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Detergent-Resistant Variants of *Bacillus subtilis* with Reduced Cell Diameter

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Variants of *Bacillus subtilis* resistant to the detergent Triton X-100 may exhibit: (i) normal cell morphology, (ii) reduced cell diameter, or (iii) helical cell shape. One variant of type ii was studied in some detail. Triton resistance, cell diameter reduction, and poor sporulation all may have resulted from a single mutation. High concentrations of Triton caused rapid lysis of wild-type cells. *B. subtilis* adapted to low Triton concentrations such that, upon subsequent exposure to higher concentrations, growth continued, although it became inhibited at very high concentrations. The variant studied retained its sensitivity to Triton-induced lysis but, after adaptation, grew at very high Triton levels. In this strain, cell diameter and cross-sectional area were reduced to about 73 and 50%, respectively, of those of wild type, yet the cells grew at normal rates, and DNA/protein/RNA ratios were largely unaltered. Peptidoglycan content per unit of cell surface area was higher in the variant than in the wild type under at least certain growth conditions.

There are several known types of mutants of normally straight, rod-shaped bacteria in which cell shape is abnormal (15, 17). Apart from those which are disturbed in the synthesis, location, or splitting of division septa (43), most of these mutants display a complete loss of rod shape, the cells being either spherical (e.g., 18, 33) or very irregularly shaped (1). In other types of shape alteration, basically rod-shaped cells grow in a helix (27, 45) or in an irregularly bent manner (12). As far as I am aware, there are only two established cases of mutations affecting cell diameter without loss of rod morphology. Both involved a diameter increase. Zaritsky and Pritchard (53) observed an increase in cell diameter of a thymine auxotroph of *Escherichia coli* when it was grown in low thymine concentrations. Shannon et al. (41) described a temperature-sensitive mutant of *Salmonella typhimurium* which grew with an increased cell diameter before dying at the nonpermissive temperature. Ogg and Zelle (28) described a large-celled, camphor-resistant mutant of *E. coli* but did not establish whether or not an increase in cell diameter was involved, as seemed to be the case in a camphor-resistant strain of *Pasteurella pestis* (52).

I describe here and discuss general features of what, to the best of my knowledge, is a new type of bacterial mutant which is both resistant to a detergent and considerably reduced in cell diameter, but which grows almost at the wild-type rates.

(A preliminary account of this work was presented previously [M. J. Tilby, Proc. Soc. Gen. Microbiol. 4:141-142, 1977].)

MATERIALS AND METHODS

Bacteria. *B. subtilis* 168 Trp⁻ (44) was obtained from the Deutsche Sammlung von Mikroorganismen, Göttingen, West Germany.

Growth conditions. Media used were Antibiotic Medium no. 3 (Pennassay broth; Difco Laboratories), nutrient broth/agar (Difco), nutrient broth sporulation medium (37), and Spizizen glucose minimal medium (44). The latter was supplemented with tryptophan (25 μg/ml) and a trace element solution (2 ml/liter) (32), which was necessary for prolonged balanced exponential growth. Liquid cultures for chemical analyses and envelope preparations were forcibly aerated and inoculated to allow growth for at least ten generations before harvesting during exponential phase. Cultures (750 ml each) grown at 30°C were used for dry weight, chemical, and cell width analyses; 250-ml cultures grown at 37°C were used for other width analyses. The effects of Triton were studied in vigorously shaken Penassay broth cultures (37°C). Growth in liquid media was followed by turbidity, read as optical density at 578 nm, samples being diluted to keep readings below 0.2. Plate cultures were incubated at 37°C. Viable counts were determined on Penassay plates.

Selection of Triton-resistant variants. Cells of strain 168 grown to stationary phase in nutrient broth containing a low concentration (0.005 to 0.01%) of

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Triton X-100 were spread (ca. 10^7 cells per plate) on nutrient agar containing Triton at 0.1 or 0.03%. Each strain described originated in completely independent cultures.

Selection of Triton-sensitive revertants. Strain TR49 was grown for about 20 h in sporulation broth, then heated at 80°C for 10 min to kill vegetative cells (37), and 0.5 ml was inoculated into 10 ml of fresh sporulation broth. This procedure was repeated three or four times before, after the final heating, the spores were spread on Penassay plates. The resulting colonies were replicated to nutrient agar containing 0.1% Triton, and Triton-sensitive colonies were isolated and retested. Each revertant strain described originated in a separate experiment.

Chemical and dry weight analyses. To minimize autolysis, cultures (750 ml each) were cooled to 0 to 2°C in about 4 min by passing through a long coil of tubing immersed in ice. All subsequent handling was carried out at 0 to 2°C. The cells were harvested by centrifugation, washed once with and then suspended in aqueous NaCl solution (1%, wt/vol; 0.17 M). Five 1-ml samples of the suspension were removed for dry weight determination, which involved collecting the cells on a predried and weighed membrane filter (0.45-μm pore size), washing with water, and drying at 60°C to constant weight. Other samples of the suspension were diluted into a 1% Formalin-1% NaCl solution for subsequent cell counting (at least 1,000 cells) with a counting chamber. Cells from the suspension were also spread on microscope slides and dried for length measurements.

The remaining cells were disrupted by sonic treatment (>99%), and a measured volume of the homogenate was fractionated (39). RNA (39) and DNA (5) were measured in the hot trichloroacetic acid-soluble fraction, and protein (23) was measured in the insoluble fraction, after solution in 1 N NaOH.

In a separate series of experiments, peptidoglycan was isolated by the procedure of Park and Hancock (30) from cells that had not been sonically disrupted. The peptidoglycan preparations were hydrolyzed (4 N HCl; 110°C for 15 h), and diaminopimelic acid was determined by using an amino-acid analyzer. The preparations were essentially free of methionine, which would have interfered with this determination.

Cell length measurements. Cell length measurements were made on dried cells stained with crystal violet by the method of Sargent (35). Such cells appeared to be about 15% shorter than living cells mounted in soft agar and observed with phase-contrast optics. This difference, which was the same for wild-type and variant TR49 cells, may have reflected shrinkage of cells during drying. Sargent (35) reported that this stain did not significantly affect cell length. However, at least part of the difference may have resulted from error due to different sample preparation methods (e.g., many short cells in the agar would have been orientated out of parallel with the focal plane, whereas dried cells adhered to the microscope slide). Because of this uncertainty, length measurements presented below have not been corrected for apparent shrinkage.

Cell width measurements. Cell width measurements were made on electron micrographs of cell walls. Cultures were rapidly cooled as described above or, for cultures grown at 37°C, by adding ice. The cells were harvested by centrifugation, suspended in 1% NaCl solution, and disrupted by shaking with glass beads in a Braun homogenizer for 2 min. Four minutes of shaking was necessary for breaking stationary-phase cells. Unbroken cells were removed by centrifugation (1,000 × g for 3 min), and the walls were then collected and washed three times with water (by centrifugation at 12,000 × g for 10 min), and finally suspended in water. Up to this stage, the specimens were kept below 4°C. They were then heated in boiling water for 10 min to destroy bound autolysin (11) and frozen until required.

The walls were prepared for electron microscopy by being collected on collodion films by the agar filtration method (21). The films were then floated on water, collected on specimen grids, and platinum-carbon shadowed. Measurements were made directly on the photographic plates. Photographs, from at least four different grids, were taken at a 2,000-fold nominal magnification and were calibrated by using a diffraction grating replica.

Triton X-100. Triton X-100 was of a grade specially purified for scintillation counting and was obtained from Serva. The minimum concentration that caused lysis of nonadapted cells varied considerably (0.007 to 0.014%) from bottle to bottle. This may or may not have been due to formation of peroxides of the detergent. Apart from resistant strain selections, in all the experiments reported here the Triton came from one bottle.

RESULTS

Selection of Triton-resistant variants. Triton-resistant variants were spontaneous and were obtained as described above. Cells preadapted to Triton (see below) were used in the selections, because preliminary experiments had shown that the strains obtained were still killed by a sudden exposure to high concentrations of Triton. The variants found could be superficially classified into three groups on the basis of cell morphology in the absence of Triton as seen under the light microscope: (i) apparently normal (≤90%); (ii) noticeably reduced in cell diameter but otherwise normal straight rods (≈5%); and (iii) basically rod-shaped cells growing in a helical form (≈5%). The helical variants, which were found after selection on 0.03% Triton, have been described briefly elsewhere (45). This communication is concerned mainly with a variant of class ii. All the variants described below were obtained by using 0.1% Triton in the selection plates. Of course, a distinction between classes i and ii may only be arbitrary. All isolates were Trp^- and therefore not contaminants and were present in the unselected population at a combined frequency of about 10^{-6} (order of magnitude).

Cell circumference measurements. Cell circumference was assessed as twice the width

of cell walls dried onto collodion films. We considered these results to be more readily interpretable than measurements of widths of dried whole cells (e.g., 51, 53). The measured width of a cell depends on its cross-sectional shape, and this is not easily predictable for dried whole cells since it depends on properties of their contents. The present approach was also used recently with *E. coli* by Meacock et al. (26).

During cell wall preparation, damage, either by homogenization or by autolysis (11), did not seem to affect significantly the results. Neither incubation of the walls at 30°C for 5 min before autolysin inactivation nor extended homogenization appreciably affected the measured circumference of wild-type cells (Table 1). The very small variance of widths (the standard deviation was typically <4% of the mean) would not have been observed if the walls had been seriously damaged.

TABLE 1. *Circumferences of cells grown exponentially in Penassay broth at 37°C*

Strain	Circumference[a]	Circumference as % wild-type circumference
Wild type (168)	2.49 ± 0.09	
	2.39 ± 0.09	
	2.48 ± 0.09[b]	
	2.42 ± 0.09[c]	
	2.39 ± 0.09[d]	
	2.38 ± 0.09[e]	
Overall mean	2.43	
Triton-resistant mutants		
TR49	1.80 ± 0.06	74
TR55	1.98 ± 0.08	81
TR50	2.09 ± 0.07	86
TR60	2.14 ± 0.10	88
TR54	2.15 ± 0.09	88
TR59	2.38 ± 0.12	98
TR51	2.42 ± 0.09	100
TR58	2.42 ± 0.10	100
Revertants of TR49		
TM1	2.23 ± 0.09	92
TM4	1.92 ± 0.08	79
TM8	2.42 ± 0.10	100

[a] Each value, in micrometers, is the mean of 100 measurements ± standard deviation, determined for a separate culture, except that walls b and c and walls d and e were from common cultures.

[b] Walls were incubated at 30°C for 5 min before inactivation of autolysins.

[c] Control for wall b.

[d] Cells were homogenized for 1.5 min.

[e] Cells were homogenized for 4.0 min.

Living cells of Triton-resistant strain TR49 were noticeably thinner than the parent strain 168 cells when observed under the light microscope. This was confirmed by measurements with the electron microscope on cell walls (also dried whole cells and sections, data not shown). Measurements were made on walls of several chosen resistant mutants grown at 37°C in Penassay broth (Table 1). Strain TR49 was the thinnest. Other strains were indistinguishable from the wild type or were of intermediate size. Therefore, further studies were centered on strain TR49. From measurements made on TR49 and on the wild type grown at 30°C (Table 2), it appears that the widths of both strains varied with growth conditions. They were thicker when grown rapidly in Penassay broth than when grown more slowly in Spizizen medium. In stationary-phase Penassay cultures, the diameters were intermediate.

Under all three growth conditions at 30°C and in Penassay broth at 37°C (Table 1), the circumference of TR49 was 70 to 75% that of the wild type. This corresponds to a reduction of cross-sectional area of the mutant to about half that of the wild type (49 to 56%). The reduced width of TR49 and its very regular rod shape are illustrated in Fig. 1.

Dry weight analyses. Dry weight per meter of cell length was calculated from the following experimental data: total dry weight per milliliter of cell suspension, cells per milliliter, and mean cell length. The results (Table 3) confirm the microscopic observations and agree well with the ratios of calculated cross-sectional areas (49 to 56%), especially since any error in width measurements will be squared in calculating cross-sectional areas. However, calculation of the dry weight per milliliter of cell volume reveals an

TABLE 2. *Cell wall circumference under various growth conditions at 30°C*

Growth condition	Circumference[a]		
	Wild type	TR49	TR49/wild type (%)
Spizizen medium, exponential	1.96 ± 0.12 1.91 ± 0.12	1.40 ± 0.06 1.32 ± 0.06	70
Penassay broth, exponential	2.31 ± 0.09 2.30 ± 0.10	1.69 ± 0.07 1.67 ± 0.08	73
Penassay broth, stationary phase for 5 h	2.07 ± 0.10	1.56 ± 0.07	75

[a] Values indicate, in micrometers, means of 100 cells ± standard deviation, each value representing a different culture.

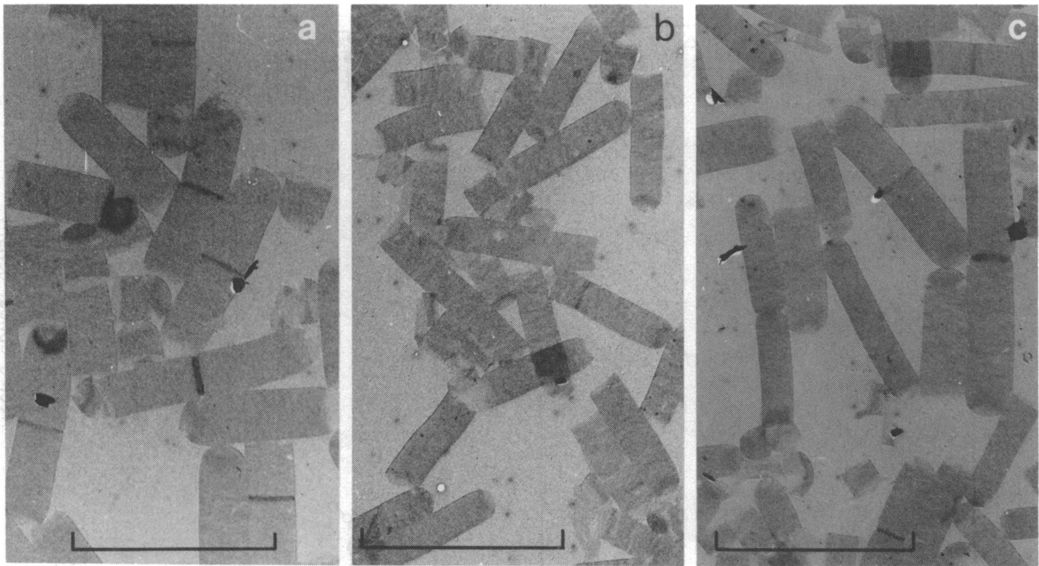


FIG. 1. *Shadowed cell walls. (a) Wild-type strain 168; (b) Triton-resistant variant TR49; (c) mixture of wild-type and resistant variant TR49. Both strains were grown exponentially in Penassay broth at 37°C. Bar indicates 5 μm. Cell walls of wild-type and thin strains showed a pronounced tendency to break transversely as described by Verwer and Nanninga (48). This phenomenon is consistent with the model of wall structure presented previously (45).*

unrealistically high value (Table 3), indicating that shrinkage occurred during preparation of specimens for microscopy.

Effects of Triton X-100 on growth. Wild-type cells growing exponentially in Penassay broth were subcultured into medium containing various concentrations of Triton, and growth was followed as the optical density at 578 nm (Fig. 2a). Above 0.008% Triton, cells lysed. At 0.007 and 0.008%, growth exhibited a lag period.

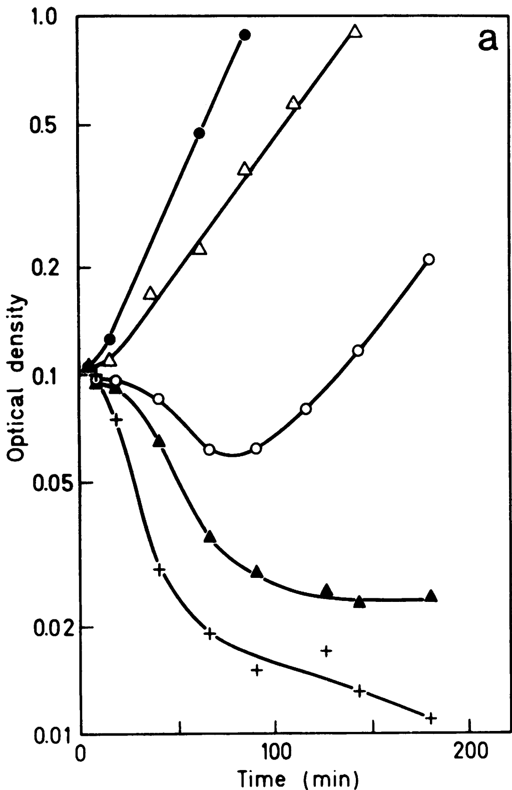
The lag phenomenon suggested that the cells adapted to the presence of Triton. This was confirmed by subculturing wild-type cells grown for three generations in a low concentration (0.006%) of Triton into higher concentrations (Fig. 2b). Growth continued slowly at concentrations that quickly killed nonadapted cells. Further confirmation of adaptation as opposed to selection was obtained by following the time course of development of resistance to high Triton concentrations by cells grown in a low Triton concentration. Also, the cells were later collected and resuspended in medium without Triton to see if they regained their sensitivity (Fig. 3).

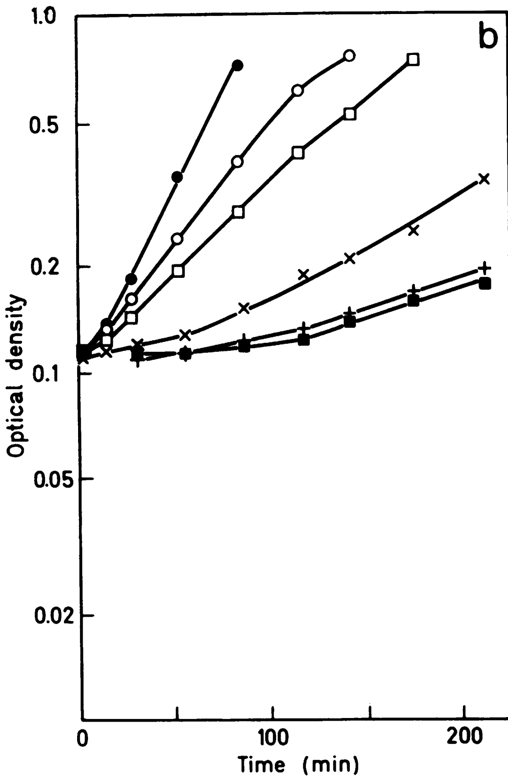
Exponentially growing wild-type cells were inoculated into Penassay broth containing 0.006% Triton. At intervals, samples were removed and added to prewarmed medium containing Triton to give a final concentration of 0.02%. Viable cells were counted before and 15 min after adding the samples to this medium. At the time indicated, cells in the main culture were removed from Triton by being collected on a warmed membrane filter and washed and suspended in warmed Penassay broth. Throughout the experiment, the culture was appropriately diluted to keep the cell density between 10^7 and 10^8 cells per ml. Within one generation from first exposure to 0.006% Triton, the cells exhibited maximum adaptation as assayed in this test. Removal of cells from Triton was followed by a slower de-adaptation, which took eight generations before Triton resistance fell to its original level. The extremely rapid development of resistance to Triton and its decline after removal

TABLE 3. *Dry weight analysis*

Growth condition	Dry wt[a]		
	Wild type	TR49	TR49/wild type (%)
Penassay broth, exponential	243 ± 39 (0.58)	136 ± 16 (0.61)	56
Spizizen medium, exponential	200 ± 14 (0.67)	110 ± 7 (0.75)	55

[a] Values represent, in nanograms per meter of cell length, the means of at least three experiments ± standard deviation; values in parentheses indicate grams (dry weight) per milliliter of cell volume, calculated by using the cell circumference estimates in Table 2.





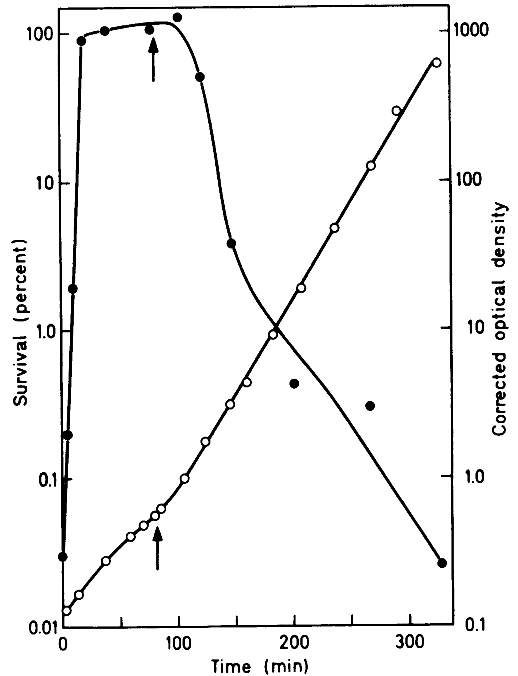


FIG. 3. *Adaptation and de-adaptation of wild-type 168 cells to Triton X-100. At zero time, cells growing exponentially in Penassay broth were subcultured into broth containing 0.006% Triton. At the time indicated (↑), the cells were collected by filtration and suspended in broth without Triton. At intervals, portions of culture were removed and viable cell counts were determined before and after 15 min of incubation in broth containing 0.02% Triton. Symbols:* ●, *percent cells surviving the incubation in 0.02% Triton;* ○, *optical density of culture, corrected for dilutions which were made to keep the actual optical density below 0.5.*

of Triton show that an adaptation process was involved.

To compare the sensitivities of wild-type and TR49 strains to lysis induced by Triton, each strain was grown exponentially in Penassay broth for about twelve generations and then subcultured into broth containing Triton at 0.02% final concentration. Lysis of the resistant strain, measured by turbidity loss, was similar to that of the wild type (data not shown).

Adapted wild-type cells growing in broth at relatively high concentrations of Triton (0.018 to 0.025%) became somewhat rounded. As

FIG. 2. *Effect of Triton X-100 on the growth of wild-type strain 168. At zero time, exponentially growing cells were subcultured into Penassay broth containing Triton at various concentrations. (a) Cells pregrown in Penassay broth; (b) cells pregrown for three generations in Penassay broth containing 0.006% Triton. Final Triton concentrations were zero* (●), *0.007%* (△), *0.008%* (○), *0.009%* (▲), *0.010%* (□), *0.015%* (×), *0.020%* (+), *and 0.025%* (■).

judged from plate cultures, they were unable to grow indefinitely at high concentrations of Triton. Adapted cells of strain TR49 were able to grow indefinitely, although slowly, at very high concentrations (up to at least 1%) without loss of rod morphology.

Growth rates and chemical composition. The ratio of surface area to volume of strain TR49 was roughly 1.4 times that of the parental strain. One might expect to find that this alteration causes deleterious effects on metabolism and growth, in view of the important metabolic and topological roles of the cell surface. However, growth of strain TR49 was only slightly, although significantly, slower than that of wild-type 168 (Table 4). Growth rates were determined turbidimetrically by using cultures that had already grown exponentially for at least ten generations. The ratios of dry weight to optical density were similar for the two strains, although the ratios of cell number to optical density differed (data not shown). This does not affect determination of doubling times, however, provided that the relationships between optical density and dry weight or cell number is linear, as is commonly found for various bacteria at low cell densities (22).

DNA, RNA, and protein contents of the two strains were compared at two growth rates (Table 5). The only significant difference between them was that strain TR49 contained a higher protein/RNA ratio than strain 168 when they were grown in Penassay broth. There were no significant differences in DNA content. In both strains, the protein/RNA ratio was much higher in cells grown in Spizizen medium than in Penassay broth, in agreement with previous results for this species (14) and *S. typhimurium* (36).

Further chemical analyses revealed that, under both growth conditions, the peptidoglycan content per total dry weight was higher in strain TR49 than in wild type (1.5 and 2.0 times as much in Penassay broth and Spizizen medium, respectively). The higher content in Penassay broth is almost completely accounted for by the high ratio of surface area to volume of strain TR49 (1.4 times the wild-type value). However, even after correction for this ratio, the peptidoglycan content of strain TR49 was 1.4 times that of wild type when both were grown in Spizizen medium. This suggests that, under this condition at least, resistant strain TR49 contains more peptidoglycan per μm^2 of cell surface area than does the parental strain.

Revertants. Strain TR49 sporulated about 100-fold less frequently than the wild type when grown to stationary phase in Schaeffer sporulation broth (37). We exploited this property to obtain spontaneous revertants of TR49 by selecting for efficient sporulation (see Materials and Methods). Colonies resulting from six separate experiments were tested for Triton sensitivity. In three cases, no sensitive colonies were found (frequency <0.05%). In the three successful experiments, they were present at frequencies of about 2, 22, and 45%. The sensitive colonies in each experiment probably represented single clones. One isolate from each experiment was grown in Penassay broth for cell circumference determination (Table 1). One strain was indistinguishable from the wild type, and another was only slightly narrower. The third was probably slightly wider than strain TR49. Thus, selection for revertants in sporulation resulted in revertants in Triton sensitivity and cell diameter.

TABLE 4. *Growth rate at 30°C*

Medium	Generation time (h)[a]		
	Wild type	TR49	TR49/wild type
Penassay	0.56	0.66 (10^{-4})[b]	1.18
Spizizen	2.12	2.32 (10^{-4})[b]	1.09

[a] Each value represents mean of at least three experiments.

[b] Value in parentheses indicates probability of obtaining this result if TR49 grew at the same rate as wild type, and is calculated from standard error of difference.

Stationary-phase TR49 cultures in sporulation broth contain about 10^9 viable cells per ml (8×10^8 to 9×10^8 cells per ml). Since only 0.5 ml was subcultured at each step of the selection, and since half of the experiments were successful, the revertants must have been present initially at a minimum frequency of 10^{-9}.

DISCUSSION

Effects of Triton X-100 on the cell. I think that at least some of the main effects of Triton X-100 on *B. subtilis* resulted from its interaction with the plasma membrane. This is supported by reports that Triton X-100 at sublytic concentrations interferes with the function or structure of eukaryotic (2, 8) and artificial lipid (20, 38) membranes. Furthermore, a wide variety of substances seem to enter cell membranes in a relatively nonspecific manner, mainly as a result of their amphiphilic chemical properties (13, 19, 40), and cause a variety of biological effects (16, 40, 42, 50). Adaptation to Triton may have involved changes in membrane fatty acid composition, as when *E. coli* adapted to aliphatic alcohols (19). It is not known whether cell lysis (Fig. 2a) resulted primarily from membrane lysis or the triggering of cell wall autolysis (11, 46).

Resistant variant TR49. Triton resistance,

TABLE 5. *Chemical composition*[a]

Growth medium	Ratio	Wild type	TR49	P
Penassay	DNA/(RNA + protein)	3.67 (±0.15) × 10^{-2}	3.58 (±0.16) × 10^{-2}	0.66
	DNA/total dry weight	2.85 (±0.15) × 10^{-2}	2.73 (±0.07) × 10^{-2}	0.78
	Protein/RNA	0.81 (±0.01)	1.10 (±0.06)	$<10^{-4}$
	(DNA + RNA + protein)/total dry weight	0.83 (±0.04)	0.78 (±0.01)	0.12
	Peptidoglycan/total dry weight	5.7 (±0.5)	8.7 (±0.5)	$<10^{-4}$
Spizizen	DNA/(RNA + protein)	3.93 (±0.16) × 10^{-2}	4.13 (±0.22) × 10^{-2}	0.76
	Protein/RNA	1.93 (±0.02)	1.90 (±0.03)	0.24
	(DNA + RNA + protein)/total dry weight	0.65 (±0.01)	0.63 (±0.02)	0.3
	Peptidoglycan/total dry weight	3.5 (±0.4)	7.1 (±0.2)	$<10^{-4}$

[a] Each value represents the mean of three experiments (± standard error). The ratios were calculated from quantities (in micrograms) of the substances per milliliter of cell suspension. Peptidoglycan was determined experimentally as nanomoles of diaminopimelic acid. The weight of peptidoglycan was calculated assuming that the average molecular weight per diaminopimelic acid residue was: (i) approximately 905 (unpublished data) and (ii) the same for both strains under both growth conditions. *P*, Probability of obtaining observed difference if strains 168 and TR49 were actually equal. Calculated from standard error of difference.

reduced cell diameter, and poor sporulation all probably resulted from a single mutation. Both the original variant and its revertants were present in wild-type and TR49 populations, respectively, at frequencies commonly found for single mutations and too high to be explained by multiple mutation. However, further genetic studies are needed before this interpretation is accepted. The poor sporulation by strain TR49 may have been a consequence of its small cell size (my unpublished data), its abnormal cell shape, or membrane alterations (cf. 3, 4).

The critical micelle concentration for Triton X-100 depends on the medium, but is approximately 0.02% (20, 47). Inhibition of growth of adapted cells became severe only at concentrations approaching the critical micelle concentration (Fig. 2b). Since the concentration of a detergent in a membrane does not increase above the critical micelle concentration (31), a slight change in cell properties could give the observed resistance to very high Triton concentrations.

Cell division. Thin mutants provide a new possibility for investigating regulation of the cell division cycle, assuming that their mutation does not significantly affect that regulation. An event in the cell cycle may occur at a certain cell length, cell surface area, and cell volume or mass. All these properties increase together during the cycle; therefore, it is difficult to determine which of them, upon attainment of a critical value, is most directly involved in initiating the event (cf. 9). At equal growth rates, the values of the critical property will be the same for both wild-type and thin mutants. However, the values of the other two properties will be different in each strain when the event occurs, because of their marked differences in cell length and surface area-to-volume ratios. In *E. coli*, initiation of DNA synthesis appears to occur upon attainment of a certain cell volume or mass (10), although a regulatory role for membrane area was suggested (25).

The data reported here indicate that in *B. subtilis* 168, overall DNA synthesis is related to cell mass or volume and not to cell surface area or length. In strain TR49, the ratios of cell surface area and cell length to volume were increased about 1.4- and 1.8-fold, respectively, compared with the wild-type strain growing at very similar rates. Nevertheless, to within experimental resolution, the ratio of DNA to total mass was not altered. The above conclusion does not necessarily apply to DNA initiation (6, 7).

Since the cell material of TR49 is spread out over nearly twice the normal length (Table 3), one might ask how nuclear segregation is achieved in this strain.

Cell diameter. Henning has discussed how bacterial cell diameter is determined (17). The cell circumference data reported here must differ from the circumferences of living cells. In vivo, the cell walls will be hydrated, stretched by osmotic pressure (24), and subject to effects of inorganic ions (24, 29). Indeed, the values of dry weight per calculated cell volume (Table 3) indicate that shrinkage of cell circumference occurred during specimen preparation. The apparent shrinkage in length (see Materials and Methods) alone was insufficient to explain these re-

sults. Nevertheless, the data on dry weight per cell length indicate that significant differential shrinkage of strains TR49 and 168 did not occur, despite their different peptidoglycan contents.

Shrinkage is also a problem with dried whole cells and sectioned cells (unpublished data), and noninterference visible light microscopy is of insufficient resolution for accurate studies (34).

Circumference and dry weight data suggest that the diameter of *B. subtilis* 168 varied with growth conditions, and that thin variant TR49 varied similarly. Sargent (35) stated that, for his derivative of *B. subtilis* 168, cell width did not vary detectably with growth rate. The reduced cell diameter of TR49 cannot be attributed to its slightly slower growth, because at a generation time of 0.66 h it was much thinner than wild-type cells growing with a generation time of 2.12 h.

Within a population of cells in balanced growth, cell width is determined quite accurately (the standard deviation of cell circumference is <4% of the mean) as in *E. coli* (51). Diameter or circumference or surface curvature could be determined by a feedback regulatory mechanism more or less directly sensitive to one of these properties, or it might be the dynamically stable outcome of interaction between many factors such as wall structure, rate and topology of wall formation, mechanism and rate of wall expansion, and rate and topology of wall turnover. If the second alternative were true, one might expect to find that a large variety of chemicals and mutations affect cell diameter (cf. 49). Such mutants may be quite numerous but not noticed, since it is not easy to judge cell diameter.

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