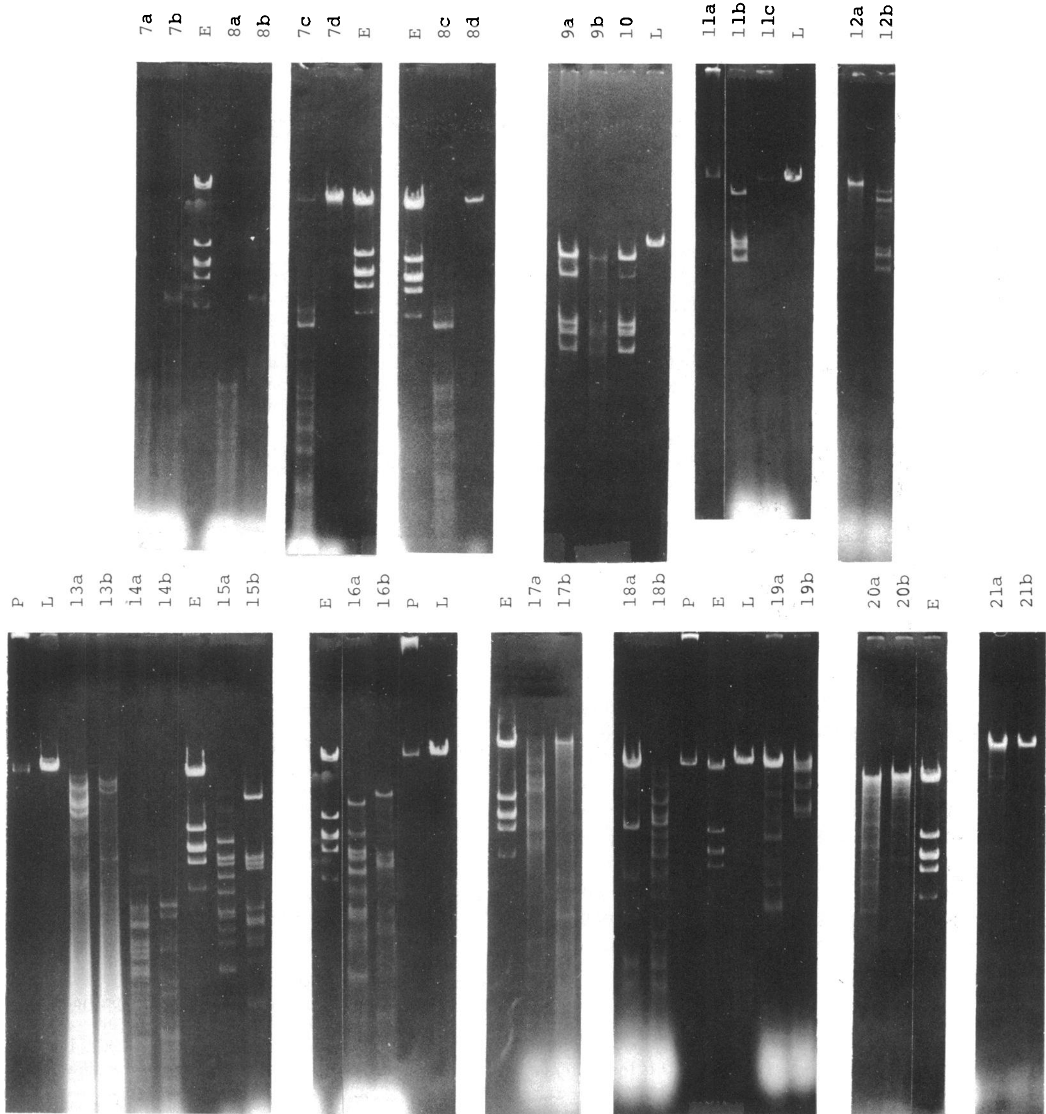


FIG. 1. *Electrophoretic patterns of phage DNAs treated with site-specific DNase from Bacillus strains. Phage DNAs were treated with a cell extract (fraction I or fraction II). The treated DNAs were purified and subjected to electrophoresis through a 0.7% agarose gel slab. P, Untreated Bacillus phage ϕ105C DNA; L, untreated E. coli phage λ DNA; E, phage λ DNA treated with Endo.R.EcoRI. (1) DNAs treated with fraction II from B. subtilis Marburg 168: a, λ DNA; b, ϕ105C·N (grown on B. amyloliquefaciens N) DNA; c, ϕ105C·168 (grown on B. subtilis Marburg 168) DNA. (2) DNAs treated with fraction I from B. subtilis ATCC 6633: a, ϕ105C·168 DNA; b, λ DNA; c, Bacillus phage SPP1·168 DNA; d, SPP1·R (grown on B. subtilis R) DNA. (3) DNAs treated with fraction II from B. subtilis IAM 1193: a, ϕ105C·168 DNA; b, λ DNA; c, SPP1·168 DNA; d, SPP1·R DNA. (4) DNAs treated with fraction II from B. subtilis IAM 1231: a, ϕ105C·168 DNA; b, λ DNA; c, SPP1·168 DNA; d, SPP1·R DNA. (5) DNAs treated with fraction II from B. subtilis IAM 1192: a, ϕ105C·168 DNA; b, λDNA; c, SPP1·168 DNA; d, SPP1·R DNA. (6) DNAs treated with fraction I from B. subtilis IAM 1145: a, ϕ105C·168 DNA; b, λ DNA. (7) DNAs treated with fraction II from B. subtilis IAM 1076: a, ϕ105C·168 DNA; b, λ DNA; c, SPP1·168 DNA; d, SPP1·R DNA. (8) DNAs treated with fraction II from B. subtilis IAM 1114: a, ϕ105C·168 DNA; b, λ DNA; c, SPP1·168 DNA; d, SPP1·R DNA. (9) DNAs treated with fraction II from B. amyloliquefaciens F: a, λ DNA; b, λ DNA previously treated with purified Endo.R.BamNI. (10) λ DNA treated with purified Endo.R.BamNI. (11) DNAs treated with fraction II from B. amyloliquefaciens K: a, ϕ105C·168 DNA; b, λ DNA; c, λ DNA previously modified into type BamHI with cell extract from B. amyloliquefaciens H. (12) DNAs treated with fraction II from B. subtilis IAM 1259: a, ϕ105C·168 DNA; b, λ DNA. (13) DNAs treated with fraction I from B. cereus ATCC 14579: a, ϕ105C·168 DNA; b, λ DNA. (14) DNAs treated with fraction II from B. cereus Rf sm st: a, ϕ105C·168 DNA; b, λ DNA. (15) DNAs treated with fraction II from Bacillus sp. 170: a, ϕ105C·168 DNA; b, λ DNA. (16) DNAs treated with fraction II from B. subtilis IAM 1247: a, ϕ105C·168 DNA; b, λ DNA. (17) DNAs treated with fraction II from B. cereus IAM 1229: a, ϕ105C·168 DNA; b, λ DNA. (18) DNAs treated with fraction II from B. megaterium B205-3: a, ϕ105C·168 DNA; b, λ DNA. (19) DNAs treated with fraction II from B. pumilus AHU 1387: a, ϕ105C·168 DNA; b, λ DNA. (20) DNAs treated with fraction II from B. megaterium 899: a, ϕ105C·168 DNA; b, λ DNA. (21) DNAs treated with fraction II from B. sphaericus IAM 1286: a, ϕ105C·168 DNA; b, λ DNA.*

12b). All strains of *B. amyloliquefaciens* tested (N, F, K, and H) possess the Endo.R.*Bam*HI-type enzyme.

The DNases from *B. subtilis* IAM 1076 and IAM 1114 exhibited the same specificity (Fig. 1: 7a through 7d, 8a through 8d) and were inactive on phage SPP1 DNA when it carried the *Bsu*R-type modification (Fig. 1: 7c, 7d, 8c, 8d). The site-specific DNases from *B. subtilis* strains ATCC 6633, IAM 1193, IAM 1231, and IAM 1192 cleaved DNA into comparatively small pieces, as does Endo.R.*Bsu*R (2) (Fig. 1: 2a through 2d, 3a through 3d, 4a through 4d, 5a through 5d), but they differ from Endo.R.*Bsu*R because the DNases from strains ATCC 6633, IAM 1193, IAM 1231, and IAM 1192 are active on phage SPP1 DNA either with or without the *Bsu*-R-type modification (Fig. 1: 2c, 2d, 3c, 3d, 4c, 4d, 5c, 5d).

The DNase from *B. subtilis* IAM 1247 is the same as that from *Bacillus* sp. 170 (which belongs to *B. cereus* [10]), judged from the electrophoretic patterns (Fig. 1: 15a, 15b, 16a, 16b). The Endo.R.*Bam*Nx-type endonuclease was not detected in cell extracts from any of the strains examined in these studies. Very weak site-specific DNase activities were detected in 11 other strains of *Bacillus*. These results indicate that *Bacillus* strains frequently possess site-specific DNases and that their specificity could be a good character for classification of microorganisms in this genus.

We thank Y. Sasaki and K. Horikoshi (this Institute) for the generous supply of strains.

This study was supported by a grant for studies on "Life Science" at this Institute and by a grant from the Ministry of Education of Japan.

LITERATURE CITED

1. **Boyer, H. W.** 1971. DNA restriction and modification mechanisms in bacteria, p. 153-176. *In* C. E. Cliftom, S. Raffel, and M. P. Starr (ed.), Annual review of microbiology, vol. 25. Annual Reviews Inc., Palo Alto, Calif.
2. **Bron, S., and K. Murray.** 1975. Restriction and modification in *B. subtilis*. Nucleotide sequence recognized by restriction endonuclease R.*Bsu*R from strain R. Mol. Gen. Genet. 143:25-33.
3. **Bron, S., K. Murray, and T. A. Trautner.** 1975. Restriction and modification in *B. subtilis*. Purification and general properties of a restriction endonuclease from strain R. Mol. Gen. Genet. 143:13-23.
4. **Hoch, J. A., M. Barat, and C. Anagnostopoulos.** 1967. Transformation and transduction in recombination-defective mutants of *Bacillus subtilis*. J. Bacteriol. 93:1925-1937.
5. **Nathans, D., and H. O. Smith.** 1975. Restriction endonucleases in the analysis and restructuring of DNA molecules, p. 273-293. *In* E. E. Snell (ed.), Annual review of biochemistry, vol. 44. Annual Reviews Inc., Palo Alto, Calif.
6. **Sharp, P. A., B. Sugden, and J. Sambrook.** 1973. Detection of two restriction endonuclease activities in *Haemophilus parainfluenzae* using analytical agarose-ethidium bromide electrophoresis. Biochemistry 12:3055-3063.
7. **Shibata, T., and T. Ando.** 1974. Host controlled modification and restriction in *Bacillus subtilis*. Mol. Gen. Genet. 131:275-280.
8. **Shibata, T., and T. Ando.** 1975. *In vitro* modification and restriction of phage ϕ105C DNA with *Bacillus subtilis* N cell-free extract. Mol. Gen. Genet. 138:269-279.
9. **Smith, H. O., and D. Nathans.** 1973. A suggested nomenclature for bacterial host modification and restriction systems and their enzymes. J. Mol. Biol. 81:419-423.
10. **Sunaga, T., T. Akiba, and K. Horikoshi.** 1976. Production of penicillinase by an alkalophilic Bacillus. Agric. Biol. Chem. (Japan) 40:1363-1367.
11. **Welker, N. E., and L. L. Campbell.** 1967. Unrelatedness of *Bacillus amyloliquefaciens* and *Bacillus subtilis*. J. Bacteriol. 94:1124-1130.
12. **Wilson, G. A., and F. E. Young.** 1975. Isolation of a sequence-specific endonuclease (*Bam* I) from *Bacillus amyloliquefaciens* H. J. Mol. Biol. 97:123-125.