COLONIAL DIMORPHISM IN NONMOTILE SALMONELLA

MASATOSHI ENOMOTO AND TETSUO IINO

National Institute of Genetics, Mishima, Japan

Received for publication 15 April 1963

ABSTRACT

ENOMOTO, MASATOSHI (National Institute of Genetics, Mishima, Japan) and TETSUO IINO. Colonial dimorphism in nonmotile Salmonella. J. Bacteriol. 86:473–477. 1963.—Two distinct colonial types, LP-type and SD-type, appeared among the nonmotile mutant clones originated from a strain of Salmonella typhimurium when they were cultivated on semisolid gelatin-agar medium (NGA) at 37°C. These colonial types reflect the nonmotile character of the mutant clones: a nonflagellated clone formed an LP-type colony which was large, translucent, and pale yellowish-gray, whereas a paralyzed flagellated clone formed an SD-type colony which was small, opaque, and dense yellowish-gray. Colonial dimorphism was also present in cells paralyzed by nonheritable change: flagellated cells, immobilized by treatment with homologous anti-H serum, formed SD-type colonies, while cells which had lost flagella, growing in the NGA medium containing 0.12% phenol, formed LP-type colonies. The distinction between the two colonial types was not impaired by addition of motility phage or various sugars to NGA, but became obscure when gelatin was omitted from the medium or the temperature of cultivation was lowered. It is concluded that the difference between the two colonial types is due to the presence or absence of flagella on the surface of the bacterial cells. Such correlation between flagellation and colonial type was observed on nonmotile mutants of several other smooth-type Salmonella strains and Escherichia coli but was not found in a rough-type Salmonella strain.

Nonmotile mutants originated from motile Salmonella are classified into two groups: one group is nonflagellated and the other produces paralyzed flagella (Stocker, Zinder, and Lederberg, 1953). Differences between these two types have been recognized by direct observation of flagella with light or electron microscopy or by agglutination tests with anti-H serum. In the present paper, it is reported that nonflagellated and paralyzed clones of smooth-type Salmonella strains form different types of colonies with each other under proper culturing conditions.

MATERIALS AND METHODS

Organisms. Four motile Salmonella strains, S. typhimurium TM2 and SW1292, S. abortus-equus SL23 (S. abortioequina), and S. abony SW1391, and nonmotile mutants derived from these strains were used for the present experiment. The characteristics of these strains have been given elsewhere (Lederberg and Iino, 1956; Iino, 1961a; Mäkelä, Lederberg, and Lederberg, 1962). A motility phage, chi, which was used for the selection of nonmotile mutants, was received from the Lister Institute of Preventive Medicine, London, England, by courtesy of B. A. D. Stocker.

Media. The composition of semisolid nutrient gelatin-agar medium (NGA) was as follows: 1% Kyokuto peptone, 1% Lender meat extract, 0.4% agar, and 8% gelatin in distilled water, the whole adjusted to pH 7.2. This medium is semisolid at 37°C and solidifies at temperatures below 25°C. It could, therefore, be used for testing bacterial motility or selecting motile bacteria out of a nonmotile bacterial culture, since, at 37°C, a motile clone forms a swarm while a nonmotile one forms a compact colony (Edwards and Bruner, 1942). In some experiments, gelatin was omitted or anti-H serum was added.

RESULTS

Detection of two colonial types among nonmotile mutants. Chi-phage attacks motile but not nonmotile Salmonella cells (Meynell, 1961). Consequently, a medium spread with chi-phage has been used for the selection of nonmotile mutants from motile Salmonella strains (Stocker et al., 1953). In an experiment to isolate nonmotile
mutants from a strain of *S. typhimurium* TM2, bacteria and chi-phage suspension were mixed in a tube containing the nutrient broth; the mixed suspension was incubated for 15 hr at 37 C, then a drop of the suspension was plated on NGA. After 24 hr of cultivation at 37 C, motile cells were mostly lysed, and temporary chi-resistant cells formed minute starlike colonies within the medium, while the nonmotile clone grew to a smooth, entire, compact colony on the medium. By detailed observation, it was found that there were two types among the nonmotile mutant colonies grown on the medium (Fig. 1A). One was large, low-convex, translucent, pale yellowish-gray (LP-type); the other was small, opaque, dense yellowish-gray (SD-type). H-antigen of these colonies was typed by slide agglutination. All LP-type colonies tested were H-negative, whereas all SD-type colonies carried an H-antigen of the parental strain, either i or 1.2. These colonial and antigenic characteristics of each clone were maintained through subsequent subcultures on NGA, even after the cultures had been freed from chi-phage. Electron microscopic study of the cells of these two colonial types showed that LP-type colonies consisted of cells without flagella whereas those of SD-type had normal but paralyzed flagella.

**Effect of medium or temperature.** On NGA, the distinction between LP- and SD-type became less clear as the temperature of cultivation was lowered. At 25 C, both nonflagellated and paralyzed clones formed SD-type colonies (Table 1, column 4). The colonial dimorphism was not apparent on a nutrient agar plate containing 1.5% agar in the nutrient broth. Omission of gelatin from NGA also resulted in the loss of colonial dimorphism. On such medium, irrespective of the temperature, all nonmotile clones formed LP-type colonies (Table 1, column 5). Addition of various sugars (galactose, arabinose, xylose, rhamnose, glucose, or lactose) to NGA in a concentration of 1.0% did not alter the two colonial types (Table 1, column 6).

**Colonial types of clones paralyzed by anti-H serum.** By addition of anti-H serum to NGA, motile *Salmonella* cells in contact with homologous H-antigen are temporarily paralyzed and form compact colonies instead of swarms (Lederberg and Ino, 1956). A diphasic strain of *S.

![Colonial types of nonmotile Salmonella mutants. Originated from (A) S. typhimurium TM2, (B) S. typhimurium SW1992, (C) S. abortus-equini SL23. Large colonies are LP-type, formed by nonflagellated mutants. Small colonies are SD-type formed by paralyzed mutants. Cultured in NGA at 37 C for 24 hr.](http://jb.asm.org/)
typhimurium, TM2 (i:1.2), and its monophagic mutant strain, SW1061 (−:1.2), were grown on NGA supplemented with corresponding anti-H serum, and the colonial types of the cells temporarily paralyzed by anti-H serum were examined. SW1061 is a mutant which is nonflagellated in antigenic phase 1 and produces normal 1.2-type flagella in phase 2 (Iino, 1961b). The results are summarized in Table 2. On NGA to which anti-phase 1 (i-type) serum was added, a phase 2 clone of either TM2 or SW1061 formed a swarm. In phase 1, TM2 was paralyzed and produced SD-type colonies in the presence of anti-phase 1 serum. On NGA to which anti-phase 2 (1.2-type) serum was added, phase 2 clones of both strains were paralyzed and grew to SD-type colonies, whereas a phase 1 clone of TM2 spread as a swarm. Nonflagellated phase 1 clones of SW1061 grew to LP-type colonies on both media as well as plain NGA. Thus, it was indicated that the flagellated clone temporarily paralyzed by anti-H serum formed an SD-type colony on NGA, whereas a nonflagellated clone formed an LP-type colony regardless of presence or absence of anti-H serum in NGA.

Colonial type of clones deflagellated by phenol. An appropriate concentration of phenol in a medium prevents the synthesis of flagella (reviewed by Lacey, 1961). When the cells of TM2 or SW1061 were cultivated overnight in a broth containing 0.12% phenol and plated on NGA containing the same concentration of phenol, the colonies composed of deflagellated cells became visible after 48 hr at 37 C. Their colonial characters were the same as those of LP-type nonflagellated mutants (Table 2).

Generality of colonial dimorphism in nonmotile Salmonella and Escherichia coli. In order to examine the generality of correlation between colonial type and flagellation in nonmotile mutant clones, of either nonflagellated or paralyzed type, further examination was made of mutants isolated from *S. typhimurium* TM2 on NGA medium. Among the mutants examined, 18 strains were nonflagellated types, of which 1 was obtained by ultraviolet-light treatment, 4 were spontaneous mutants isolated from NGA cultures, and the others were selected by chi-phage. All formed LP-type colonies on NGA at 37 C. The remaining 65 strains were paralyzed mutants of spontaneous origin isolated with or without chi-phage selection. These paralyzed mutants all formed SD-type colonies. The differences in the colonial type between nonflagellated and paralyzed clones were also confirmed for the nonmotile mutants derived from *S. typhimurium* SW1292, *S. abortus-equus* SL23 (Table 1 and Fig. 1B, C), and three *Escherichia* strains, W3094,

---

**Table 1. Colonial type of nonmotile Salmonella mutants grown on NGA media**

<table>
<thead>
<tr>
<th>Original motile strain</th>
<th>Nonmotile mutant*</th>
<th>Colonial type on NGA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. typhimurium</em> TM2</td>
<td>SJ78 (mot−)</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>SL478 (mot− ath− phe−)</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>SJ374 (fla−)</td>
<td>LP</td>
</tr>
<tr>
<td></td>
<td>SL480 (fla− ade− pro−)</td>
<td>LP</td>
</tr>
<tr>
<td><em>S. typhimurium</em> SW1292</td>
<td>SJ498 (mot−)</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>SJ398 (fla−)</td>
<td>LP</td>
</tr>
<tr>
<td><em>S. abortus-equus</em> SL23</td>
<td>SJ186 (mot−)</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>SJ149 (fla−)</td>
<td>LP</td>
</tr>
</tbody>
</table>

* Symbols: mot−, paralyzed mutant; fla−, nonflagellated mutant; ath−, phe−, ade−, pro−; mutants requiring adenine-thiamine, phenylalanine, adenine, or proline, respectively.
† Galactose, arabinose, xylose, rhamnose, glucose, or lactose was added in 1% concentration.

---

**Table 2. Colonial type of Salmonella clones paralyzed by anti-H serum or deflagellated by phenol**

<table>
<thead>
<tr>
<th>Strain</th>
<th>H-antigen</th>
<th>Colonial type with NGA</th>
<th>Anti-i serum-NGA</th>
<th>Anti-1.2 serum-NGA</th>
<th>Phenol-NGA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. typhimurium</em> TM2</td>
<td>Phase 1 i</td>
<td>Swarm</td>
<td>SD</td>
<td>Swarm</td>
<td>LP</td>
</tr>
<tr>
<td></td>
<td>Phase 2 1.2</td>
<td>Swarm</td>
<td>SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. typhimurium</em> SW1061</td>
<td>Phase 1 —</td>
<td>LP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phase 2 1.2</td>
<td>Swarm</td>
<td>SD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
W3096, and W3106, derived form E. coli K-12. An exception was a strain of S. abony SW1391. This strain forms rough colonies, and the distinction of colonial type between nonflagellated and paralyzed mutants was not clear: both mutant types formed colonies similar to SD-type on NGA.

**DISCUSSION**

There are many instances in which colonial characteristics are attributed to certain morphological, physiological, or serological features of the bacterial cells composing a colony. Especially, a change in bacterial cell surface antigen is often noticed as a change in colonial type. In *Salmonella*, the colonies which contain Vi-positive cells are more opaque than those composed from Vi-negative cells (Craigie and Brandon, 1936), and the loss of O antigen accompanies the change in colonial type from smooth to rough (Arkwright, 1921). The relationship of another bacterial surface antigen, flagellar antigen, to colonial type has been noted in connection with locomotive function. A clone of motile flagellated bacteria forms a swarm, while a nonmotile bacterial clone forms a compact colony on a semisolid medium (Edwards and Bruner, 1942). The present study demonstrated that a semisolid nutrient agar medium containing gelatin (NGA) differentiates not only a colonial type of motile from nonmotile cell but, among the latter, distinguishes two types in smooth *Salmonella* strains. The two types are composed of nonflagellated and paralyzed clones and form different colonial types, referred to in this paper as LP- and SD-types, respectively. The colonial type on NGA is constant, regardless of whether the nonmotile character is caused by mutation or by an environmental factor such as anti-H serum or phenol. Presence or absence of flagella on nonmotile cells also determines these colonial types. An exception was observed on a rough-type strain of S. abony SW1391. The colonial type of this strain may be affected strongly by other factors than flagella. The cells of this strain are filamentous and cling together. Therefore, it is possible that the clinging together of the filamentous cells produces a condition similar to that of paralyzed flagellated cells. It is likely that the difference in color between the two colonial types is due to the density of the component cells of the colonies, since SD-type colonies are smaller than those of LP-type though there is no significant difference between nonflagellated and paralyzed clones in rate of cell division or size of a cell. The intertwisting of the flagella of the cells may restrict mechanically the spreading on the medium, or the electric charge of flagellar protein may interfere with the mode of aggregation of the cells in a colony. It is worth noting that the colonies of paralyzed mutants grown on semisolid medium without gelatin show the LP-type which is characteristic of nonflagellated clones grown on NGA.

NGA has been used for detection of motile mutants from nonmotile bacteria (Stocker et al., 1953). On this medium, progenies of a motile mutant cell among more than 10⁶ nonmotile cells can be detected as a swarm spreading out from a compact mass of nonmotile cells. For the isolation of a nonmotile clone from motile bacterial culture, NGA is not suitable. For this purpose, motility phage has been used as the most effective selective agent. For example, chi-phage plated with *Salmonella* cells on a cultural medium kills only motile cells and allows the nonmotile cells to grow to form colonies. As reported in the present paper, chi-phage does not affect the distinctive colonial characteristics of both nonflagellated and paralyzed clones on NGA. Therefore, by using NGA and chi-phage combined, it is now possible to isolate either nonflagellated or paralyzed mutants in *Salmonella* with high efficiency.

Whether colonial morphology is useful for identification of nonflagellated and paralyzed bacteria other than *Salmonella* is an interesting question. The finding that a colonial dimorphism as in *Salmonella* is also present in nonmotile mutants originated from three motile clones of *E. coli* K-12 suggests the generality of the phenomenon.

**ACKNOWLEDGMENT**

This investigation was supported by research grant AI-02872 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

**LITERATURE CITED**


Edwards, P. R., and D. W. Bruner. 1942. Seto-