Some Properties of a Sporulating *Bacillus subtilis* Mutant Containing Heavy DNA

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SUMMARY

A mutant (Cbl-1) was isolated from *Bacillus subtilis* 168 wild type (wt) sporulating cultures grown on nutrient agar. This mutant differs from the parental strain as follows. (1) In nutrient broth the mutant excretes a substance which kills the wt cells. Therefore when grown in a mixed culture (Cbl-1 plus wt), this property leads to a take-over pattern by the Cbl-1 mutant, and the spores obtained from such a culture are entirely of the mutant type. (2) The DNA of the Cbl-1 strain has a buoyant density in CsCl of 1.714 g./cm.³ while that of the normal *B. subtilis* 168 is 1.703 g./cm.³ although both strains have a G+C content of 42 moles %. An explanation for the higher buoyant density of the Cbl-1 DNA has not yet been found. In addition to the base ratio data, transformation and transduction experiments indicate that Cbl-1 is a mutant of *B. subtilis* 168.

INTRODUCTION

The interaction between populations in bacterial cultures is a well-known phenomenon which leads frequently to unpredictable results. The phenotypic expression and the proportions of types of bacteria in a microbial population is strictly controlled through selective mechanisms by the interaction of the bacteria in a given environment. One such typical example is the rough—smooth variation of *Brucella abortus* (Braun et al. 1951). The proportions of these two types of cells in the culture is regulated by the concentration of alanine. As the smooth inoculum grows it excretes alanine, which stimulates its further growth but not that of the rough cells. As a result a progressive selection of the smooth cells ensues, so that during the stationary phase they are the predominant type in the population.

Another example of cellular interaction was recently reported by Meers & Tempest (1968). Their studies concern the influence of extracellular products on the behaviour of mixed microbial populations in magnesium-limited cultures. The ability of *Bacillus subtilis* and *Bacillus megaterium* to outgrow each other or outgrow yeast in a magnesium-limited medium depends on the concentration of a specific extracellular factor produced in the Bacillus cultures.

The experiments presented here demonstrate an unexpected predominance of a mutant of *Bacillus subtilis* 168 when grown in a mixed culture with the parental
wild-type strain. The results to be described provide also an explanation of the presence of 'heavy' DNA in the spores of *B. subtilis*, previously reported from this laboratory (Halvorson, Szulmajster, Cohen & Michelson, 1967). This spore DNA was reported to differ from that isolated from the vegetative forms of this organism by its higher buoyant density in cesium chloride and by a higher Tm, although the base composition of these two DNAs was found to be identical. Although a biological role was not assigned to this heavy 'spore' DNA (Halvorson *et al.* 1967), the reported data was suggestive of a difference between spore DNA and vegetative cell DNA. This impression was also supported by similar findings of Douthit & Halvorson (1966) in *B. cereus*. However, this interpretation seems to be erroneous, at least in *B. subtilis*, in the light of the unexpected physiological behaviour and properties of a spontaneous mutant found in the same *B. subtilis* cultures, used in the previous work (Halvorson *et al.* 1967). Thus the 'heavy' DNA appears to be that of the spontaneous Cbl-1 mutant rather than specific for spores.

**METHODS**

**Organisms.** Derivatives of *Bacillus subtilis* strain MARBURG were used throughout these studies. The genotype source and method of isolation are shown in Table 1. To minimize heterogeneity, all of the markers studied were introduced into *B. subtilis* 168 by Reilly (Young, Smith & Reilly, to be published). The Cbl-1 strains were isolated from several aged spore stock cultures of *B. subtilis*, 168 and SMY. These strains are the same as those used previously (Halvorson *et al.* 1967). Evidence will be presented that Cbl-1 is most likely a mutant of the 168 strain. This organism can hardly be detected in *B. subtilis* populations when plated on minimal agar media or in overnight cultures on complex media. After 2–4 days of growth on nutrient medium, the small white mutant colonies are readily distinguished from the larger dark brown wt colonies.

An erythromycin-resistant derivative from the Cbl-1 mutant was isolated by plating 0·1 ml. of a heavy spore suspension (about 1–2 x 10^8 spores/ml.) after heating
Bacillus subtilis mutant containing 'heavy' DNA

10 min. at 80°, on nutrient broth agar (Difco) containing 1 μg. erythromycin/ml. After 2 days at 37°, eleven colonies which still formed spores (Spo+) were isolated from 25 plates. These clones retained albino phenotype and were erythromycin-resistant.

**Culture media and bacterial assays.** The sporulation medium used in most of the experiments has been described previously (Kerjan, Marchetti & Szulmajster, 1967). In some experiments the nutrient broth was replaced by Bacto-peptone (Difco). Each new batch of the above nutrients was checked to determine whether it provided a high rate of sporulation (at least 80% of the population in 24 hr) before serial experiments were undertaken.

Liquid cultures were always grown in Fernbach flasks containing a volume of medium not exceeding one-tenth of the volume of the flask. Cultures of 250 ml. were usually inoculated with exponential cultures. The inocula of the parent strain were prepared by inoculating 10 ml. of medium with spores from agar slopes. For Cbl-I, cells grown for 18 hr on Tryptose Blood Agar base (TBAB, Difco) were used instead of spores. All the cultures were incubated at 37° with vigorous aeration. Growth was followed by determining the extinction at 650 mμ in a Zeiss spectrophotometer.

Viable counts were made by plating the organisms on nutrient broth agar (Difco) after suitable dilutions in sterile 0.05 M phosphate buffer (pH 7.0). The number of spores was counted after heating the suspension for 10 min. at 80°. In some experiments the number of spores was determined in a Petroff-Hausser counting chamber with a phase-contrast microscope.

**Isolation of DNA.** DNA from vegetative forms was prepared according to Marmur (1961) and from spores by the method described previously (Halvorson et al. 1967).

Sedimentation equilibrium measurement in CsCl was performed in the Spinco Model E ultracentrifuge (Meselson, Stahl & Vinograd, 1957) using ultraviolet absorption photographs scanned with a Joyce-Loebel recording micro densitometer. The density of DNA was calculated by using the 2 C phage DNA (a gift from Dr P. May) as a reference.

Base ratios were determined as previously described (Halvorson et al. 1967).

**Transduction.** Modification of the procedures of Takahashi (1961), and Hoch Barat & Anagnostopoulos (1967) were used to prepare lysates and obtain transduction (Young, Smith & Reilly, to be published). After transduction of a motile variant of BR 19 (carrying hisA1 and trp-2) the His+ clones were picked and subsequently replicated on TBAB plates in the absence and presence of phage ø 25 to test for phage resistance.

**Transformation.** The procedures for preparing competent bacteria were similar to those described previously (Young & Spizizen, 1961) with the exception that bacteria were grown for 5 hr in medium 1 and 65 min. in medium 2. Auxotrophic requirements were satisfied by adding 50 μg./ml. of the necessary amino acids.

Transformation was also achieved by treating the recipient bacteria at T₃ (3 hr after exponential growth) with donor DNA for 30 min. and terminating the reaction by adding 100 μg. (0.1 ml.) of pancreatic deoxyribonuclease (Worthington) (Bott & Wilson, 1967). Shaking was continued for 15 min. and the cells centrifuged down. Suitable dilutions of the cells were then made in minimal medium and plated either on Spizizen minimal medium agar plus or minus tryptophan the required amino acid or on nutrient broth agar plus or minus erythromycin. According to Takahashi (1965)
the erythromycin-resistant transformants were plated in 10 ml of melted nutrient broth agar (Difco), kept at 45°C, left for 3 to 4 hr at 37°C, at which time the plates were overlaid with 10 ml of the same medium containing erythromycin 2 μg./ml. The numbers of erythromycin-resistant colonies were counted after 2 days. All the recipient strains utilized for transformation contained the mutant trpC2 locus as well as other mutations (Table I). Thus, it was possible to compare all data to the frequency of transformation for trp-2 markers.

RESULTS

Physiological behaviour of the Cbl-1 mutant

Figure 1 shows that the 168 strain of Bacillus subtilis or an artificial mixture of this strain and the mutant Cbl-1 (1:1 ratio) initiated growth immediately at an exponential rate. Under the same conditions growth of the Cbl-1 strain started with a lag which lasted about 4 hr. In some experiments the lag was as long as 5 hr. Growth in the mixed culture was due to the 168 strain as observed by plating samples at different time intervals on nutrient broth agar after suitable dilutions.

Figure 2 shows the proportion of the two types of cell in a mixed culture. The mixture was made with exponentially growing cells of the two cultures in proportions shown in the figure at time 0. Cells of each type are readily distinguished by their
Bacillus subtilis mutant containing 'heavy' DNA

phenotype on nutrient agar. After 35 hr of incubation the surviving \( wt \) organisms were almost entirely spores.

As shown in Fig. 3 the ability of Cbl-1 to take over the population depended on the relative amounts of each bacterial type in the culture after mixing the two populations.

Development of spores in separate and mixed cultures of \( wt \) and Cbl-1. Three flasks, \( a, b \) and \( c \), each containing 25 ml. of nutrient broth (Difco) were inoculated with equal proportions of exponentially growing cells of (a) strain 168 \( M \), (b) Cbl-1 and

![Graph](image)

Fig. 3. Dependence of the number Cbl-1 cells in a final mixed culture on the Cbl-1/168 \( M \) ratio present in the initial culture after mixing.

Fig. 4. Buoyant density of Cbl-1 DNA. Micro densitometer tracings of samples equilibrated in a CsCl density gradient formed by centrifugation at 42,040 rev./min. at 20°. The band on the right is the standard 2 \( C \) phage DNA.

Table 2. Evolution of spore formation in separate and mixed culture of 168 \( M \) and Cbl-1 strains

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>168 ( M )</th>
<th>Cbl-1</th>
<th>168 ( M )</th>
<th>Cbl-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_0 )</td>
<td>( C ) ( 1.6 \times 10^8 )</td>
<td>( 7.7 \times 10^7 )</td>
<td>( 1 \times 10^8 )</td>
<td>( 7 \times 10^7 )</td>
</tr>
<tr>
<td>( S )</td>
<td>( - )</td>
<td>( - )</td>
<td>( - )</td>
<td>( - )</td>
</tr>
<tr>
<td>( T_5 )</td>
<td>( C ) ( 2.6 \times 10^8 )</td>
<td>( 3.2 \times 10^8 )</td>
<td>( 6.5 \times 10^7 )</td>
<td>( 6 \times 10^7 )</td>
</tr>
<tr>
<td>( S )</td>
<td>( 3.5 \times 10^8 )</td>
<td>( 7.5 \times 10^7 )</td>
<td>( &lt; 10^6 )</td>
<td>( 3.5 \times 10^8 )</td>
</tr>
<tr>
<td>( T_7 )</td>
<td>( C ) ( 3.5 \times 10^8 )</td>
<td>( 2.8 \times 10^8 )</td>
<td>( 7 \times 10^7 )</td>
<td>( 7 \times 10^7 )</td>
</tr>
<tr>
<td>( S )</td>
<td>( 7.5 \times 10^7 )</td>
<td>( 7 \times 10^7 )</td>
<td>( &lt; 10^6 )</td>
<td>( 2 \times 10^7 )</td>
</tr>
<tr>
<td>( T_9 )</td>
<td>( C ) ( 3.6 \times 10^8 )</td>
<td>( 2.5 \times 10^8 )</td>
<td>( 3 \times 10^7 )</td>
<td>( 1 \times 10^8 )</td>
</tr>
<tr>
<td>( S )</td>
<td>( 1.2 \times 10^8 )</td>
<td>( 1 \times 10^8 )</td>
<td>( 2 \times 10^7 )</td>
<td>( 4.5 \times 10^7 )</td>
</tr>
<tr>
<td>( T_{24} )</td>
<td>( C ) ( 2.9 \times 10^8 )</td>
<td>( 3 \times 10^8 )</td>
<td>( 5 \times 10^7 )</td>
<td>( 1.2 \times 10^9 )</td>
</tr>
<tr>
<td>( S )</td>
<td>( 2.2 \times 10^8 )</td>
<td>( 3.5 \times 10^8 )</td>
<td>( &lt; 10^6 )</td>
<td>( 1 \times 10^8 )</td>
</tr>
<tr>
<td>( T_{40} )</td>
<td>( C ) ( 3 \times 10^8 )</td>
<td>( 2.9 \times 10^8 )</td>
<td>( &lt; 10^6 )</td>
<td>( 9.5 \times 10^7 )</td>
</tr>
<tr>
<td>( S )</td>
<td>( 3.5 \times 10^8 )</td>
<td>( 3 \times 10^8 )</td>
<td>( &lt; 10^6 )</td>
<td>( 1.2 \times 10^9 )</td>
</tr>
</tbody>
</table>

\( C \) = bacteria before heating. \( S \) = bacteria after heating 10 min. at 80° C. (spores). \( T_0 \) designates the end of exponential growth; \( T_5, T_7, \) etc., indicate time in hr after \( T_0 \).
(c) an equal volume of wt+Cbl-I. Growth was followed by increase in extinction and by plating samples on agar plates in suitable dilution at different time intervals. T₀ (the end of exponential growth) was normalized for the three cultures by taking into account the 5-hr lag phase observed with the mutant strain in this experiment. The experiment shows (Table 2) that sporulation of the bacterial populations of the two strains in separate cultures occurs at the normally observed rate in nutrient broth medium. This is also true for the Cbl-I strain in the mixed culture where the sporulation rate of the Cbl-I bacteria was comparable to that in the corresponding separate culture. On the contrary, sporulation of the wild-type population in the mixed culture was extremely low throughout the entire incubation period and these bacteria were continuously lysing. Consequently, after 24 hr, almost the entire bacterial population was composed of Cbl-I spores.

Table 3. Effect of 168/Cbl filtrate on 168 M cells

<table>
<thead>
<tr>
<th>Cbl-I filtrates from cultures:</th>
<th>Cells*</th>
<th>Spores*</th>
</tr>
</thead>
<tbody>
<tr>
<td>168 M bacteria, harvested at T₃ and transferred to:</td>
<td>Exponential</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>T₅</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>T₇</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>T₉</td>
<td>2.2</td>
</tr>
<tr>
<td>Control 168 M bacteria at T₃ transferred to:</td>
<td>168 M filtrate from a culture at T₃</td>
<td>100</td>
</tr>
</tbody>
</table>

* Relative values after 24 h.
† The end of exponential growth is designated as T₀; T₃, T₇, T₉, etc., indicate time in hr after T₀.
‡ In this experiment 100 is equal to 1.9 × 10⁸ bacteria and to 1.8 × 10⁸ spores.

Effect of Cbl-I filtrates

It appeared that the Cbl-I strain was excreting a substance toxic to and perhaps lytic for the wild type. Although the nature of this substance is not as yet known it was of interest to determine the time during the growth cycle when the excretion takes place. For this purpose sterile (Millipore) filtrates were prepared from a Cbl-I culture at various stages of growth. These filtrates were kept overnight at room temperature. A 100 ml. culture of wt was grown, the bacteria harvested at T₃ and suspended in 2 ml. of fresh nutrient broth. Each sterile filtrate was inoculated with 0.2 ml. of the 168 suspension and incubated at 37°C for 24 hr with vigorous shaking. In the control experiment the 168 suspension was transferred to its homologous filtrate. Viable bacteria were counted by plating before and after heating (spores) on nutrient broth agar as described in ‘Methods’.

A typical experiment is summarized in Table 3. The filtrate prepared from exponential Cbl-I bacteria supported growth and had no effect on the sporulation of the wt. When the 168 cells were inoculated in a T₃ filtrate, most of the bacteria remain viable but only about 15% of them formed spores. The most dramatic effect was observed when wild-type bacteria were inoculated in a Cbl-I filtrate prepared from T₃ or later cultures. Very few bacteria remain viable and consequently very few spores were formed. A change in the number of bacteria or spores was not observed in the control which had not been transferred to filtrates of Cbl-I. These results suggest that the substance lysing or killing the wild-type bacteria is excreted by the Cbl-I bacteria into the medium.
**Bacillus subtilis mutant containing ‘heavy’ DNA**

during the stationary phase of growth. It was also observed that the Cbl-1 filtrate had no effect on bacteria of *Bacillus cereus, B. megaterium, B. licheniformis* or *Escherichia coli* harvested at the post-logarithmic growth phase.

**Buoyant density of Cbl-I DNA in CsCl**

The physiological experiments cited above directed our attention to the earlier observations of the appearance of a satellite band of DNA in sporulating cultures of *Bacillus subtilis* 168, which differed from that found in the vegetative forms by its higher buoyant density in CsCl equilibrium centrifugation and by its higher Tm (Halvorson et al. 1967).

Figure 4 shows the buoyant density in CsCl equilibrium centrifugation of the DNA isolated either from the vegetative forms or from the spores of the Cbl-I mutant strain: \( e = 1.714 \, \text{g./cm}^3 \). This is precisely the density previously reported for the ‘heavy’ DNA found in the *Bacillus subtilis* spores. In addition, the base ratio (Table 6) of the Cbl-I DNA (vegetative or spore) was found to be identical to that reported for the ‘heavy’ DNA (G+C = 42 to 43\%)(Halvorson et al. 1967).

**Genetic homology of Cbl-I and *B. subtilis* 168**

Is Cbl-I a contaminant or a mutant derived from *Bacillus subtilis* 168? Three different types of experiments support the contention that Cbl-I is a mutant of *B. subtilis* 168.

**Table 4. Transformation of *Bacillus subtilis* 168 auxotrophs with DNA *B. subtilis* wt and Cbl-I**

<table>
<thead>
<tr>
<th>Donor DNA</th>
<th>lys-3</th>
<th>trp-2</th>
<th>metB10</th>
<th>thy</th>
<th>trp-2</th>
<th>Ery*</th>
</tr>
</thead>
<tbody>
<tr>
<td>168 wt</td>
<td>7.0 x 10⁶</td>
<td>6.7 x 10⁶</td>
<td>7.0 x 10³</td>
<td>3.0 x 10⁴</td>
<td>2.3 x 10⁴</td>
<td>ND</td>
</tr>
<tr>
<td>Cbl-I Ery* (vegetative)</td>
<td>3.3 x 10⁶</td>
<td>1.3 x 10⁶</td>
<td>1.3 x 10³</td>
<td>ND</td>
<td>ND</td>
<td>5.2 x 10⁴</td>
</tr>
<tr>
<td>Cbl-I (spore)</td>
<td>10⁴</td>
<td>&lt; 10⁴</td>
<td>&lt; 10⁴</td>
<td>&lt; 10⁴</td>
<td>&lt; 10⁴</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Viable count varied from 9 x 10⁴ to 2 x 10⁶ in these experiments with all the recipient strains used. ND: not determined. In these experiments the concentration of wt, Cbl-I vegetative and spore DNA was 1, 0.5 and 0.1 µg/ml. respectively.

Firstly, the transformation experiments shown in Table 4 demonstrate that the DNA isolated from Cbl-I is capable of transforming mutants of *Bacillus subtilis* 168, although with a lower efficiency than *wt* DNA. It can also be seen in that Table that when the *thy, trp* auxotroph of *B. subtilis* 168 was used, transformation by the Cbl-I spore DNA was limited only to the *thy* phenotype. At present no explanation can be given to this surprising observation.

Secondly, traits from Cbl-I can also be transferred to strains of *Bacillus subtilis* by PBS-I mediated transduction. In these experiments lysates of PBS-I were prepared on motile vegetative populations of Cbl-I. These lysates were used to transduce three strains of *B. subtilis* 168, *BR19, BR85* and *BR151*. Successful transduction of the mutant loci in these strains was obtained in all experiments. As shown in Table 5, the locus-governing phage resistance in Cbl-1 is linked to hisAr. The percentage
co-transfer of Pha' with hisA1 is similar to that observed previously with class A and class B phage-resistant mutants of \textit{B. subtilis} 168 (Young, Brown & Reilly, 1968).

Thirdly, as shown in Table 6, the base ratio of the Cbl-1 spore or vegetative form DNA is consistent with that obtained by other authors for \textit{B. subtilis} 168 (Schildkraut, Marmur & Doty, 1962).

Table 5. \textit{Linkage of phage-resistance markers to his A1* in PBS-1 transduction}

<table>
<thead>
<tr>
<th>Donor (PBS-1 lysates)</th>
<th>Phenotype of recombinants</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTA412</td>
<td>His+ 400</td>
<td>Pha' 245</td>
</tr>
<tr>
<td>GTA290</td>
<td>His+ 600</td>
<td>Pha' 267</td>
</tr>
<tr>
<td>Cbl-1</td>
<td>His+ 400</td>
<td>Pha' 201</td>
</tr>
</tbody>
</table>

* Recipient BR19 (hisA1, trp-2).

Table 6. Base ratios of Cbl-1 spore and vegetative form DNA

<table>
<thead>
<tr>
<th>Source of DNA</th>
<th>A</th>
<th>G</th>
<th>C</th>
<th>T</th>
<th>G+C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative forms</td>
<td>28.2</td>
<td>21.8</td>
<td>21.7</td>
<td>28.3</td>
<td>43.5</td>
</tr>
<tr>
<td>Spores</td>
<td>28.3</td>
<td>21.6</td>
<td>21.0</td>
<td>29.3</td>
<td>42.6</td>
</tr>
<tr>
<td>Spores*</td>
<td>28.2</td>
<td>21.9</td>
<td>20.5</td>
<td>29.4</td>
<td>42.4</td>
</tr>
</tbody>
</table>

* From Halvorson, Szulmajster, Cohen & Michelson (1967).

**DISCUSSION**

The results presented here force a reinterpretation of the previously reported work from this laboratory (Halvorson \textit{et al.} 1967), which led to the suggestion that \textit{Bacillus subtilis} spores contained a 'heavy' DNA. In fact the spores from which the 'heavy' DNA was isolated derived from the Cbl-1 mutant present in the cultures. We have shown here that through its physiological properties the Cbl-1 strain, when present in a culture with 168 strain in relatively small numbers, may lead to a complete inversion of the bacterial type in the population.

Furthermore, because of the long lag of the Cbl-1 strain, these bacteria will be undetectable in such a mixed culture during exponential growth which will be dominated by the wild-type population (168 strain). Therefore, the DNA extracted from the bacteria during the latter period will have the properties of the normal \textit{Bacillus subtilis} DNA with its known density in CsCl, \( e = 1.703 \text{ g./cm.}^3 \). When exponential growth of the \textit{wt} stops, the Cbl-1 mutant starts to develop (Fig. 1) and continues to grow partially at the expense of the \textit{wt} which lyses. This lysis seems to affect the wild-type population during its entire presporulation period. This phenomenon leads rapidly to an increase of the proportion of Cbl-1 bacteria in the total population. As a result, the DNA extracted at this stage \((T_s-T_a)\) will show, in CsCl gradient centrifugation, a satellite 'heavy' DNA band in addition to the main band characteristic of the normal \textit{B. subtilis} DNA. Thus, a complete take-over by the Cbl-1 bacteria occurs in the culture and spores produced after 24 to 36 hr are derived predominantly from the Cbl-1 population. Consequently, if DNA is isolated from the dormant spores...
Bacillus subtilis mutant containing 'heavy' DNA

of such a culture, 85 to 95% of the DNA will have the density of 1.715 ± 0.002 g./cm.3, characteristic of the Cbl-1 mutant. In addition, it has also been observed that DNA is extracted from the Cbl-1 lyophilized spores by the method described previously (Halvorson et al. 1967) with an efficiency 2 to 3 times higher than from those of B. subtilis 168.

Base ratio analysis, transformation and transduction experiments, all indicate that Cbl-1 is a mutant of Bacillus subtilis 168 and not a contaminant. The accumulation of sporulation mutants in old cultures of bacilli is a well-known phenomenon. This is explained by the fact that after sporulation is completed, autolysis of the sporangia provides a new nutrient supply for the non-committed bacteria present in the population (Michel, Cami & Schaeffer, 1968). It is quite conceivable that the new nutrient supply may, in some cases, also favour the selection of a particular mutant similar to that reported here. The Cbl-1 mutants, as already mentioned, can easily escape detection in overnight cultures plated on nutrient agar, which are generally used as inoculum for liquid media. It is therefore not surprising that a given number of unrevealed mutant cells are introduced in liquid cultures together with the wild-type bacteria.

The problem which still remains unsolved is the cause of the high density of the Cbl-1 DNA despite its base ratio being identical to the normal Bacillus subtilis DNA. Chemical analyses (P, N, amino acids, abnormal bases and metals) have so far given no answer to the question.

Another interesting and perhaps fundamental aspect of the Cbl-1 mutant concerns its resistance to phages which infect Bacillus subtilis. Previous experiments have demonstrated that glucosylated teichoic acid is essential for the adsorption of many of the phages which infect B. subtilis (Young, 1967). These phage-resistant strains can be subdivided into three classes. (1) Class A (gtaA) mutants are defective in UDPG:polyglycerol teichoic acid glucosyl transferase and are stimulated by galactose. (2) Class B (gtaB) mutants do not have any demonstrable defects in the enzymes involved in the biosynthesis of glucosylated teichoic acid yet fail to glucosylate this polymer. The growth of these mutants is not affected by galactose. (3) Class C (gtaC) mutants are defective in phosphoglucomutase and lyse in a medium containing galactose. Morphologically, Cbl-1 resembles gtaB290, a strain which grows slightly slower than wt and produces small colonies on nutrient agar (Reilly, 1965). It is possible that the primary defect in Cbl-1 is an alteration of the association of enzymes with the structural protein of the cytoplasmic membrane analogous to the mutants of Neurospora studied by Woodward (1968). Studies are in progress to determine the enzymic defect in Cbl-1 mutant. It may be important to note that the genes regulating surface structures such as flagella (Grant & Simon, 1968) glucosylation of teichoic acid (Young et al. 1968) and the rough-smooth variation of B. subtilis 168 (Young & Haywood, unpublished observations) are all located in one region of the chromosome.

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