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Physical Maps of the Genomes of Three *Bacillus cereus* Strains

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***Not*I restriction maps of the chromosomes from *Bacillus cereus* ATCC 10876, ATCC 11778, and the *B. cereus* type strain ATCC 14579 have been established and compared with the previously established map of *B. cereus* ATCC 10987. Between 10 and 14 *Not*I fragments were observed, ranging from 15 to 1,300 kb, in digests of DNA from the various strains. The sizes of the genomes varied between 5.4 and 6.3 Mb. The maps were constructed by hybridization of 42 random probes, prepared from *B. cereus* ATCC 10987 libraries, to fragments from partial and complete *Not*I digests, separated by pulsed-field gel electrophoresis. Nine probes were specific for ATCC 10987 only. Probes for five *B. subtilis* and five *B. cereus* genes were also used. The *Not*I restriction fragment patterns of the four strains were strikingly different.**

The genus *Bacillus* is generally recognized as being more heterogeneous than most other bacterial genera. For instance, species of the same genus of most bacteria usually do not differ more than 10 to 15% in their DNA base composition, whereas *Bacillus* strains have a range of 32 to 69 mol% G+C content (17).

Classification of *Bacillus* species is based on the study of about 50 supposedly independent phenotypic properties (21). Even if all of these phenotypes resulted from the interaction of several gene products, they would hardly make up more than 5 to 10% of the total genome. An alternative strategy for comparison of strains might be to obtain an overall view of the whole genome by making physical maps.

Bacillus cereus has a G+C content of 36%, making it suitable for analysis with the restriction endonuclease *Not*I, which recognizes the sequence GCGGCCGC. By using pulsed-field gel electrophoresis (PFGE), a physical map may be constructed without a previous genetic map of the organism. We have recently established the complete physical map of the *B. cereus* ATCC 10987 chromosome by aligning its 11 *Not*I fragments (9). We have now analyzed the *Not*I restriction fragment patterns of three additional *B. cereus* strains obtained from American Type Culture Collection. Although the size of the genome and the number of *Not*I fragments were very similar in all four strains, the *Not*I fragment patterns obtained by PFGE were strikingly different.

MATERIALS AND METHODS

Bacterial strains. *B. cereus* ATCC 10987, ATCC 10876, ATCC 11778, and ATCC 14579 were obtained from the American Type Culture Collection. Strain ATCC 14579 is the type strain (20). Strain ATCC 10876 is also called *B. cereus* 569 (2), and this strain and its derivative have been widely used for the study of β-lactamases (1, 13, 14). Strain ATCC 11778 has been used as a test organism for tetracycline antibiotics (3). It has been claimed to be a mycoides subspecies of *B. cereus*, although it is not grouped in the *B. cereus* subsp. *mycoides* subcluster (18). Strain ATCC 10987 is a xylose-positive variant that we have previously worked with because of its high production of phospholipase C (PLC) (15).

Preparation of DNA. Bacteria were grown at 37°C, cast in agarose blocks, and further treated to remove all bacterial components except DNA, as described previously (9).

Digestion of DNA in agarose blocks with *Not*I. The DNA was digested with *Not*I essentially as described previously (9), except for an overnight preincubation period at 0°C after the addition of *Not*I. This step was included to ensure sufficient diffusion of the enzyme mixture into the agarose block. For complete digests 20 U of *Not*I for 8 h was used per agarose block, whereas for partial digests 5 U for 10 min was used.

Electrophoresis. (i) Method. A PFGE instrument (Beckman) was routinely used. Apparatuses from Bio-Rad Laboratories, Richmond, Calif., and from Pharmacia, Uppsala, Sweden, with hexagonal electrodes were used in some experiments. In all experiments the electrophoresis buffer was 25 mM Tris-borate buffer (pH 8.0)–0.05 mM EDTA (0.25× TBE). Pulse times of 5 to 120 s were used. Regular electrophoresis was run in 1× TBE at 20 to 100 V in 0.8% agarose.

(ii) Size markers. *Saccharomyces cerevisiae* chromosomes (size range, 260 to 1,300 kb) were obtained from Pharmacia or Bio-Rad. Other markers were phage λ concatemers (Pharmacia) and a mixture of λ concatemers and *Hin*dIII fragments (New England Biolabs). The sizes of the *Not*I fragments were calculated from the sizes of the yeast chromosomes.

Probes. The Bc probes were DNA fragments from the *B. cereus* ATCC 10987 genome, isolated from *B. cereus* libraries in pUC18, λgt10, and λgt11 vectors. *Hin*dIII (New England Biolabs) was used to construct the library in pUC18, and *Eco*RI (New England Biolabs) was used for the lambda libraries. Probes numbered Bc6 to Bc204 were from a pUC18 library, and 36 of them were used in these experiments.

B. cereus PLC probe (8) was donated by T. Johansen, Tromsø, Norway; *B. cereus* phosphatidylinositol-specific phospholipase C (PI-PLC) (10) was donated by A. Kuppe, University of Oregon, Eugene; *B. subtilis* pyruvate dehydrogenase (6) was donated by H. Hemilä, University of Tromsø, Helsinki, Finland; *B. cereus* β-lactamases I and II (14) were donated by J. O. Lampen, The State University of New Jersey, Piscataway; pAKI covering *recF*, *gyrA*, and *gyrB*

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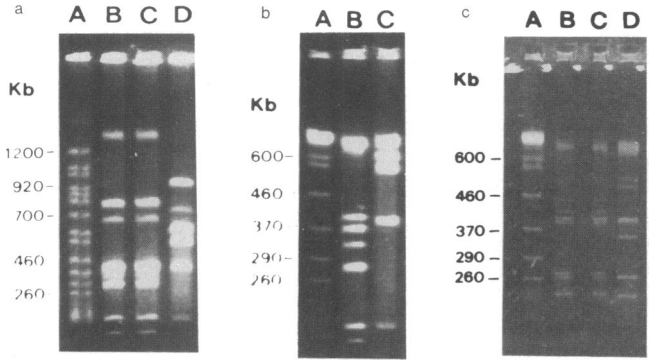

FIG. 1. PFGE of DNA from *B. cereus* strains digested with *Not*I. A Beckman apparatus was used with a 60-s pulse for 18 h at 150 mA for panel a and a 30-s pulse for 20 h at 150 mA for panels b and c. (a) Lanes: A, *S. cerevisiae* chromosomes (standard); B and C, *B. cereus* ATCC 14579; D, *B. cereus* ATCC 10876. (b) Lanes: A, *S. cerevisiae* chromosomes (standard); B, *B. cereus* ATCC 14579; C, *B. cereus* ATCC 10876. (c) Lanes: A, *S. cerevisiae* chromosomes (standard); B and C, *B. cereus* ATCC 10987; C, *B. cereus* ATCC 11778. A partial *Not*I fragment of 550 kb is visible in lane C.

(origin region) (11) as well as genes for the rRNA operon (p14B8) (22) from *B. subtilis* was donated by K. Bott, University of North Carolina, Chapel Hill; *B. subtilis* TerC was donated by R. G. Wake, University of Sydney, Sydney, Australia; and *B. subtilis* AbrB was donated by O.-M. Gulliksen, Oslo, Norway.

Southern blotting and hybridization. The gels were blotted overnight in 20× SSC (1× SSC is 0.3 M NaCl plus 0.03 M sodium citrate) onto Hybond-C Extra (Amersham Corp., Amersham, United Kingdom) as described previously (9) and hybridized at 65°C overnight in 3× SSC–0.1% sodium dodecyl sulfate (SDS)–10× Denhardt solution (1× Denhardt solution is 0.02% polyvinylpyrrolidone, 0.02% Ficoll 400, and 0.02% bovine serum albumin) containing 10% dextran sulfate (Pharmacia) (9). The filters were washed twice for 30 min at 65°C in 3× SSC–0.1% SDS, twice in 1× SSC–0.1% SDS, and twice in 0.3× SSC–0.1% SDS. When less stringent conditions were used, some of the washes were omitted. In some experiments a temperature of 55°C was used for hybridization and washing.

RESULTS

***Not*I fragments.** Whereas the *B. subtilis* genome has been reported to give more than 72 fragments after digestion with *Not*I (7), *B. cereus* ATCC 10987 has only 11 *Not*I sites (9). The three other *B. cereus* strains have 10 (ATCC 10876), 13 (ATCC 14579), and 14 (ATCC 11778) *Not*I sites. Several electrophoretic runs with different pulse times were necessary to obtain good separation of the fragments (Fig. 1). The pulse time varied from 2 to 90 s. For each strain more than 40 gels were analyzed. The fragment sizes for the four strains are given in Table 1. The size of the total genomes varied from 5,440 to 6,260 kb. Since fragments below 20 kb may not be visible in PFGE, we also analyzed *Not*I fragments from the four strains by regular agarose gel electrophoresis. No fragments below 20 kb were detected by ethidium bromide staining, except for a plasmid band of apparent size 15 kb in strain ATCC 14579 (data not shown). Strains ATCC 14579, ATCC 10876, and ATCC 10987 contained plasmids that were easily visible in PFGE gels when one-half to one plug was used, without restriction enzyme treatment.

TABLE 1. Sizes of *Not*I fragments in four *B. cereus* strains

Fragment	Size[a] (kb) in strain:			
	ATCC 14579	ATCC 10987	ATCC 11778	ATCC 10876
A	1,300	1,300	1,000	1,030
B	840	940	980	1,000
C	820	840	790	780
D	700	460	770	670
E	400	430	500	600
F	370	410	420	580
G	340	400	420	390
H	290	260	360	390
I	280	250	270	50
J	50	210	270	40
K	40	200	200	
L	40		200	
M	15		50	
N			40	
Total	5,485	5,700	6,270	5,530

[a] Without plasmids.

Hybridization. The filters obtained after Southern blotting of the DNA fragments in pulsed-field gels were hybridized to the probes, and at least two different pulse times were used for each probe. Some of the probes hybridized to fragments of similar size in all the strains, whereas other probes hybridized to large fragments in some strains and to smaller fragments in other strains. The PLC probe always hybridized to one of the largest *Not*I fragments. The probes for PLC and PI-PLC never hybridized to the same *Not*I fragment. PI-PLC appeared to be absent in strain ATCC 10876.

An example of the hybridization results is shown in Fig. 2; pAKI hybridized to a 420-kb fragment in ATCC 11778 and to a 600-kb fragment in ATCC 10876, and Bc48 hybridized to a 770-kb fragment in ATCC 11778 and to a 400-kb fragment in ATCC 14579. Hybridization to these fragments was verified in other experiments with shorter pulse times.

The plasmid probe p14B8 from *B. subtilis*, containing one set of rRNA genes (22), hybridized to a large fragment in all strains and to an additional fragment in all strains except the type strain, ATCC 14579. High-stringency conditions were used in these experiments (washing at 65°C in 0.3× SSC–0.1% SDS), yet very strong signals were observed with this

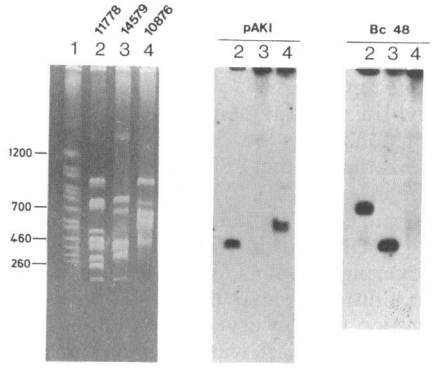

FIG. 2. *Not*I fragments of DNA from three *B. cereus* strains separated by PFGE in a Beckman apparatus with a 60-s pulse for 20 h at 150 mA. Lane 1 contains *S. cerevisiae* chromosomes. The gel was blotted onto nitrocellulose, hybridized with pAKI, and rehybridized with probe Bc48.

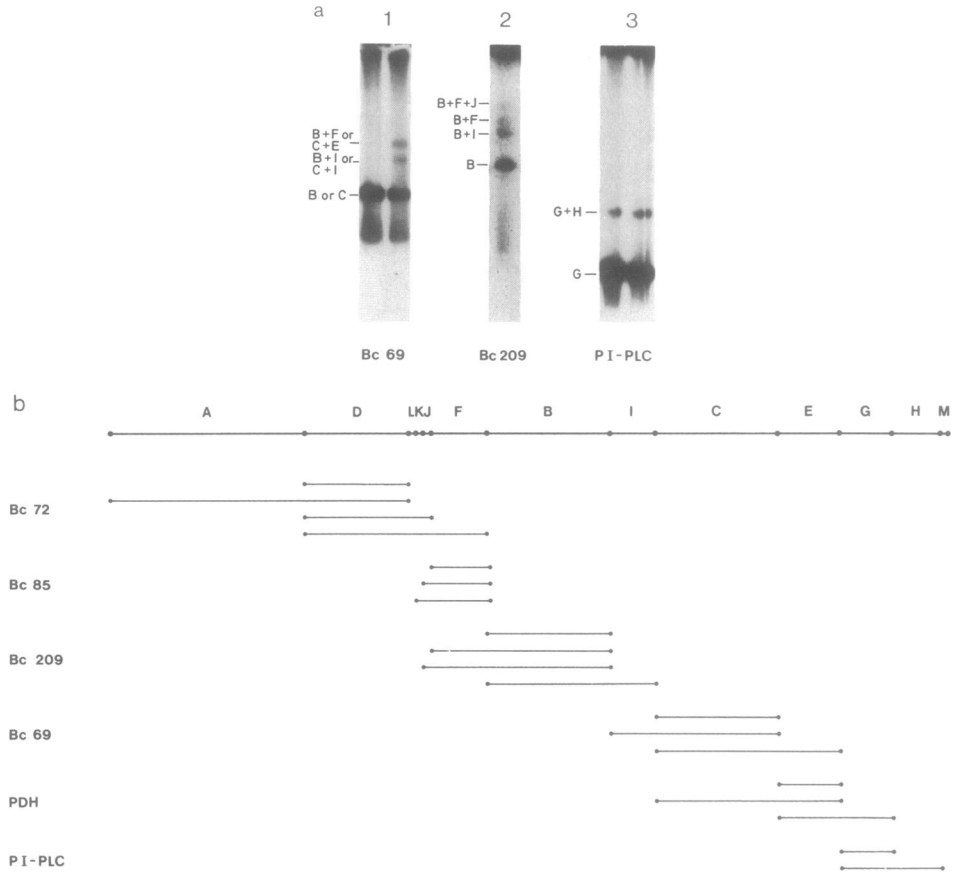

FIG. 3. Construction of the physical map of the strain ATCC 14579 chromosome. (a) Fragments after partial *Not*I digests separated at 60-s pulses for 20 h at 150 mA (Beckman apparatus). The gel was blotted and hybridized to the probes indicated. (b) Assignments of fragments of the strain ATCC 14579 chromosome. Results are from more than 30 hybridization experiments on 20 separate gels.

probe, probably owing to the high similarity between the rRNA genes of *B. cereus* and *B. subtilis* (21) and to the high copy number of these genes (22). These results were confirmed by hybridization with Bc88, which, by sequencing, was identified as part of the gene for 23S rRNA, starting at nucleotide 100 with respect to the sequence from *B. subtilis*. The sequence homology was 91%.

For strain ATCC 10987 probe Bc37 was a linker clone spanning a *Not*I site between fragment A (1,300 kb) and fragment K (200 kb).

No hybridization to any *Not*I fragment of strain ATCC 10987 was obtained with the *B. subtilis* plasmid probe pAKI (covering *recF*, *gyrA*, *gyrB*, and the replication origin) (11) even at a hybridization temperature of 55°C and less stringent washing conditions (3× SSC–0.1% SDS at 55°C). Very weak hybridization to the 1,300-kb fragment was observed for this probe in strain ATCC 14579 (Fig. 2). To test whether pAKI was localized on a small *Not*I fragment in strain ATCC 10987, 10 μg of DNA from strain ATCC 10987 was digested with *Not*I overnight, subjected to regular electrophoresis, blotted onto a nitrocellulose filter, and hybridized to pAKI. No hybridization was observed, even under low-stringency conditions. Hybridization was obtained for strains ATCC 10876 and ATCC 11778 at a stringency of 0.3× SSC–0.1% SDS at 65°C. The *B. subtilis* TerC probe, did not hybridize to strains ATCC 10987 and ATCC 14579 even under low-stringency conditions, whereas it hybridized, although weakly, to the two other strains at a stringency of 1× SSC–0.1% SDS at 65°C. Hybridization to smaller fragments was excluded after regular electrophoresis for strains ATCC 10987 and ATCC 14579.

The smallest fragments (15 to 50 kb) were separated by using 5-s pulses for 18 h. No probes hybridized to any of these fragments.

Construction of the maps. By hybridizing blots from partial *Not*I fragments, we determined the sizes of the neighboring fragments (see Fig. 3 to 5). The filters were reprobed four to six times with different probes. The physical maps are based on more than 150 hybridization experiments.

(i) ***B. cereus* ATCC 14579.** For strain ATCC 14579 the alignment of the fragments is illustrated in Fig. 3, which shows the hybridization results of six selected probes. When Bc72 was used to probe partial *Not*I digests, bands of 1.2 Mb (fragments D, L, K, J, and F), 830 kb (fragments D, L, K, and J), and 700 kb (fragment D) were seen. In addition, a band of >1.5 Mb was seen, interpreted as fragments A+D. Further experiments with higher resolution in the appropriate size range showed that the difference between the 830- and the 700-kb fragments consisted of three smaller fragments of 50 kb (fragment J) and 40 kb (fragments K and L) (Fig. 3b). Similarly, when Bc85 was used as a probe on a blot in which the DNA fragments had been separated at a 20-s pulse time, the 370-kb fragment (fragment F) was flanked by a 50-kb fragment (fragment J) followed by a 40-kb fragment (fragment K) (Fig. 3b). Fragments K and L (40 kb) have never been separated, but in the gels where the 40- and 50-kb

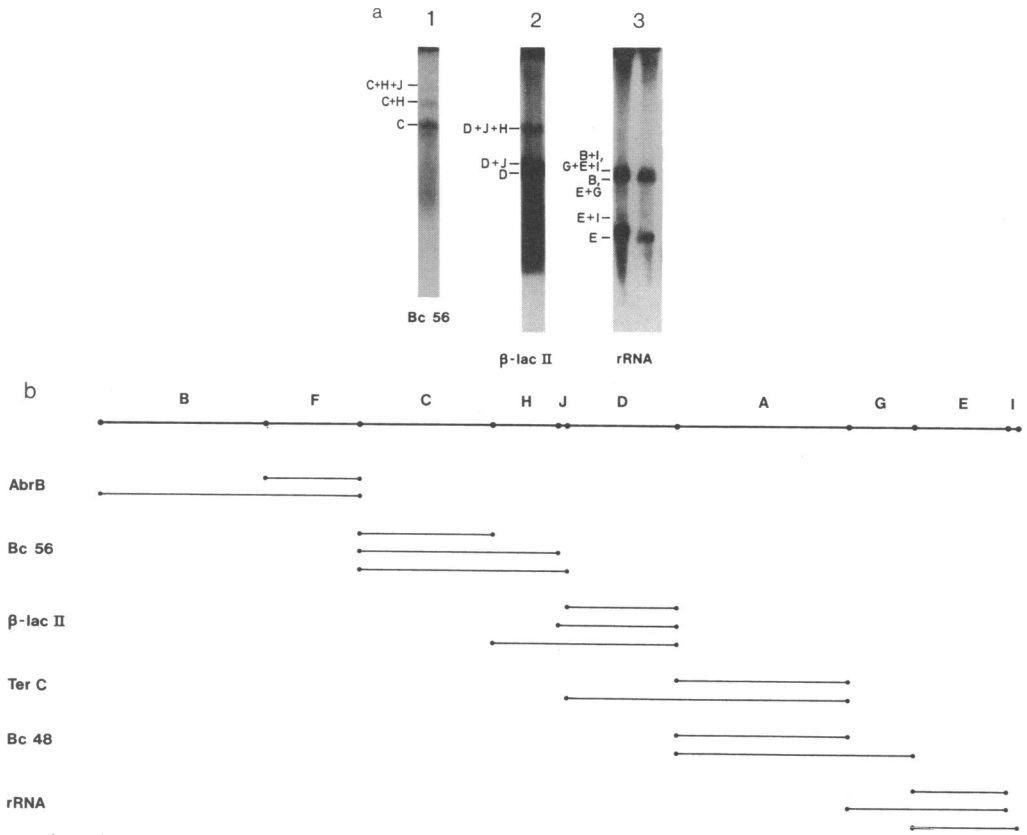

FIG. 4. Construction of the physical map of the strain ATCC 10876 chromosome. (a) Fragments after partial *Not*I digests separated at 60-s pulses for 20 h at 150 mA (Beckman apparatus) and hybridized to the probes indicated. (b) Assignments of fragments of the strain ATCC 10876 chromosome. Results are from more than 30 hybridization experiments on 20 separate gels.

fragments were well separated, the 40-kb band was wider and showed more intense staining, suggesting a double band.

Fragments B (840 kb) and C (820 kb) of strain ATCC 14579 were difficult to separate. Probes that hybridized to one of these fragments gave similar signal patterns (Fig. 3) because both fragments were neighbors of fragment I (280 kb). Separation was obtained only after long pulse times (60 to 90 s). The fragments could then be ordered in partial *Not*I digests linking on one side fragments B, F, and J and on the other side fragments B and I (probe Bc209; Fig. 3).

Fragment E (400 kb) was flanked by fragments C and G, whereas hybridization with the probe for PI-PLC showed that fragment G (340 kb) was flanked by fragments E and H (290 kb) (Fig. 3).

When *Not*I-digested DNA from strain ATCC 14579 (one-sixth of a block) was run in parallel with undigested DNA from the same strain (one-half of a block), a 15-kb band corresponding to the plasmid harbored by this strain was seen in both lanes. In the lane with *Not*I-digested DNA, however, the intensity and area of the band were markedly increased, although less DNA was applied, indicating the presence of a double band and thus a genomic fragment of about this size. This was in accordance with results of partial digests, when where a weak band was seen just above the 280/290-kb double band (data not shown).

(ii) *B. cereus* ATCC 10876. The *Not*I fragments of strain ATCC 10876 were aligned in the same way as for strain ATCC 14579. Hybridization with a probe for β-lactamase II gave strong signals corresponding to the double band of fragments D and D+J (670 kb plus 40 kb), and weaker signals corresponding to the partial fragment D+J+H (Fig. 4). PFGE of undigested DNA showed a distinct band of a plasmid of apparent size 650 kb, to which the β-lactamase II probe did not hybridize. The two smaller fragments of 40 kb (fragment J) and 50 kb (fragment I) were separated by using a 5-s pulse time. The 40-kb fragment was sometimes more distinct than the 50-kb fragment, but no hybridization experiments indicated more than two small fragments. By hybridization of partial *Not*I digests (probe Bc56), the fragment order was determined to be C-H-J (Fig. 4). The rRNA gene sets hybridized to fragments B and E. Hybridization of partial *Not*I digests placed the 50-kb fragment between fragments B and E (Fig. 4). Hybridization experiments with probes for fragment F always showed strong hybridization signals in the compression region, suggesting that fragment F was flanked on either side by fragments of 780 kb or larger. The fragment order was thus determined to be B-F-C.

(iii) *B. cereus* ATCC 11778. In strain ATCC 11778 the two bands in the 200-kb doublet (fragments L and K) could not be separated, and nor could the 270-kb doublet (fragments I and J) or the 420-kb doublet (fragments F and G), although shorter pulse times were used. Hybridization of partial digests verified, however, the presence of two fragments of 200 kb, two of 270 kb, and two of 420 kb. The smallest fragments (fragments M and N) were located next to fragment A by hybridization with the PLC probe (Fig. 5). From all these data, physical maps of strains ATCC 10876, ATCC 14579, and ATCC 11778 (Fig. 6) were constructed. The map for strain ATCC 10987 (9) is included for comparison (Fig.

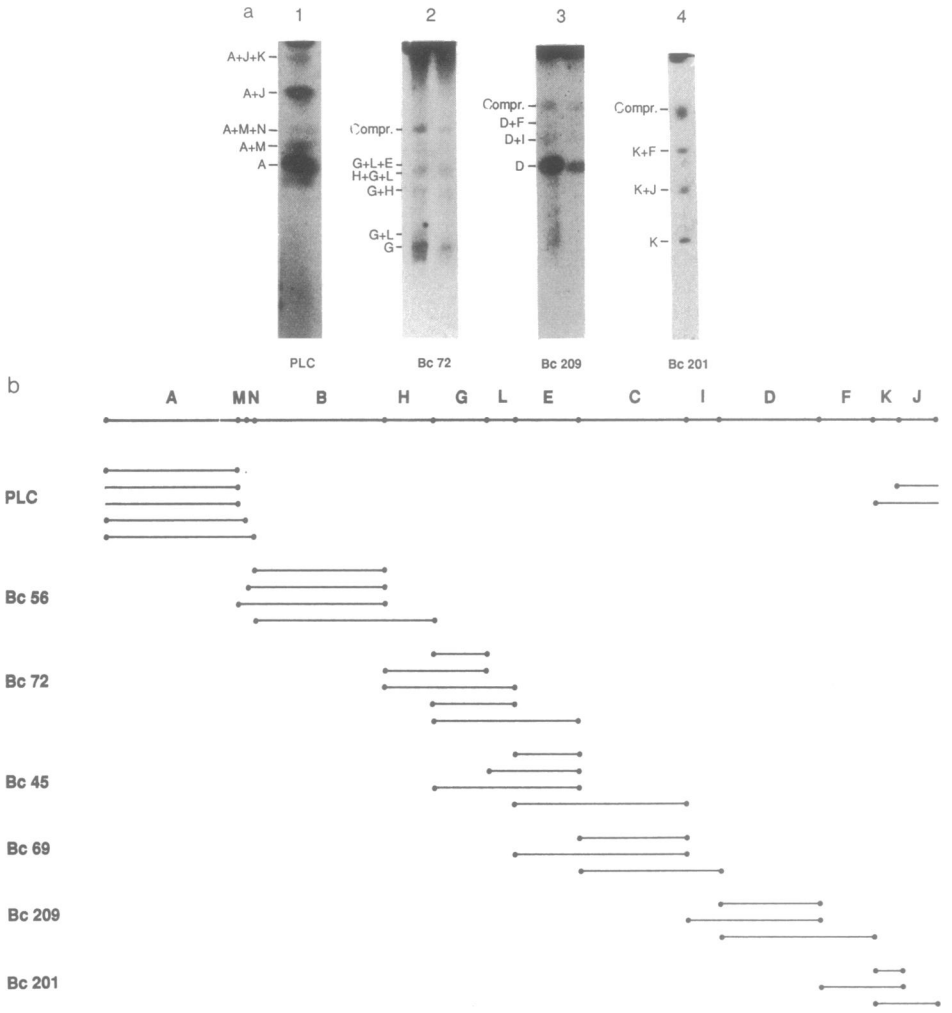

FIG. 5. Construction of the physical map of the strain ATCC 11778 chromosome. (a) Fragments after partial *Not*I digests separated at 100-s pulses for 40 h at 11 V/cm (Bio-Rad apparatus) (lane 1), 60-s pulses for 20 h at 150 mA (Beckman apparatus) (lanes 2 and 3) and 30-s pulses for 20 h at 150 mA (Beckman apparatus) (lane 4) and hybridized to the probes indicated. (b) Assignments of fragments of the strain ATCC 11778 chromosome. Results are from more than 30 hybridization experiments on 20 separate gels.

6). We used 42 probes from *B. cereus* ATCC 10987, of which 27 hybridized with all four strains. Nine probes from strain ATCC 10987 did not react with any other strain.

DISCUSSION

PFGE is a powerful method in the study of genome size and organization. However, to obtain reliable results when several fragments of similar sizes are present, it is necessary to run several gels under different conditions and to rely on genomic probes.

The *B. cereus* strains analyzed in this study contained 10, 13, or 14 *Not*I fragments, and the sizes of their chromosomes were similar (Table 1). They were all considerably larger than the *Escherichia coli* chromosome of 4.7 Mb (19). They were also significantly larger than the *B. subtilis* chromosome, which has been determined to be 4.2 Mb (7, 16).

The *Not*I fragment patterns were strikingly different when the four *B. cereus* strains were compared (Fig. 1, 2, and 6). This is in contrast to results obtained with *Campylobacter jejuni* (4) and *Lactococcus lactis* (23), for which the fragment patterns observed by PFGE in various strains were similar. Analysis of *Haemophilus influenzae* strains, on the other

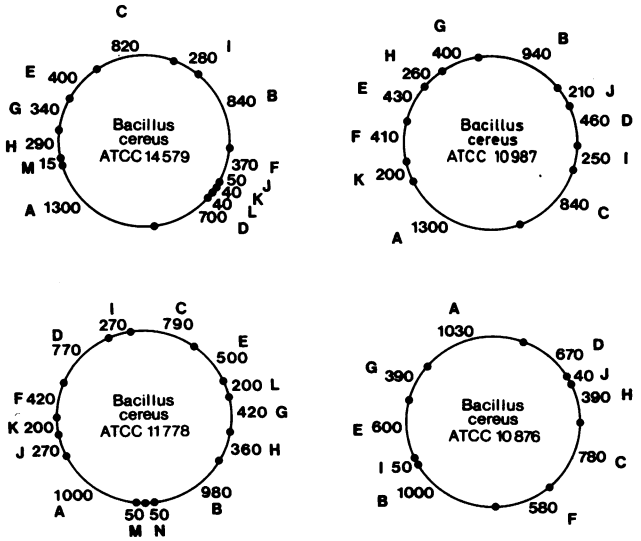

FIG. 6. Physical maps of the chromosomes of *B. cereus* ATCC 14579, ATCC 10876, and ATCC 11778 based on *Not*I fragments. The map of strain ATCC 10987 is included for comparison (9).

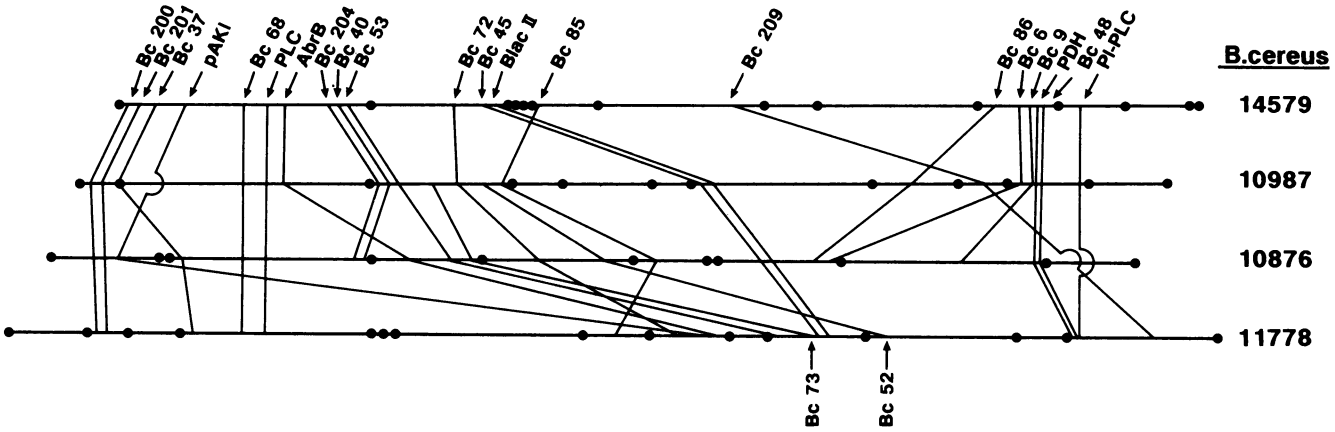

FIG. 7. Comparison of the maps of the four *B. cereus* strains. Probes assigned to one *Not*I fragment in three or four strains are included. The position of the probes within the fragment is arbitrary.

hand, showed identical fragment patterns for some strains, whereas others differed markedly (12). The observed difference between the *B. cereus* strains is in agreement with their well-documented heterogeneity (18) and raises the question of new criteria for bacterial taxonomy.

The maps of the *Not*I fragments of the four *B. cereus* strains were aligned to obtain maximum apparent similarity (Fig. 7). Probes that reacted with one fragment in each of three or four strains are marked in the figure by lines between the corresponding *Not*I fragments. The exact position and order of the probes within each *Not*I fragment have not been determined. Although there are certain overall similarities, the relationships appeared to be complex, possibly involving deletions, additions, and inversions.

Nine of the probes from ATCC 10987 appeared to be specific for this strain. For instance, probe Bc16 harbors the 3′ end of the β-lactamase I gene, followed by 1.8 kb of downstream sequence, of which at least the first 200 bases have no homology to the downstream sequence of any other β-lactamase I gene, and this probe does not hybridize to any of the other strains.

On the basis of the 16S rRNA oligonucleotide cataloging approach, *B. cereus* and *B. subtilis* are closely related (21). The p14B8 probe (rRNA operon) contains the origin region of the *B. subtilis* chromosome (22). It is possible that the origin of replication is likewise close to the rRNA genes in *B. cereus*. The rRNA genes are present on one or two fragments only, and for all strains p14B8 hybridized to one of the largest *Not*I fragments. The number and location of rRNA genes in *B. cereus* have not been determined. In *B. subtilis*, about 80% of the DNA which hybridizes to rRNA replicates very early (22). The rRNA genes on the large *B. cereus* fragment may represent these early-replicating genes, close to the origin. Consistent with this interpretation, two additional probes from the *B. subtilis* origin region (AbrB and pAKI [*recF*, *gyrA*, and *gyrB*] [11]) both hybridized to the largest fragment in ATCC 14579. AbrB has previously been localized to the 1,300-kb fragment in ATCC 10987 (9), whereas pAKI did not hybridize. A series of control experiments showed that pAKI did not hybridize to any fragment of ATCC 10987 under the conditions used.

In contrast, probe pAKI hybridized to smaller fragments in strains ATCC 11778 (420 kb) and ATCC 10876 (600 kb). Thus, one may conclude that the origin may be localized to the 420-kb fragment in ATCC 11778, to the 600-kb fragment in ATCC 10876, and to the 1,300-kb fragment in ATCC 10987 and ATCC 14579. For all strains the ribosomal gene probe hybridized to these fragments as well.

TerC did not hybridize to strain ATCC 10987 or to strain ATCC 14579, even at low stringency. The two positive *B. cereus* strains also showed very weak signals. It is possible that the DNA sequences in *B. subtilis* and in the *B. cereus* strains are not similar enough to hybridize under the conditions used and that strains ATCC 10987 and ATCC 14579 are more distantly related to *B. subtilis* than are strains ATCC 10876 and ATCC 11778.

It is interesting that the gene for PI-PLC is more than 350 kb away from the PLC gene. PLC is part of the cytolytic determinant cereolysin AB, which is made up of linked genes for PLC and sphingomyelinase (5).

Data from the four *B. cereus* strains analyzed here will, in future experiments, be compared with those from other *Bacillus* strains by the combination of PFGE and hybridizations used here. Such experiments will allow conclusions about the usefulness of this method for taxonomic studies. At this stage it is obvious that it is possible to establish a useful framework for comparison of related strains.

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REFERENCES

1. **Ambler, R. P., M. Daniel, J. Fleming, J. Hermoso, C. Pang, and S. G. Waldey.** 1985. The amino acid sequence of the zinc-requiring β-lactamase II from the bacterium *Bacillus cereus* 569. FEBS Lett. **185**:207–211.
2. **Benedict, R. G., W. H. Schmidt, and R. D. Coghill.** 1945. Penicillin. VII. Penicillinase. Arch. Biochem. **8**:377–384.
3. **Bowman, F. W.** 1957. Test organisms for antibiotic microbial assays. Antibiot. Chemother. **7**:639–640.
4. **Chang, N., and D. E. Taylor.** 1990. Use of pulsed-field agarose gel electrophoresis to size genomes of *Campylobacter* species and to construct a *Sal*I map of *Campylobacter jejuni* UA580. J. Bacteriol. **170**:5211–5217.
5. **Gilmore, M. S., A. L. Cruz-Rody, M. Leimeister-Wächter, J. Kreft, and W. Goebel.** 1989. A *Bacillus cereus* cytolytic determinant, cereolysin AB, which comprises the phospholipase C

and sphingomyelinase genes: nucleotide sequence and genetic linkage. J. Bacteriol. **171:**744–753.

6. **Hemilä, H., A. Palva, L. Paulin, S. Staffan, and I. Palva.** 1990. Secretory S complex of *Bacillus subtilis*: sequence analysis and identity to pyruvate dehydrogenase. J. Bacteriol. **172:**1–12.
7. **Itaya, M., and T. Tanaka.** 1991. Complete physical map of the *Bacillus subtilis* 168 chromosome constructed by a gene-directed mutagenesis method. J. Mol. Biol. **220:**631–648.
8. **Johansen, T., T. Holm, P. H. Guddal, K. Sleten, F. B. Haugli, and C. Little.** 1988. Cloning and sequencing of the gene encoding the phosphatidylcholine-preferring phospholipase C of *Bacillus cereus*. Gene **65:**293–304.
9. **Kolstø, A.-B., A. Grønstad, and H. Oppegaard.** 1990. Physical map of the *Bacillus cereus* chromosome. J. Bacteriol. **172:**3821–3825.
10. **Kuppe, A., L. M. Evans, D. A. McMillen, and O. H. Griffith.** 1989. Phosphatidyl-specific phospholipase C of *Bacillus cereus*: cloning, sequencing, and relationship to other phospholipases. J. Bacteriol. **171:**6077–6083.
11. **Lampe, M. F., and K. F. Bott.** 1985. Genetic and physical organization of the cloned *gyrA* and *gyrB* genes of *Bacillus subtilis*. J. Bacteriol. **162:**78–84.
12. **Lee, J. J., H. O. Smith, and R. J. Redfield.** 1989. Organization of the *Haemophilus influenzae* Rd genome. J. Bacteriol. **171:**3016–3024.
13. **Madonna, M. J., Y. F. Zhu, and J. O. Lampen.** 1987. Nucleotide sequence of the β-lactamase I gene of *Bacillus cereus* 569/H and 5/B. Nucleic Acids Res. **15:**1877.
14. **Nielson, J. B. K., and J. O. Lampen.** 1983. β-Lactamase III of *Bacillus cereus* 569: membrane lipoprotein and secreted protein. Biochemistry **22:**4652–4656.
15. **Otnaess, A.-B., C. Little, K. Sletten, R. Wallin, S. Johnsen, R. Flengsrud, and H. Prydz.** 1977. Some characteristics of phospholipase C from *Bacillus cereus*. Eur. J. Biochem. **79:**459–468.
16. **Piggot, P. J.** 1989. Revised genetic map of *Bacillus subtilis* 168, p. 1–41. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), The aerobic endosphore-forming bacteria. American Society for Microbiology, Washington, D.C.
17. **Priest, F. G.** 1981. DNA homology in the genus *Bacillus*, p. 33–57. *In* R. C. W. Berkeley and M. Goodfellow (ed.), The aerobic endospore-forming bacteria. Academic Press Ltd., London.
18. **Priest, F. G., M. Goodfellow, and C. Todd.** 1988. A numerical classification of the genus *Bacillus*. J. Gen. Microbiol. **134:**1847–1882.
19. **Smith, C. L., J. G. Econome, A. Schutt, S. Klco, and C. R. Cantor.** 1987. A physical map of the *Escherichia coli* K 12 genome. Science **236:**1448–1453.
20. **Smith, N. R., T. Gibson, R. E. Gordon, and P. H. A. Sneath.** 1964. Type cultures and proposed neotype cultures of some species in the genus *Bacillus*. J. Gen. Microbiol. **34:**269–272.
21. **Stackebrandt, E., W. Ludwig, M. Weizenegger, S. Dorn, T. J. McGill, G. E. Fox, C. R. Woese, W. Schubert, and K.-H. Schleifer.** 1987. Comparative 16S rRNA oligonucleotide analyses and murein types of round-spore-forming Bacilli and non-spore-forming relatives. J. Gen. Microbiol. **133:**2523–2529.
22. **Stewart, G. C., F. E. Wilson, and K. F. Bott.** 1982. Detailed physical mapping of the ribosomal RNA genes of *Bacillus subtilis*. Gene **19:**153–162.
23. **Tanskanen, E. I., D. L. Tulloch, A. J. Hillier, and B. E. Davidson.** 1990. Pulsed-field gel electrophoresis of *Sma*I digests of lactococcal genomic DNA, a novel method of strain identification. Appl. Environ. Microbiol. **56:**3105–3111.