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# Division Mutants of *Bacillus subtilis*: Isolation and PBS1 Transduction of Division-Specific Markers[1]

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A procedure for the isolation of *Bacillus subtilis* mutants that appear to be defective in septum synthesis has been described. Fourteen mutants isolated with this technique were found to be located at four distinct loci on the *B. subtilis* chromosome. These have been designated *divA*, *divB*, *divC*, and *divD*. The four mutants in the *divA* group synthesize septa; however, they do so with a high frequency of error resulting in minicell production. Mapping data were obtained by scoring cotransduction frequencies using PBS1-transducing lysates. Thus, some of the genes apparently involved in septum synthesis and their order on the genome have been established. The results of a study by electron microscope of some of the mutants is also presented.

The process of cell division involves a great number of steps. Division is intimately associated with the synthesis of a septum, duplication and segregation of genetic material, concomitant growth of cell wall and membrane, and the separation of the two daughter cells. It is difficult, therefore, to isolate mutants that only involve the terminal steps in the division process and do not also affect the general metabolism and growth of the cell. In an attempt to obtain such mutants, we selected cells that form filaments at high temperature but grow well at low temperature. These strains were then screened to eliminate mutations that affected division because of defects in deoxyribonucleic acid (DNA) replication, nutritional requirements, or steps in the final separation of the daughter cells. The remaining mutants appeared to have some defect in the process of septum formation, and we have therefore classified them as division mutants. However, until we know the biochemical sequence involved in septum formation and the steps which are blocked in the mutants, this classification remains tentative.

Many of the genetic studies on cell division have been carried out in *Escherichia coli* (e.g., references 2, 9, 16, 17; J. N. Reeve and D. J. Clark, Bacteriol. Proc., p. 71, 1970). There are reports of mutants with impaired division in *Bacillus subtilis* (e.g., references 4, 11, 12, 13) and in *Erwinia* (7). Recent work with *Streptococcus faecalis* (8) has led to a clearer picture of the steps involved in crosswall formation and division. The division mutants described in this paper fall into four distinct genetic and morphological classes. They may allow further analysis of the sequence of events involved in cell division in *B. subtilis*.

[1] From a dissertation submitted by D. Van Alstyne in May 1971 to the University of California, San Diego, in partial fulfillment of the requirements for the Ph.D. degree.

## MATERIALS AND METHODS

A *B. subtilis* auxotroph, BR77 *thr5*, *trpC2* (requiring threonine and tryptophan), was used as the parent strain from which all division mutants were derived.

As shown in Table 1, several auxotrophs were used as recipients for transformation and transduction studies. GSY712, SB26, C50, SR168, GSY1036, GSY384, SB120, and the JH10 strain were all obtained from J. Hoch (Scripps Clinic and Research Foundation, La Jolla, Calif.); the BD81 and BD92 auxotrophs were obtained from D. Dubnau; G15 was constructed by G. Grant in M. I. Simon's laboratory; the BR auxotrophs were constructed by B. Reilly in J. Spizizen's laboratory (Scripps Clinic and Research Foundation, La Jolla, Calif.). The series of strains with the prefix VA was prepared during this study by mutagenesis of BR77.

**Media.** All cultures were grown on Spizizen's minimal medium supplemented with 0.5% glucose, 0.1% casein hydrolysate, and 20 μg of appropriate growth requirements per ml or on Tryptose blood-agar base (TBAB; Difco Laboratories, Detroit, Mich.). The medium used for transformations was that described by Spizizen (14).

**Mutagenesis.** All division mutants were isolated after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine at 100 μg/ml (approximately 50% survival) by the procedure of Adelberg et al. (1).

**Mutant isolation.** After mutagenesis, the cells were washed twice in minimal medium suspended in 1 ml of Penassay broth (Difco), and incubated at 30 C for a minimum of 8 hr. The culture was diluted 1:10 in fresh Penassay broth to a final volume of 10 ml and incubated at 45 C for 4 hr. One milliliter of the culture was then layered on a 26-ml sucrose gradient (2 ml of 35%, 12 ml of 30%, and 12 ml of 15% sucrose) and centrifuged at 4,000 × *g* at 4 C for 5 min in the swinging bucket rotor of a Sorvall RC-2 centrifuge. The bottom 2 ml of sucrose was collected, and 2 ml of Penassay broth was added. Cells in this sample were incubated at 30 C overnight. This procedure was repeated four times. After the fifth enrichment, the cells were collected from the 26-ml sucrose gradient, diluted with 10 ml of minimal medium, pelleted at 10,000 rev/min for 10 min and resuspended in 1 ml of minimal medium. The 1-ml sample was layered on a second 30-ml sucrose gradient (6 ml of 35%, 12 ml of 30%, and 12 ml of 15% sucrose). The centrifugation was carried out as before. Ten-drop (about 0.5 ml) fractions were collected from the bottom of the gradient. Each fraction was plated directly on minimal media containing 20 μg of threonine and 20 μg of tryptophan. Ten plates were made from each gradient.

The *divA* mutants were found by direct observation by microscope of the clones derived from the gradient. The mutants appeared to form both filaments and minicells. They were able to form colonies at 45 C. They also formed minicells when grown in liquid culture at 30 and 45 C. The other colonies were then picked onto duplicate TBAB plates and incubated at 45 and 30 C to find temperature-sensitive mutants. It was found that smaller colonies on the minimal plates were more often temperature-sensitive division mutants. These mutants also tended to have a generation time significantly longer than that of wild-type cells. Possible temperature-sensitive mutants were then again checked by microscope for filament formation and lack of septation when grown on minimal medium or Penassay broth at 45 C. Mutants satisfying these requirements were then assayed by the Burton (5) procedure for DNA synthesis at 45 C. Those mutants unable to synthesize DNA at the nonpermissive temperature were excluded from this study. All the *divB*, *divC*, and *divD* mutants were selected this way.

**Transformation.** Transforming DNA was isolated by the procedure of Massie and Zimm (10) with the use of lysozyme and Pronase. Transformation was carried out by the method of Anagnostopoulos and Spizizen (3).

**Transduction.** PBS1 (15) was used as the donor virus throughout. Plaque assays were carried out using *B. licheniformis* (ATCC 8480) as the indicator strain. Stock virus was prepared by infection of *B. licheniformis* in Penassay broth. After lysis, the phage were purified and concentrated by differential centrifugation. The preparation of transducing lysates was carried out by the procedures of Reilly and Spizizen (*personal communication*). Donor strains were inoculated into Penassay broth from overnight TBAB plates and were grown to an optical density of approximately 150 Klett units, or until late log phase. The cultures were then infected with stock PBS1 at a multiplicity of five. The infected lysate was incubated with shaking for 1 hr at which time 50 μg of chloramphenicol per ml was added. The cultures were shaken for an additional 2 to 3 hr. The cells were then incubated at 37 C without shaking overnight to undergo autolysis. The lysate was treated with 1 μg of deoxyribonuclease per ml and centrifuged at 6,000 × *g* for 10 min. The supernatant fluid was sterilized by passage through a 0.45-μm membrane filter. Recipient strains were streaked on TBAB plates and grown overnight. They were then heavily inoculated (about $5 \times 10^7$ cells/ml) into Penassay broth and grown for 5 hr, or until maximum motility was obtained. The recipient culture and the transducing lysate were mixed in equal volumes, diluted 1:2 into fresh Penassay broth, and incubated for 20 min at 37 C with shaking. The infected cells were washed once in minimal salts by centrifugation, and were plated on selective medium and incubated at the appropriate temperature (30 C for the transduction of a temperature-sensitive marker). Recombinant clones carrying a division mutation were scored by picking colonies onto duplicate minimal medium plates (containing 20 μg of each of the appropriate requirements per ml), which were incubated at 45 and 30 C.

The *uvr* marker was scored by growth on minimal medium containing 20 μg of the appropriate requirements and 0.05 μg of mitomycin C per ml. The *uvr*⁻

TABLE 1. *Strains of Bacillus subtilis*

| Strain | Markers |
|---|---|
| BR62 | *ade*16, *trpC*2 markers |
| BD81 | *cysA*14 |
| BR27 | *ade*6, *trpC*2 |
| BD92 | *cysB*, *hisA*1, *trpC*2 |
| BR19 | *hisA*1, *trpC*2 |
| BR85 | *argC*, *trpC*2 |
| GSY712 | *metC* |
| SB26 | *metA*, *trpC*2 |
| C50 | *asp*, *trpC*2 |
| SR168 | *cysC*, *trpC*2 |
| GSY1036 | *ura*, *metA*, *hisA*1 |
| GSY384 | *argA*, *leu* |
| GR44 | *leu*, *trpC*2 |
| SB120 | *aroD*, *trpC*2 |
| BR76 | *lys*, *trpC*2 |
| BR50 | *metB*, *trpC*2 |
| JH10 | *gap*-1, *trpC*2 |
| G15 | *hisA*1, *uvr* |
| BR77 | *thr*5, *trpC*2 |
| VA321 | *thr*5, *trpC*2, *divB*32(ts) |
| VA71 | *thr*5, *trpC*2, *divB*71(ts) |
| VA472 | *thr*5, *trpC*2, *divB*47(ts) |
| VA435 | *thr*5, *trpC*2, *divB*43(ts) |
| VA268 | *thr*5, *trpC*2, *divC*26(ts) |
| VA61 | *thr*5, *trpC*2, *divC*61(ts) |
| VA55 | *thr*5, *trpC*2, *divC*55(ts) |
| VA242 | *thr*5, *trpC*2, *divC*24(ts) |
| VA322 | *thr*5, *trpC*2, *divD*32(ts) |
| VA97 | *thr*5, *trpC*2, *divD*97(ts) |
| VA51 | *thr*5, *trpC*2, *divA*51 |
| VA356 | *thr*5, *trpC*2, *divA*35 |
| VA27 | *thr*5, *trpC*2, *divA*27 |

strains are sensitive to mitomycin; under these conditions the *uvr*$^+$ colonies grow up rapidly.

**Electron microscopy.** One mutant was chosen from each genetic group for examination by microscope. These mutant strains were VA356, VA321, VA268, and VA322. The temperature-sensitive mutants were preincubated at 30 C for 30 min before the temperature was shifted to 45 C. After growing to late log phase (about 3 hr), the cells were collected by centrifugation. The VA356 strain was grown at 37 C. BR77, the parent strain, was used as the normal control and was grown at 45 C.

Fixing and embedding of the samples were carried out as follows. Cells were embedded in 2% agar, and the solidified block was cut into appropriate-size strips; the blocks were then fixed in 2% glutaraldehyde in 0.1 M phosphate, *p*H 7.2, for 2 hr at 4 C, washed three times (15 min each) in buffer, and then fixed in 2% osmium tetroxide in the same buffer. The blocks were washed for 15 min with distilled water and then were dehydrated for 15 min in 30, 50, 70, 90, and 100% ethanol at 4 C. After being brought to room temperature, the blocks were washed twice for 15 min in 100% ethanol in a dessicator; the ethanol was then diluted 1:2 with propylene oxide for 20 min. After a 20-min wash with undiluted propylene oxide in the dessicator, the fluid was changed to 1:2 propylene oxide. Epon monomer and samples were left in the dessicator (with no vacuum) until the propylene oxide evaporated. The blocks were then placed in fresh Epon and kept at room temperature for two days, with a change to fresh Epon each day. Polymerization was carried out under vacuum at 60 C for 2 days.

## RESULTS

**Properties of ts div$^-$ Mutants.** All temperature-sensitive mutants isolated share certain characteristics. Without exception these mutants have doubling times varying from 1 to 2 hr in Penassay broth at 30 C, significantly longer than that of the parent strain, BR77, which has a generation time of 40 min under the same conditions. In all cases, filament formation is extensive, even at the permissive temperature. Once late log phase is reached at 30 C, these filaments form septa and begin to divide; at 45 C, the filaments make few septa and finally lyse. Shifting a culture from 30 to 45 C in the middle of log phase or in late log phase results in only partial lysis. These growth characteristics are independent of the medium used [Spizizen's minimal salts, TBAB, tryptone broth, antibiotic medium (3)]. Furthermore, when these mutations are transduced into other strains, the same behavior is observed. Figure 1 illustrates the morphology of one strain carrying the temperature-sensitive mutant, *divD*32 (ts), grown at 45 C. The cells grow as long filaments.

The three *divA* mutants described here are not temperature-sensitive. They continue to form cells of irregular lengths at any temperature and on either rich or minimal medium. Presumably, enough viable cells are formed under any growth conditions to allow the survival of the culture. These mutants begin to grow by forming long filaments which separate at irregular intervals, at times pinching off small spherical cells at the tips of the filaments. It is not known whether these spherical cells contain DNA. The strains carrying these mutations (*divA*51, *divA*27, *divA*35) appear to make "mistakes" in the position at which the septum is formed. Figure 2 illustrates the morphology of the strain with the *divA*51 mutation. Many septa can be seen to be forming, and small cells are released from the ends of the filament.

Colony morphologies of the temperature-sensitive mutants, when grown at 30 C, vary from opaque, brittle, compact colonies of mutants at the *divB* locus to diffuse, almost translucent colonies of mutants at the *divC* and *divD* loci. The mistake mutant colonies closely resemble those of BR77 when grown at 37 or 30 C.

DNA synthesis after shifting from 30 to 45 C was not impaired in any of the mutants reported here. A representative result is shown in Fig. 3, where the strain carrying *divD*32(ts) was grown at 30 C and shifted to 45 C after 45 min. At 45 C, DNA synthesis continued for 2.5 hr. During the 1.75 hr at 45 C, the viable count decreased, whereas at 30 C the number of colony formers increased slowly.

All the mutants described were also competent when transformed by using a slight modification of the procedure reported by Anagnostopoulos, i.e., the *div*$^-$ mutants were grown to competence for 6 to 7 hr rather than the 4 to 5 hr, the growth period usually used for *B. subtilis* strains.

**Mapping.** The approximate locations of the *div* loci were initially determined by transducing a number of test strains with markers that represented different regions of the *B. subtilis* map and looking for linkage of a given marker with the temperature-sensitive characteristic of the *div* strains. Table 2 shows the linkage of the temperature-sensitive markers with the auxotrophic markers tested. In each case, the recipient contained at least two auxotrophic markers, usually *trp* C2 and the test marker. Linkage was further demonstrated by growing recombinants for the test marker on selective medium and then picking them onto duplicate plates which were incubated at 30 and 45 C to test for linkage with the *div* locus (Table 3).

The linkage of the mistake mutants was determined by direct examination by microscope of recombinants picked on selective media and inoculated directly into 1 ml of Penassay broth. There was no cotransduction with the *ade*16 or

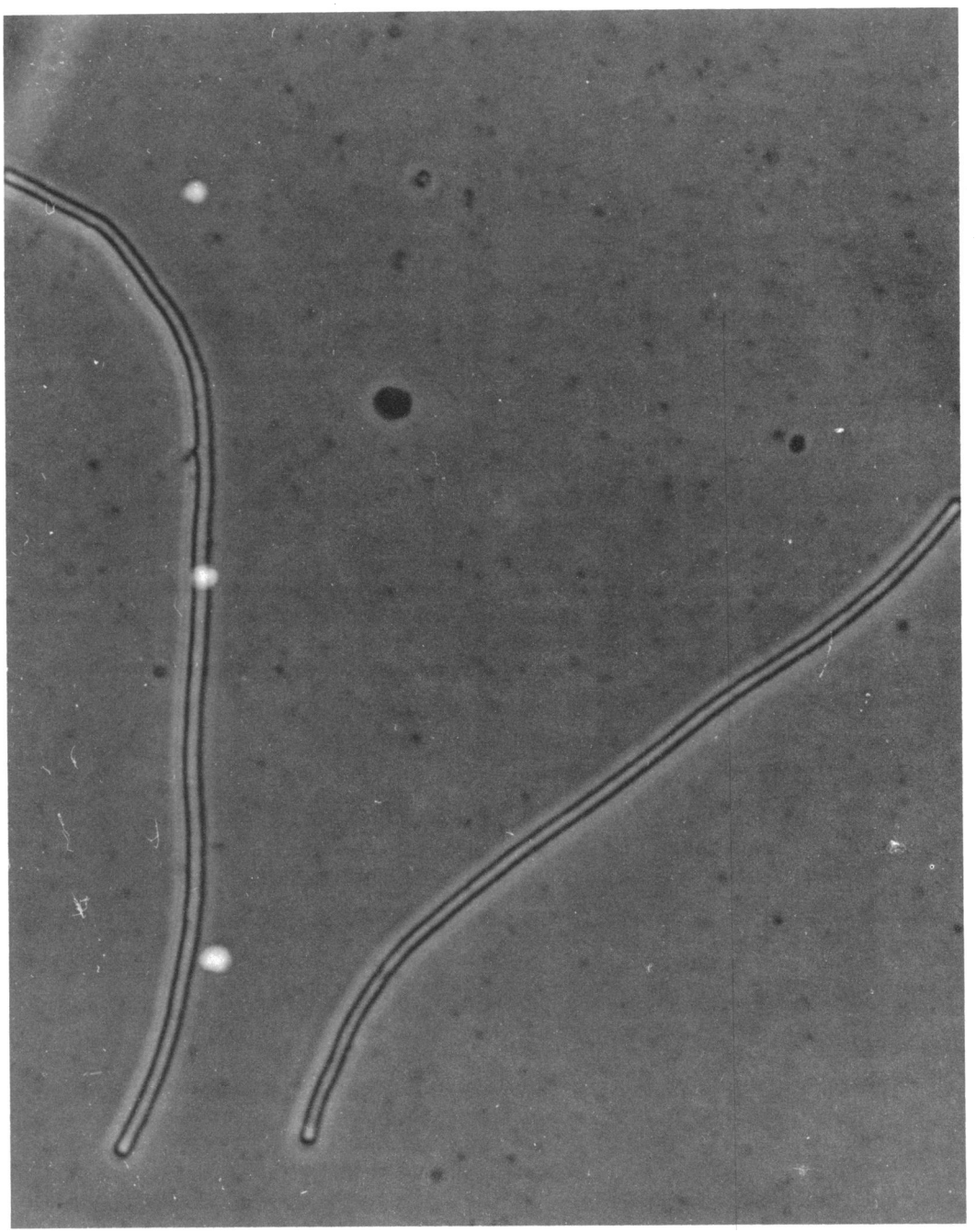

FIG. 1. *Typical filamentous growth at the nonpermissive temperature in liquid medium. VA322 grown at 45 C for 2 hr. ×1,000.*

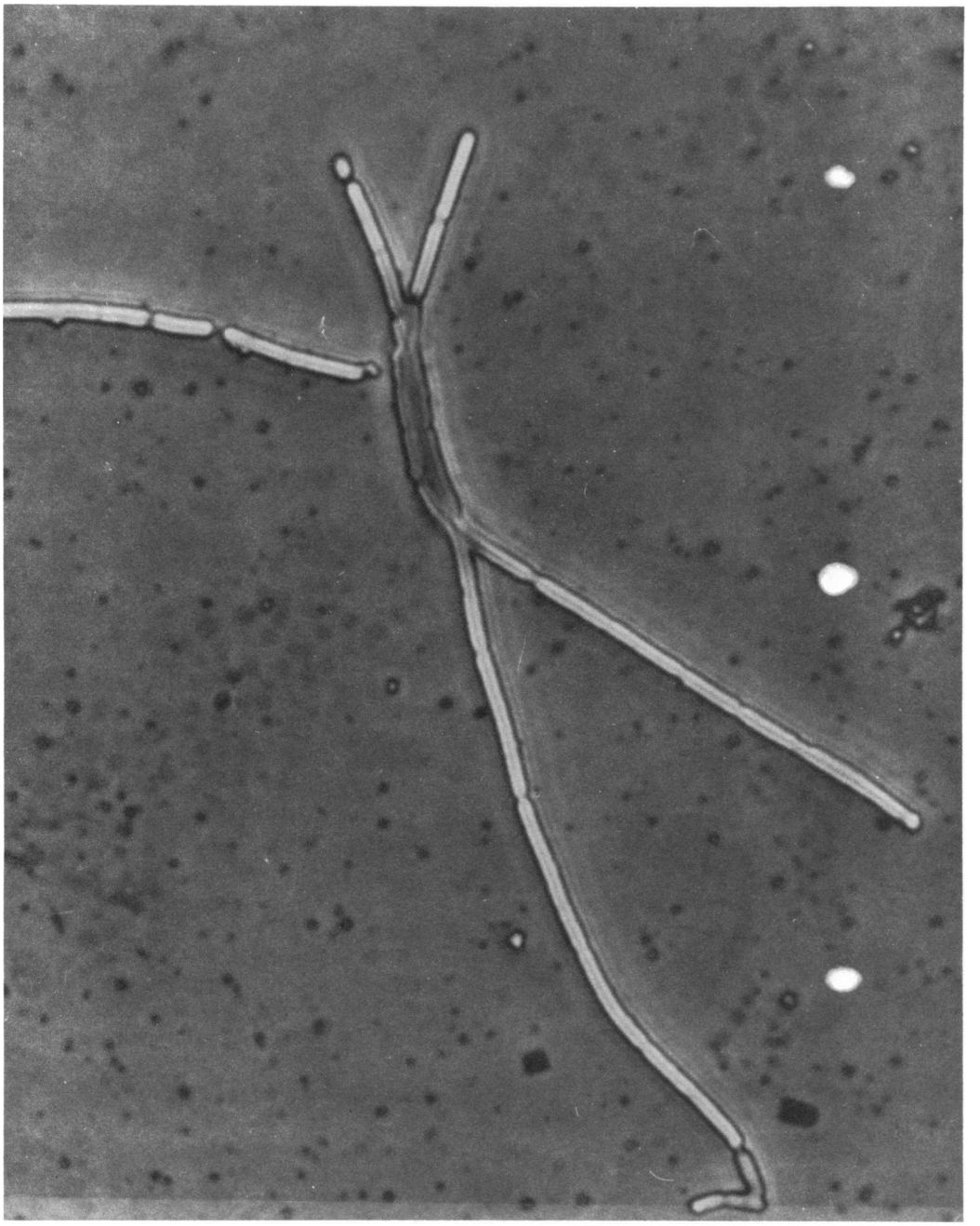

FIG. 2. *Typical growth of a mutant in the divA group. VA51 grown at 37 C for 3 hr in liquid medium.* ×*1,000.*

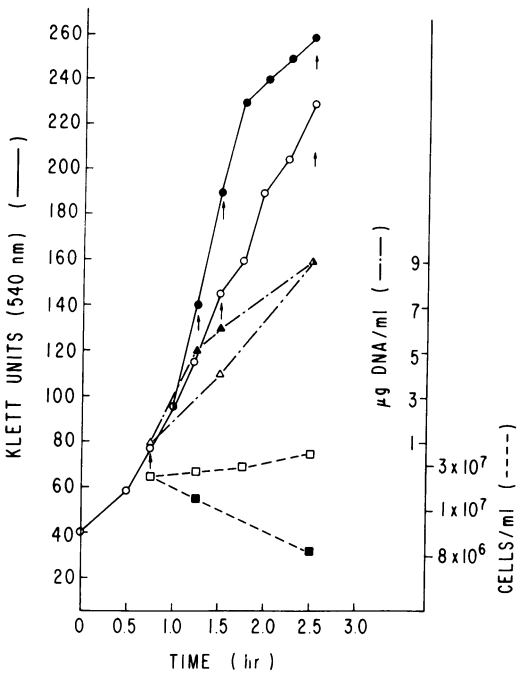


FIG. 3. *DNA synthesis and change in cell number of a temperature-sensitive septum mutant VA322 grown at 30 C for 45 min and shifted to 45 C. Symbols:* (●) *Klett units at 45 C,* (○) *Klett units at 30 C,* (▲) *μg of DNA/ml at 45 C,* (△) *μg of DNA/ml at 30 C,* (■) *colony-forming units* × $10^6$/ml at 45 C, (□) *colony-forming units* ×$10^6$*/ml at 30 C.*

*ade*6 markers. However, strong linkage with the *cysA*14 marker was found. Only a small number of recombinants was examined. Table 3 summarizes the linkage data for the *divA* mutants.

To correctly position the *divC* locus to the left or right of the *his* A1 marker, the position of *divC*26(ts) relative to both the *his* and *uvr* markers was determined. G15 (*hisA*1$^-$ *uvr*$^-$) was transduced with a lysate made on VA268, and *his*$^+$ recombinants were tested for those which were *his*$^+$ *divC*, *his*$^+$ *uvr*$^+$, and *his*$^+$ *divC uvr*$^+$. These results (Table 4), as well as the absence of linkage with the *cysB* marker, clearly position the *divC* locus.

Figure 4 summarizes the linkage data for the different loci, and Fig. 5 shows the positions of the four *div* markers on the *B. subtilis* chromosome (6).

**Electron microscopy.** Examination by electron microscope of the strain which carries the *divA*35 mutation revealed a much higher frequency of errors in the position of the septum than was evident when only the light microscope was used. Figure 6A shows a typical field in which three cells have two septa placed very close together. Figure 6B clearly shows septa synthesized at the poles of cells. Although the position of these septa is inappropriate, the septa themselves appear to be normal structures, as shown in Fig. 6C.

Mutants in the *divB* group tend to form twisted filaments. Figure 7C illustrates this and shows that septa are made irregularly, i.e., there are many long, twisted filaments with no apparent septa. Some of the septa that are found appear quite normal, but others are abnormal in several respects: they vary in thickness; they are initiated several times within a very small region; and often they are not synthesized directly across the cells but grow out from the membrane at angles, causing the structure to end as a membranous mass in the interior of the cell. A striking example of such errors is shown in Fig. 7B.

Figure 8B illustrates that the *divC*26(ts) mutant makes very few septa but does make what appear to be many large thickenings along the membrane. Figure 8A and C shows higher magnifications of these thickenings, some of which appear to have balloon-like structures protruding from them into the interior of the cell.

The *divD*32(ts) mutant appears to make very few septa (Fig. 9). Figure 9B shows a higher magnification of a typical septumless filament. There are no invaginations or changes in thickness along the membrane, although some small circular structures are evident. Normal, dividing bacteria are illustrated in Fig. 10.

TABLE 2. *Cotransduction of temperature-sensitive markers*

| Recipient strain | Selected markers | Cotransduction of temperature-sensitive markers | | |
|---|---|---|---|---|
| | | Donor lysates | | |
| | | VA321 | VA268 | VA322 |
| BR62 | Ade$^+$ | 0 | 0 | 0 |
| BD81 | Cys$^+$ | 0 | 0 | 0 |
| BR27 | Ade$^+$ | 0 | 0 | 0 |
| BD92 | Cys$^+$ | + | 0 | 0 |
| BR19 | His$^+$ | 0 | + | 0 |
| BR85 | Arg$^+$ | 0 | 0 | 0 |
| GSY712 | Met$^+$ | 0 | 0 | 0 |
| SB26 | Met$^+$ | 0 | 0 | 0 |
| SR168 | Cys$^+$ | 0 | 0 | + |
| GSY384 | Arg$^+$ | 0 | 0 | 0 |
| BR44 | Leu$^+$ | 0 | 0 | 0 |
| SR120 | Aro$^+$ | 0 | 0 | 0 |
| BR76 | Lys$^+$ | 0 | 0 | 0 |
| BR50 | Met$^+$ | 0 | 0 | 0 |
| JH10 | Glu$^+$ | 0 | 0 | 0 |

TABLE 3. *Linkage relationships of division markers*

| Recipient | Donor lysate | Selected marker | Unselected marker | Fraction of *ts* recombinants | Cotransduction (%) |
|---|---|---|---|---|---|
| BD92 | VA321 | Cys$^+$ | *divB*32(ts) | 5/64 | 8 |
| | | | | 22/200 | 11 |
| BD92 | VA71 | Cys$^+$ | *divB*72(ts) | 25/200 | 12 |
| | | | | 22/164 | 13 |
| BD92 | VA472 | Cys$^+$ | *divB*47(ts) | 35/200 | 18 |
| | | | | 23/200 | 15 |
| BD92 | VA435 | Cys$^+$ | *divB*43(ts) | 15/188 | 8 |
| | | | | 10/99 | 10 |
| BR19 | VA268 | His$^+$ | *divC*26(ts) | 142/590 | 24 |
| | | | | 14/100 | 14 |
| BR19 | VA61 | His$^+$ | *divC*61(ts) | 42/200 | 21 |
| | | | | 41/200 | 20 |
| BR19 | VA55 | His$^+$ | *divC*55(ts) | 15/118 | 13 |
| | | | | 36/200 | 18 |
| BR19 | VA242 | His$^+$ | *divC*24(ts) | 18/95 | 19 |
| | | | | 55/198 | 28 |
| C50 | VA322 | Asp$^+$ | *divD*32(ts) | 86/200 | 43 |
| | | | | 89/219 | 41 |
| C50 | VA97 | Asp$^+$ | *divD*97(ts) | 75/200 | 37 |
| | | | | 42/110 | 38 |
| SR168 | VA322 | Cys$^+$ | *divD*32(ts) | 92/200 | 46 |
| | | | | 59/105 | 56 |
| | | | | 42/106 | 43 |
| SR168 | VA97 | Cys$^+$ | *divD*97(ts) | 24/70 | 34 |
| | | | | 101/200 | 50 |
| GSY1036 | VA322 | Ura$^+$ | *divD*32(ts) | 139/190 | 73 |
| | | | | 148/177 | 84 |
| GSY1036 | VA97 | Ura$^+$ | *divD*97(ts) | 128/200 | 64 |
| | | | | 90/118 | 79 |
| BD81 | VA51 | Cys$^+$ | *divA*51 | 23/60 | 38 |
| BD81 | VA356 | Cys$^+$ | *divA*35 | 38/50 | 76 |
| BD81 | VA27 | Cys$^+$ | *divA*27 | 29/50 | 58 |

TABLE 4. *Linkage relationship of divC locus*

| Recipient strain: G15<br>Donor lysate: VA268 | | | |
|---|---|---|---|
| Selected marker | Unselected marker | Fraction of recombinants | Cotransduction % |
| His$^+$ | *uvr*$^+$ | 486/590 | 82 |
| His$^+$ | *divC* | 142/590 | 24 |
| His$^+$ | *divC*, *uvr*$^+$ | 104/590 | 17 |

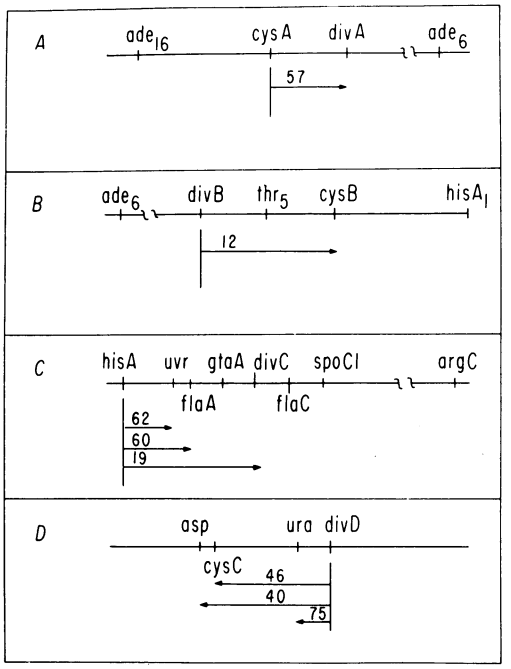


FIG. 4. *Linkage relationships of division loci divA (A), divB (B), divC (C), and divD (D). Linkage is expressed as per cent cotransduction in PBS-1; the average value for the crosses shown in Table 3 is given in A, B, and D. The averages given in C include three other transduction experiments not shown above.*

## DISCUSSION

Four different genetic loci have been shown to be concerned in some way with the synthesis of septa in *B. subtilis*. It is possible that other loci are also important in the synthesis of the septum or in the regulation of its synthesis. Nukushina and Ikeda (12) have reported three other mutants (*ts*-76, *ts*-12, and *ts*-1) described as having no septa at high temperature. The *ts*-76 marker was cotransduced with *met* B while the remaining two markers were cotransduced with *lys*. However, they also reported that their *lys* marker was closely linked to *ura*. It seems possible that their *ts*-76 and *ts*-12 actually fall into

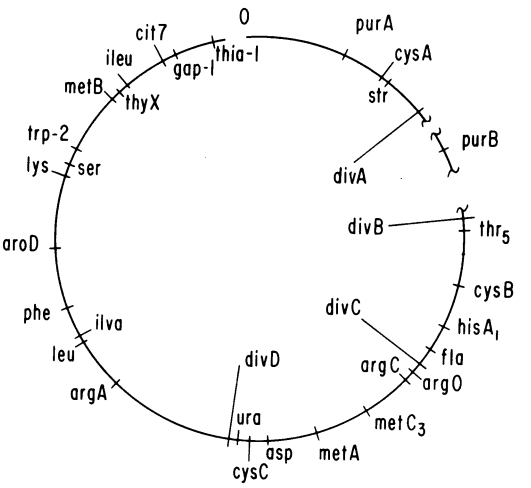


FIG. 5. *Linkage of div markers to the B. subtilis chromosome.*

the *divD* locus presented here. No mutants isolated in this study were found to cotransduce with *met* B.

One problem was encountered in establishing the linkage relationship of the *divB* marker. After obtaining a low cotransduction frequency with the *cysB* marker and finding no contransduction of *divB* with *hisA*1, it was assumed that the division mutation would be tightly linked to *thr5*. However, it was not possible to achieve any cotransduction of *divB* with *thr5* by using a donor lysate prepared on a *thr*$^+$ revertant, with BR77 as the recipient. This may be a consequence of the proximity of the *divB* marker to a discontinuous region on the map. In any event, the position assigned to the *divB* locus is the one most consistent with the available data and markers.

The electron microscope studies reinforce the mapping data and suggest that each group of mutants affects a different function involved in the process of septum formation. The only mutant group which does not appear to initiate septum synthesis at the nonpermissive temperature is the *divD* group. Membranous structures which were seen in all three remaining groups may somehow be involved in septum formation.

Rogers (13) has reported the isolation of mutants with disturbed morphology and cell division. Electron microscope studies of these mutants revealed some abnormal membrane structures similar to those seen in our *divB* mutant. However, since our mutants are all quite different in their morphology and in their osmotic properties, it is unlikely that they are defective in the same gene.

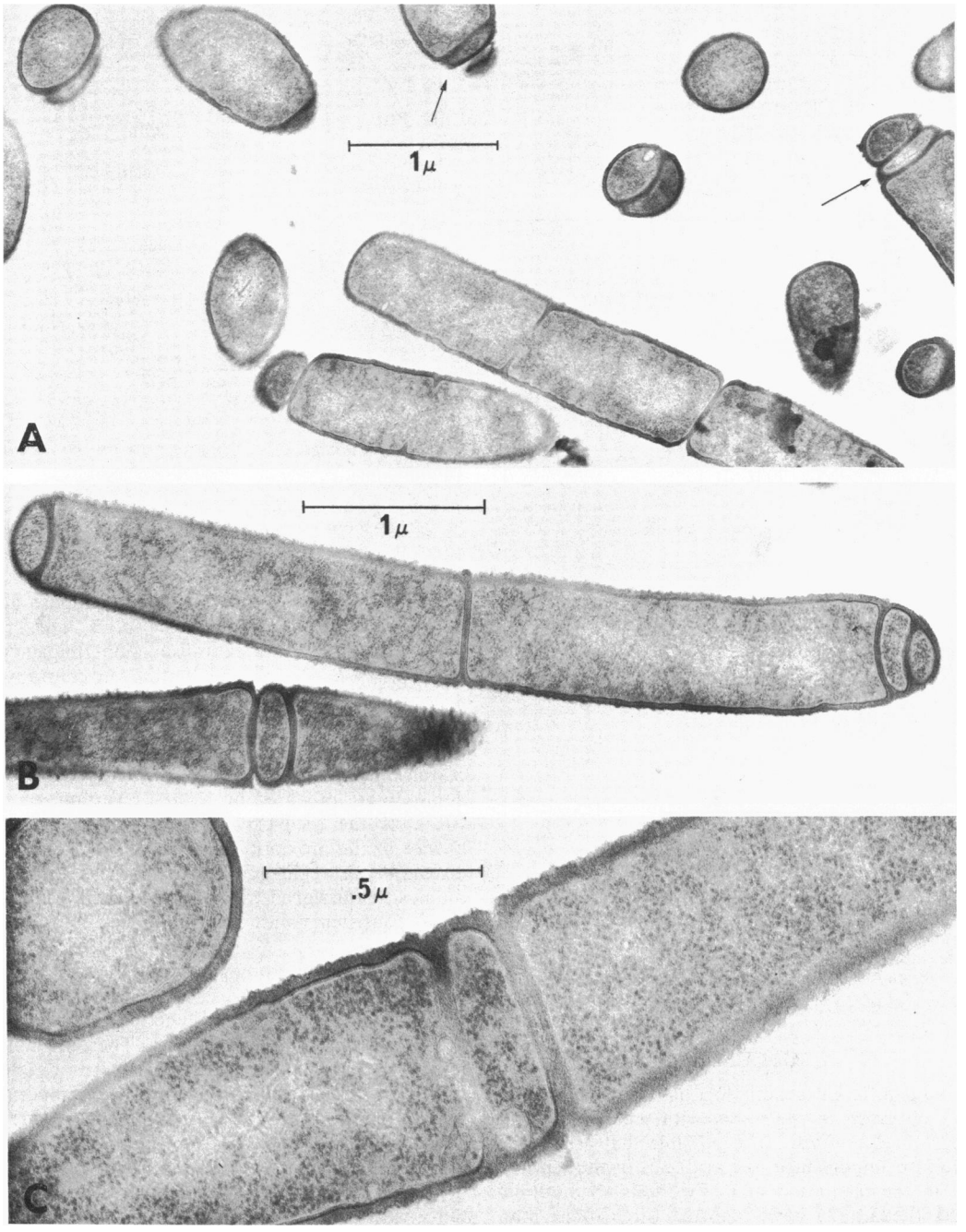


FIG. 6. *A, General field of a dividing divA mutant; B, divA mutant forming septa incorrectly; C, incorrectly placed septa.*

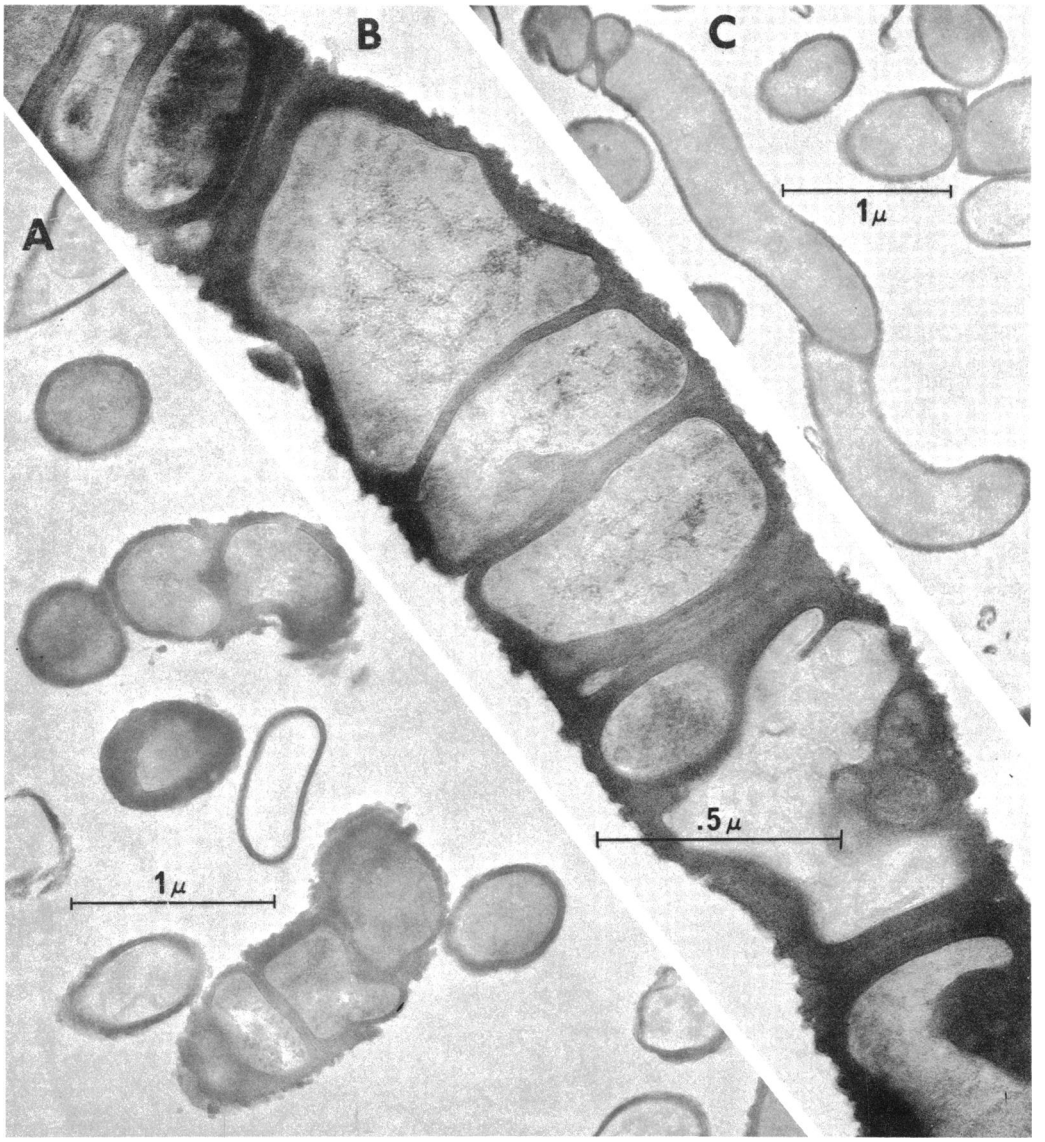


FIG. 7. *A, Abnormal septa made in divB mutant; B, a particularly extreme example of abnormal septa made at 45 C by divB mutants; C, typical filament formed by divB mutants at 45 C.*

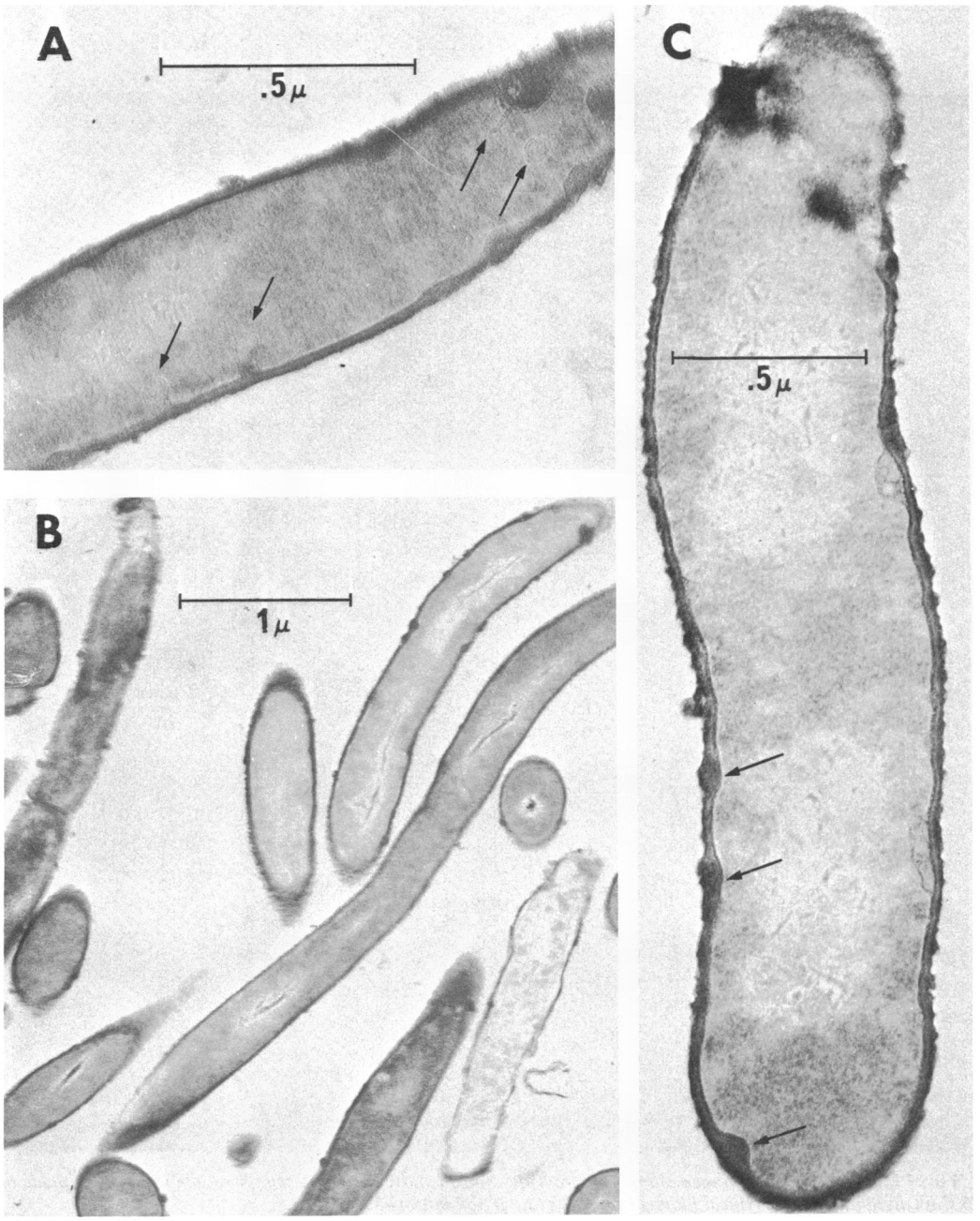


FIG. 8. *A, Example of balloon-like structures protruding from membrane thickenings in a divC mutant grown at 45 C; B, a general field of a divC mutant grown at 45 C; C, membrane thickenings characteristic of the divC mutant.*

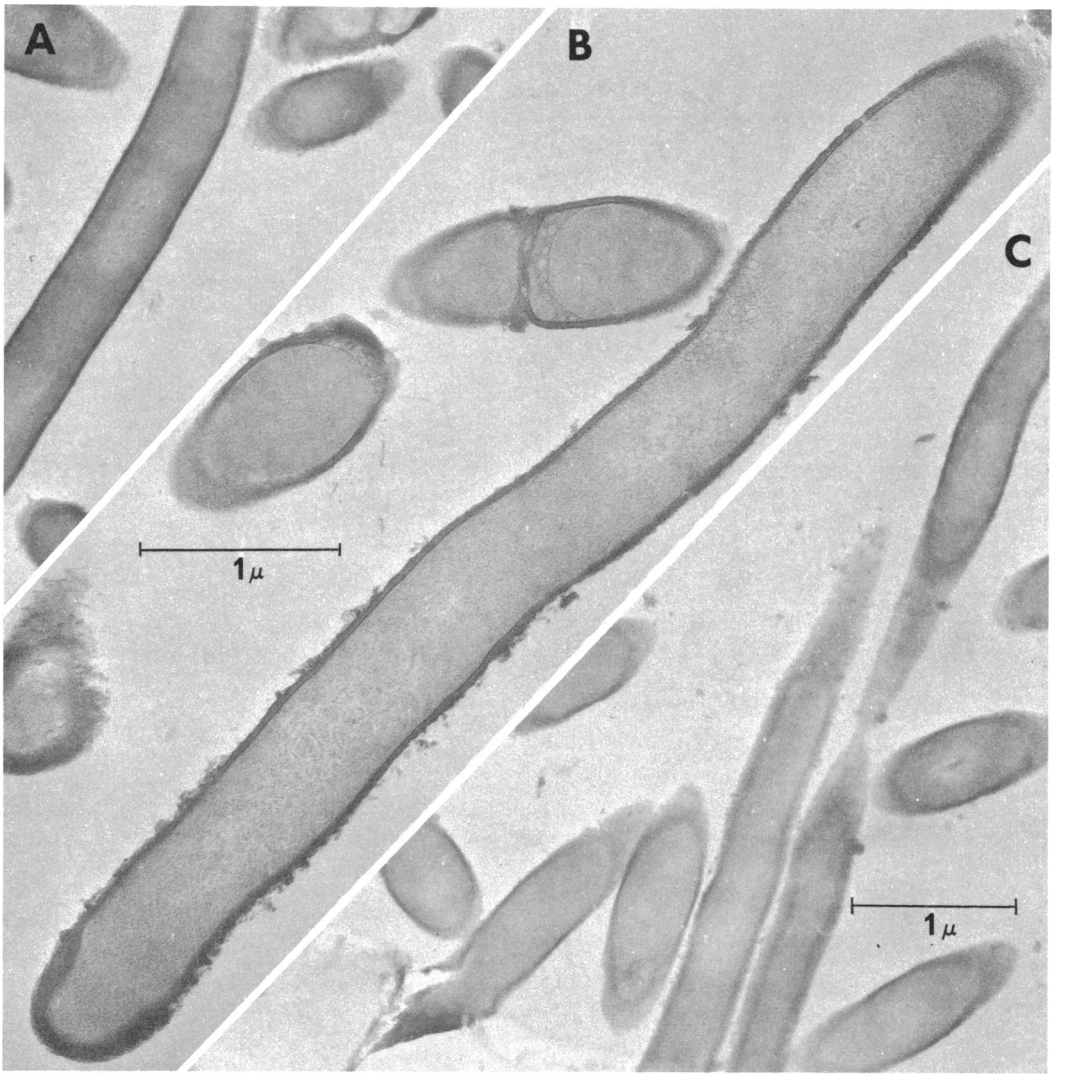


FIG. 9. *A and C, septumless filaments formed by a divD mutant grown at 45 C; B, higher magnification photograph of a typical divD filament.*

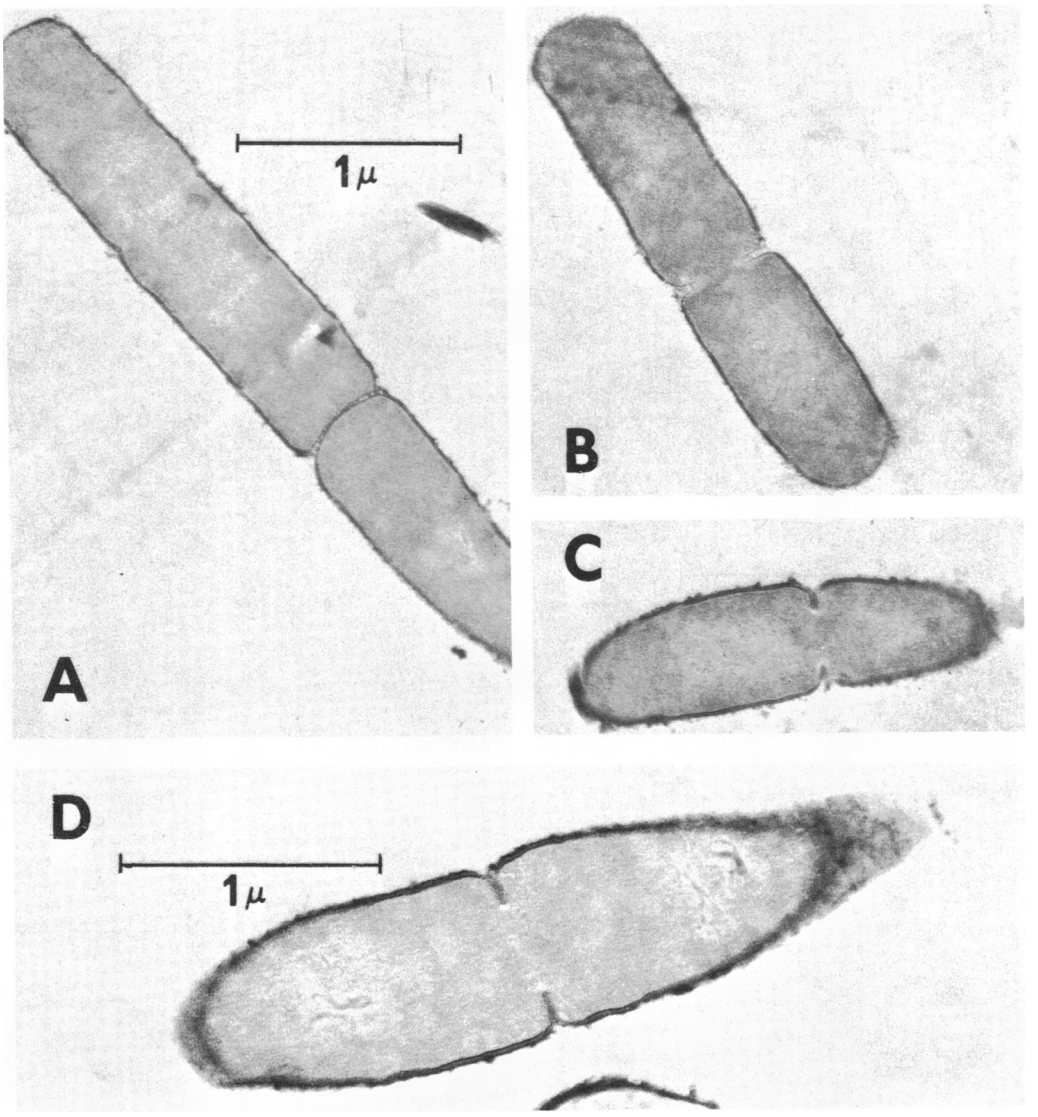


FIG. 10. *Normal dividing BR77, the parent strain, grown at 45 C.*

These four genetic groups probably represent interruptions in a sequential process that determines the presence of the septum, its position in the cell, and its form. One may speculate on the nature of this series of events. It is not clear at this time whether they are concerned with the process of synthesizing septa or whether they control the initiation of septum synthesis. It is hoped that further characterization of these mutants will provide a more complete understanding of this aspect of cell division.

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