HETEROCARYOSIS-LIKE PHENOMENON IN BACILLUS SUBTILIS

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ABSTRACT

Ikeda, Y. (University of Tokyo, Tokyo, Japan) and T. IIjima. Heterocaryosis-like phenomenon in Bacillus subtilis. J. Bacteriol. 83:1025–1028. 1962.—When a mixture of methionine-dependent, histidine-dependent, streptomycin-resistant cells and tryptophan-dependent, streptomycin-sensitive cells of Bacillus subtilis K is plated on minimal medium supplemented with asparagine and streptomycin, tiny colonies consisting of both component cells and X-type cells appear at a frequency of about $10^{-4}$. Because the X-type cell segregates persistently both components and X-type, even after spore formation, it is considered to be a heterocaryon or a syncaryon possessing two kinds of genomes in a common cytoplasm.

It was reported in a previous paper (Kohiyama and Ikeda, 1959) that a mixed plating of two double auxotrophs of Bacillus subtilis K onto an appropriate medium gave tiny colonies consisting of both components and what appeared to be recombinants. Although the organisms of the intermediate step giving the recombinants were considered to be either heterocaryons or heterozygous diploids (Kohiyama, 1959), no concrete evidence was presented. In this paper, a heterocaryosis-like phenomenon observed in the same strain of B. subtilis is described.

MATERIALS AND METHODS

Strains. Two mutants of B. subtilis K were employed. M-12 is a methionine- and histidine-dependent and streptomycin-resistant strain (met'his' str-), which reverted spontaneously to met'his' str- and met'his' str- types at frequencies of $1 \times 10^{-4}$ and less than $1 \times 10^{-4}$, respectively. T-1 is a tryptophan-dependent and streptomycin-sensitive strain (try' str-s). The strain reverted to the wild type at a frequency of $2-5 \times 10^{-4}$.

Media. Minimal (MM) and complete (CM) media were described previously (Kohiyama and Ikeda, 1959). HM refers to MM supplemented with L-methionine and L-histidine, and T refers to L-tryptophan supplementation. Each amino acid was added to MM to a level of 100 $\mu$g/ml. The medium used for crossing (EM) consisted of constituents of MM and 100 $\mu$g/ml of L-asparagine. When streptomycin is added (100 $\mu$g/ml), each medium is described as CMS, MMS, and so on.

Method of crossing. Two mutants were incubated separately at 37 C in nutrient broth in shaken tubes, and transferred to fresh media on the next day. After 2 hr, cells were collected by centrifugation and suspended again in nutrient broth. The cell suspensions thus prepared from the two mutants were mixed and held at 37 C for 2 hr. The cell mixture was washed twice by centrifugation and plated on EMS. Colonies that appeared on this medium were counted after 3 or 4 days. All platings were carried out in triplicate. The number of viable cells in the suspension was determined by plating samples on nutrient agar after dilution.

Determination of segregants. The phenotypes of segregants from a tiny colony or an X-type colony (see below) were determined morphologically as well as nutritionally. For example, segregants capable of growing on CMS and HMS were judged as M-12 type, and those on CM and T as T-1 type. Furthermore, colony morphology of M-12 and T-1 (Fig. 1) was also employed for the determination. The X type is a possible heterocaryon or syncaryon which grows scantly on MM and segregates both M-12 and T-1 types on CM. The colony type is characteristic.

RESULTS

Appearance of tiny colonies on EMS. To investigate the combination of two auxotrophs capable of giving prototrophic colonies on EMS, the following crosses were carried out: M-12 (met'his') × T-1 (try'), M-12 (met'his') × T-12 (try'-thi'), M-12 (met'his') × A-17 (ade-ura'), M-12 (met'his') × T-11 (try'-ade'), T-12 (try'-thi') ×
A-15(ade-his-), M-12(met-his-) × A-1(ade-), and M-12(met-his-) × U-1(ura-). (One of the two components was streptomycin resistant.) It was found, however, that no cross except M-12 × T-1 could give colonies on EMS.

The progeny, recovered at a frequency of about $10^{-4}$ (Table 1), grew poorly on MM and MMS. Therefore, they are not prototrophs in a strict sense. Their colony morphology on EMS was quite similar to that reported previously (Kohiyama, 1959).

**Analysis of tiny colonies.** Four and seven tiny colonies obtained in experiments 1 and 2, respectively (Table 1), were analyzed. For the analysis, cells were picked up carefully with a platinum needle from the center region of each colony and streaked on MMS. The growth on this medium was very poor. After 3 days, whole cells were harvested into sterile water and homogenized by vigorous bubbling and agitation. Paired cells were quite few (less than 1%) in this suspension. Samples were plated on MM, and no prototrophic colonies appeared when $1.5 \times 10^4$ to $2.4 \times 10^5$ cells were tested (Table 2). Besides this, plating on MM supplemented with either L-methionine or L-histidine produced no colonies (not shown in Table 2).

When the cells were plated on CM after high dilution, three types of colonies appeared (Fig. 1) The largest grew on T and the medium on HMS. Therefore, they are T-1 type and M-12 type, respectively. The smallest, (X type) grew poorly on MM, HM, and T, and consisted of both component types (M-12 and T-1) and X type (see Table 2).

**Table 2. Analysis of tiny colonies**

<table>
<thead>
<tr>
<th>Colonies</th>
<th>Cells*</th>
<th>Segregants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M-12</td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>1-b</td>
<td>$2.7 \times 10^4$</td>
<td>44.0</td>
</tr>
<tr>
<td>1-c</td>
<td>$1.5 \times 10^4$</td>
<td>35.5</td>
</tr>
<tr>
<td>1-d</td>
<td>$5.4 \times 10^4$</td>
<td>30.6</td>
</tr>
<tr>
<td>1-e</td>
<td>$6.0 \times 10^4$</td>
<td>32.9</td>
</tr>
<tr>
<td>2-a</td>
<td>$10^5$ to $10^7$</td>
<td>36.3</td>
</tr>
<tr>
<td>2-b</td>
<td>$10^6$ to $10^7$</td>
<td>47.8</td>
</tr>
<tr>
<td>2-c</td>
<td>$10^6$ to $10^7$</td>
<td>41.7</td>
</tr>
<tr>
<td>2-d</td>
<td>$1.4 \times 10^5$</td>
<td>34.6</td>
</tr>
<tr>
<td>2-e</td>
<td>$2.5 \times 10^6$</td>
<td>37.5</td>
</tr>
<tr>
<td>2-f</td>
<td>$5.2 \times 10^6$</td>
<td>44.7</td>
</tr>
<tr>
<td>2-g</td>
<td>$2.4 \times 10^7$</td>
<td>43.0</td>
</tr>
</tbody>
</table>

* No prototrophic colonies developed.  
† Zero among 100 to 200 colonies tested.
Successive segregation of component types from X type. By selecting each X-type colony from clones 1-b, 1-c, 1-d, and 1-e, their segregation patterns were examined. Experiments were performed by plating homogenized cells on CM. Subsequently, colonies that appeared were classified by the morphological and the nutritional methods. Every X type examined gave rise to M-12, T-1, and X types (Table 3). Moreover, the second and the third X types also gave the same segregation patterns. Among them, the segregants from the third X types were examined carefully under a microscope. No large cells were found; that is, X-type cells appeared to be of normal size.

Segregation of component types from a single spore of an X-type cell. To eliminate the possibility that persistent association of two cells, one M-12 cell and one T-1 cell, might have resulted in the X type, a culture consisting of a large number of X-type cells and small numbers of M-12 and T-1 cells (the asterisked culture in Table 3) was transferred to sporulation medium (potato agar medium). When this spore suspension, in which more than 99% of the spores were single, was plated on CM, 98.6% of the colonies were X-type, 0.8% M-12 type, and 0.6% T-1 type. Since no significant difference was found between the culture with vegetative cells and the culture with spores, the result can be taken as evidence disproving the possibility mentioned above.

To produce more concrete evidence, a single spore was isolated by a micromanipulator. As a result, the first spore culture gave X, M-12, and T-1 types in a ratio of 99.90:0.05:0.05, and the second spore culture in a ratio of 99.56:0.30:0.14. These ratios are almost equal to that of the mother culture (97.0:0.3:2.7).

DISCUSSION

It can be concluded that an X-type cell (and spore) comprises two kinds of genomes. If genetic complementation were perfect between the two genomes, the cell would grow on minimal medium. Because this was not the case with the X-type cell, it is abnormal in any sense. Similar phenomena have been reported in B. subtilis (Kohiyama, 1959) and Streptomyces coelicolor (Bradley, 1958). Moreover, it is known that anomalous heterocaryons or syncaryons occasionally produce what appear to be recombinants. It might be because the selection of genetic markers was not adequate that no recombinant was recovered in this work. It must be noted, however, that the heterogeneity persisted even after spore formation. This fact suggests bipartite structure of one nucleus in a spore. If this is true, the term syncaryon may be better than heterocaryon.

With regard to the origin of X-type cells, there are three possibilities. Two of these, deoxyribonucleic acid-mediated transformation and phage-mediated transduction, are not probable. The culture filtrate contributed nothing to the formation of X-type cells. To prove the third possibility, cell conjugation between an M-12 cell and a T-1 cell, fertility of B. subtilis was studied. Although the problem has not yet been solved, we suggest the third mechanism as the most probable one. The phenomenon of cell conjugation has been demonstrated directly in Escherichia coli K-12 (Anderson, Wollman, and Jacob, 1957) and indirectly in Pseudomonas aeruginosa (Holloway and Fargie, 1960), Vibrio cholerae (Bhaskaran and Iyer, 1961), and S. coelicolor (Sermonti and Spada-Sermonti, 1956). Recently, the presence of episomic factors with different grades of fertility has been shown (Ozeki, Howarth, and Clowes, 1961).

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LITERATURE CITED


