Colonial Morphology of *Escherichia coli* on Tergitol-7 Medium

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**ABSTRACT**

SCHERER, RHODES K. (National Animal Disease Laboratory, Ames, Iowa). Colonial morphology of *Escherichia coli* on Tergitol-7 medium. Appl. Microbiol. 14:152-155. 1966.—*Escherichia coli* cultures grown on Tergitol-7 medium, with 2,3,5-triphenyltetrazolium chloride added, produced three main types of colonies: rough, intermediate, and mucoid. These colonies were yellow to amber in color and produced slight yellow zones in the medium. Rough colonies were flat, dry, and spreading, with a cut-glass appearance. Intermediate-type colonies varied considerably, but could be divided into two general subtypes. Intermediate-rough colonies had the cut-glass appearance characteristic of rough colonies, but were much more compact and raised, with irregular edges. Intermediate-smooth colonies had a slight cut-glass appearance, but were smooth and entire. Mucoid-type colonies also appeared in two subtypes. Mucoid A colonies were mucoid hemispheres. Mucoid B colonies, after incubation at 37 C for 24 hr, appeared as small, intermediate colonies. However, during a 24-hr holding period at room temperature, mucilaginous material proliferated around the colonies. A fourth type of colony was red with blue surrounding medium. Only mucoid-type cultures could not be serologically O-grouped.

Blood-agar is widely used for culturing milk in the diagnosis of bovine mastitis. When coliform organisms occur, further differentiation with a minimal number of tests is desirable. Various selective media have been described for this purpose, their use depending mainly on the fermentation of lactose. An agar medium utilizing the selective bactericidal property of Tergitol-7 was described by Pollard (7). Chapman (1, 2) modified the medium by the addition of 2,3,5-triphenyltetrazolium chloride, thus making it easier to distinguish among the various coliform organisms. Wiseman and Sarles (9) used the medium as a screening method for differentiating the intestinal coliform bacteria in chickens.

While using this medium for characterizing coliform organisms isolated from bovine udder infections, we observed that the *Escherichia coli* isolates produced more than one type of colony. This finding was confirmed with isolates from other animal sources. The purpose of this report is to describe the different colonial types observed on Tergitol-7 medium.

**MATERIALS AND METHODS**

*Cultures.* *E. coli* cultures (251) were isolated from 92 animals; 138 cultures were from enteric infections in 41 calves, 57 were from feces in 19 cows, 39 were from udder infections in 21 cows, 8 were from vaginal swabs in 5 heifers, 4 were from an aborted fetus, 1 was from a pig, 2 were from 2 guinea pigs, and 2 were from a lamb. One culture (no. 11775) was obtained from the American Type Culture Collection.

After isolation, each culture was grown in beef infusion broth, then stored at -70 C until it was used in the studies described.

*Media.* Difco Tergitol-7 agar was used, with 2,3,5-triphenyl-tetrazolium chloride (TTC) added to give a concentration of 40 mg per liter of medium. The tetrazolium solution was sterilized by Seitz filtration and added to the melted and cooled (45 C) medium prior to pouring into petri dishes. The plates were dried by incubating overnight at 37 C and were then stored at 4 to 5 C.

*Morphology studies.* The colonial characteristics were studied with a Bausch & Lomb stereoscopic wide-field binocular microscope by use of reflected and transmitted light. Observations were made after the plates were incubated for 18 to 24 hr at 37 C, and again after another 18 to 24 hr at room temperature (25 C).

*Serology.* Seventy-two cultures of *E. coli* were serologically grouped according to their somatic antigens by Paul J. Glantz, Department of Veterinary Science, Pennsylvania State University, University Park, whose cooperation is gratefully acknowledged.
**E. coli on Tergitol-7 Medium**

Fig. 1. *Escherichia coli* colonial types on Tergitol-7 medium and blood-agar. (A) Rough (24 hr), 4.3 X; (B) intermediate-rough (24 hr), 4.3 X; (C) intermediate-smooth (24 hr), 4.1 X; (D) mucoid A (24 hr), 7.1 X; (E) mucoid B (24 hr), 5.9 X; (F) mucoid B (48 hr), 4.4 X; (G) tetrazolium reducer (24 hr), 10.8 X on Tergitol-7 medium. A mixture of the rough, intermediate, and mucoid types is shown on blood-agar (H) and Tergitol-7 medium (I) after 24 hr of incubation. The dense, lighter colored colonies on blood-agar are mucoid type A; the others are the rough and intermediate types. On Tergitol-7 medium, the colony types are (1) rough, (2) intermediate, and (3) mucoid type A.
RESULTS

Colonial morphology on Tergitol-7 medium with TTC. Four types of E. coli colonies were found. Three types, rough, intermediate, and mucoid, were yellow to amber and produced slight yellow zones in the medium. The fourth, tetrazolium-reducing type, was red with blue zones in the medium.

Rought-type colonies were flat, dry, and spreading, with irregular edges and a sharp, cut-glass appearance. These colonies measured 7 to 15 mm in diameter and were the largest of all types. Their color was yellow or amber (Fig. 1A).

Intermediate-type colonies varied considerably, but were divided into two general subtypes, intermediate-rough and intermediate-smooth.

Intermediate-rough colonies had the cut-glass appearance of rough colonies, but they were much more compact and raised and not as flat, dry, or spreading. The edges were irregular. The interstices of the cut-glass portion appeared to be filled with a translucent, moist material (Fig. 1B).

Intermediate-smooth colonies were much more compact, with slightly raised, darker yellow or amber centers, and more entire, lighter yellow edges. These colonies were smoother, with a slight cut-glass surface, the interstices of which appeared to be filled with a moist, translucent material (Fig. 1C).

Mucoid colonies also appeared in two forms, A and B.

Mucoid type A colonies were yellow, amber, or sometimes peach-colored mucoid hemispheres or globules. In some instances, these colonies produced long, mucuslike strings when teased with a small wire loop (Fig. 1D).

Mucoid type B colonies appeared as small (2.5 to 3 mm) intermediate-type colonies after incubation at 37 C for 24 hr (Fig. 1E). However, during a 24-hr holding period at room temperature, mucuslike material proliferated in a ring around the periphery of the colonies, giving the appearance of a doughnut (Fig. 1F). When cultures of this type were incubated continuously at room or lower temperature for 48 hr, mucoid-type colonies developed without the initial appearance of the small, intermediate form. However, when cultures of this type were incubated continuously for 48 hr at 37 C, only the intermediate-type colonies with no mucuslike material were formed (Fig. 1E). Mucoid type A strains always produced mucoid hemispheres, no matter what the temperature of incubation. Neither did temperature of storage have any effect on our mucoid types A or B. None of the rough- or intermediate-type cultures grown at 37 C and then held at room temperature, nor the six rough- and intermediate-type strains grown at 10 and 20 C, showed this phenomenon.

Six cultures of bovine fecal origin were unlike the other types in that the colonies were red, with clear, colorless entire edges of varying widths. They were moist, but not mucoid, and were smaller than any other type (1 to 2 mm). The surrounding medium was blue.

A mixture of rough-, intermediate-, and mucoid-type A cultures is shown plated on blood-agar (Fig. 1H), and the same mixture plated on Tergitol-7 medium with TTC after 24 hr of incubation (Fig. 1I). On blood agar, the rough and intermediate types appeared gray, granular, and translucent, except for the centers, which were more dense. The mucoid type A colonies appeared whiter and denser than the rough and intermediate types. The tetrazolium-reducing cultures formed white, smooth, entire colonies that were much more dense and generally smaller than all other types.

Biochemical reactions. The rough and intermediate-type cultures gave comparable biochemical reactions, with few exceptions. Mucoid-type cultures were less active biochemically than cultures of the rough or intermediate types, whereas the tetrazolium-reducing type was the least active of all. The latter organisms fit the description of alkaliscens, except for the production of gas in lactose and mannitol.

Serological reactions. The results of the serological O-group typing are presented in Table 1. Half of the rough colonial-type cultures, 56% of the intermediate-type cultures, none of the mucoid-type cultures, and all of the tetrazolium-reducing type cultures tested could be O-grouped. Only one O group (135) was shared by the rough- and intermediate-type strains. The culture in the

<table>
<thead>
<tr>
<th>Colonial type</th>
<th>No. of cultures</th>
<th>No. typable</th>
<th>O groups found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rough</td>
<td>10</td>
<td>5</td>
<td>4, 5ab, 9, 49, 135</td>
</tr>
<tr>
<td>Intermediate</td>
<td>48</td>
<td>27</td>
<td>2a, 6, 8, 10, 17, 22, 32, 82, 83, 108*</td>
</tr>
<tr>
<td>Mucoid A and B</td>
<td>8</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>Tetrazolium reducers</td>
<td>6</td>
<td>6</td>
<td>108</td>
</tr>
</tbody>
</table>

* This culture was only related to O group 108.
† Two cultures could be a mixture of O groups 22 or 130.
rough group was isolated from an udder infection in a cow in one herd, and the culture in the intermediate group was isolated from an udder infection in a cow in another herd.

**DISCUSSION**

Rough, smooth, and mucoid forms of *E. coli* have been described by Dulaney (3) and Parr (6). Dulaney's (3) description of the R form is applicable to our description of rough types on Tergitol-7 medium with TTC, and her S form is similar to our mucoid type A. The phenomenon of secondary mucoid proliferation found in mucoid type B incubated at 37°C and then at 23°C was described by Webster and Burn (8) in colonies of *Salmonella enteritidis* and for coli-aerogenes organisms by Parr (6). It was not necessary to hold our cultures at low temperatures (10 to 20°C) to maintain the mucoid properties, as was indicated by Morgan and Beckwith (5).

Rough- and intermediate-type cultures appeared to be more closely related biochemically than either type was to the mucoid- or tetrazolium-reducing types. The latter organisms may be similar to the red colonies classified by Wiseman and Sarles (9) as *Paracolobactrum coliforme* and *P. intermedium*.

Only the mucoid-type cultures could not be typed serologically, and only one of the 21 O groups was shared by the rough and intermediate colonial types. The fact that only one O group was shared, plus the fact that the mucoid strains were not typable, suggests that the differences in colonial morphology on Tergitol-7 medium with TTC might be of value for screening purposes in epizootiological studies of *E. coli* infections.

**LITERATURE CITED**