TAXONOMIC STUDIES ON CORYNEFORM BACTERIA

I. DIVISION OF BACTERIAL CELLS

KAZUO KOMAGATA, KAZUHIKO YAMADA AND HIROMOCHI OGAWA

Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan

(Received December 20, 1968)

Cell division of the so-called eubacteria and coryneform bacteria was studied by the time lapse microscopy and microcinematography. The rod-shaped bacteria were divided into the three types of simple type, snapping type, and bending type on the basis of the mode of cell division. From the results obtained the mode of bacterial cell division is considered to indicate a taxonomic significance, and eubacteria capable of growing by simple type division are clearly distinguished from coryneform bacteria which multiply by snapping or bending type.

The aerobic, Gram-positive, not acid-fast, non-sporeforming, rod-shaped bacteria which taxonomically belong to the genera of Corynebacterium, Microbacterium, Cellulomonas, Arthrobacter, etc., are called "coryneform bacteria" (1). Furthermore, the genus Brevibacterium established by BREED (2) is considered to belong to this category in a broad sense (3). Coryneform bacteria are widely distributed in soil, water, sewage, food, and plant and animal materials, and some have been employed for the commercial production of amino acids such as glutamic acid, lysine, etc. However, these bacteria have not been easily identified since their generic concepts were not established clearly. Mode of cell division of bacteria has been regarded as an interesting subject from the viewpoint of taxonomy, and a characteristic cell division of Corynebacterium diphtheriae has been reported (4) but details have been little known to date. This may be due to the difficulty encountered in observing the living bacterial cells which are small in size and optically less contrasting.

This paper deals with the cell division of the so-called eubacteria (hereafter designated as the bacterial group belonging to Pseudomonadales and Eubacterales except families Corynebacteriaceae and Brevibacteriaceae according to Bergey's Manual of Determinative Bacteriology, 7th Ed. (5)) and coryneform bacteria observed under the time lapse microscopy and microcinematography, and it was concluded that the rod-shaped bacteria are divided into

1 Present Address: The Institute of Applied Microbiology, University of Tokyo, Tokyo.
three groups, and that coryneform bacteria are distinguished from eubacteria on the basis of the mode of cell division.

MATERIALS AND METHODS

*Microorganisms.* Tested bacteria comprised 37 strains belonging to 35 species and 17 genera as shown in Table 1, and they were confirmed to be pure.

<table>
<thead>
<tr>
<th>Tested bacteria</th>
<th>Tested bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alcaligenes faecalis</em> ATCC(^a) 8750</td>
<td><em>Corynebacterium fascians</em> ATCC 12974</td>
</tr>
<tr>
<td><em>Arthrobacter atrocyaneus</em> CCM(^b) 1645</td>
<td><em>Corynebacterium hydrocarbolectas</em> IAM 1484</td>
</tr>
<tr>
<td><em>Arthrobacter citreus</em> CCM 1647</td>
<td><em>Corynebacterium xerosis</em> ATCC 7711</td>
</tr>
<tr>
<td><em>Arthrobacter globiformis</em> ATCC 8010</td>
<td><em>Enterobacter aerogenes</em> ATCC 13084</td>
</tr>
<tr>
<td><em>Arthrobacter ramosus</em> ATCC 13727</td>
<td><em>Erwinia amylovora</em> CCM 1114</td>
</tr>
<tr>
<td><em>Arthrobacter simplex</em> ATCC 6946</td>
<td><em>Escherichia coli</em> ATCC 9637</td>
</tr>
<tr>
<td><em>Arthrobacter tumescens</em> ATCC 6947</td>
<td><em>Escherichia coli</em> ATCC 11775</td>
</tr>
<tr>
<td><em>Arthrobacter tumescens</em> IAM(^c) 1447</td>
<td><em>Flavobacterium heparinum</em> ATCC 13125</td>
</tr>
<tr>
<td><em>Arthrobacter ureafaciens</em> ATCC 7562</td>
<td><em>Microbacterium flavum</em> ATCC 10340</td>
</tr>
<tr>
<td><em>Bacillus megaterium</em> IAM 1030</td>
<td><em>Microbacterium lacticum</em> ATCC 8180</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> IFO(^d) 3035</td>
<td><em>Micrococcus glutamicus</em> ATCC 13032</td>
</tr>
<tr>
<td><em>Brevibacterium ammoniagenes</em> ATCC 6872</td>
<td><em>Micrococcus luteus</em> ATCC 398</td>
</tr>
<tr>
<td><em>Brevibacterium lactofermentum</em> ATCC 13869</td>
<td><em>Nocardia minima</em> IAM 374</td>
</tr>
<tr>
<td><em>Brevibacterium linens</em> ATCC 8377</td>
<td><em>Proteus vulgaris</em> IAM 1025</td>
</tr>
<tr>
<td><em>Brevibacterium stationis</em> ATCC 14403</td>
<td><em>Pseudomonas aeruginosa</em> ATCC 7700</td>
</tr>
<tr>
<td><em>Cellulomonas biazea</em> ATCC 486</td>
<td><em>Pseudomonas fluorescens</em> ATCC 13525</td>
</tr>
<tr>
<td><em>Cellulomonas fim</em> ATCC 8183</td>
<td><em>Serratia marcescens</em> IAM 1105</td>
</tr>
<tr>
<td><em>Corynebacterium aquaticum</em> ATCC 14665</td>
<td><em>Vibrio tyrogenes</em> ATCC 7085</td>
</tr>
<tr>
<td><em>Corynebacterium diptheriae</em> ATCC 11913</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) ATCC: American Type Culture Collection, Rockville, Maryland, U.S.A.  
\(^b\) CCM: Czechoslovak Collection of Microorganisms, J.E. Purkyne University, Brno, Czechoslovakia.  
\(^c\) IAM: The Institute of Applied Microbiology, University of Tokyo, Japan.  
\(^d\) IFO: Institute for Fermentation, Osaka, Japan.

*Preparation of microculture.* A microculture of the bacteria was carried out on a thin agar film placed on a slide glass as shown in Fig. 1. An agar patch of 5 mm square and 0.5 mm thick was cut off from the agar film solidified in a Petri dish, and transferred to the center of a clean slide glass. A small amount of diluted bacterial suspension was inoculated in the center of the agar film with a capillary pipette. After diffusion of water into the agar, the film was covered with a cover slip and immediately sealed with
solid paraffin. Nutrient and brain-heart infusion agars were employed and incubated at 30° or 37° on a heating stage of a microscope (Leitz) or in a plastic chamber.

Time lapse microscopy. A microscope (Ortholux, Leitz) equipped with a Heineian phase-contrast condenser was used for the time lapse microscopy and microcinematography. Bacterial septa could be seen clearly under a dark phase-contrast state. An apochromatic objective lens (Leitz) of 40 or 90 magnification, and a Periplanish eye-piece (Leitz) of 10 magnification were used. For microphotography, Orthomat (Leitz) or Aristophoto (Leitz) was employed, and a hard contrast film (Fuji Minicopy) was used and developed for 4 to 6 min at 20° with Copinal (Fuji). A field showing 3 to 5 bacterial cells under the microscope was adequate for the observation. Microphotographs were taken every 10 to 15 min during the incubation.

Microcinematography. The cell division which occurred within a few seconds was observed more satisfactorily by microcinematography than time lapse microscopy. For cinematographic observation, a 16-mm movie camera (Bolex 16-mm, H 16 Reflex, Bolex) equipped with a slow motion motor and a time-regulator was used. Panchromatic negative films (RP, ASA 64, Fuji, and Tri-X, ASA 160, Kodak) were used. The film was exposed at 5- to 30-sec intervals.

RESULTS

The cell division of bacteria took place after cell elongation, by fission and post-fission movement. The tested bacteria could be divided into the following three groups on the basis of the mode of cell division.
As shown in Fig. 2, a cell of *Escherichia coli* ATCC 9637 became about twice longer than the starting cell within 30 min at 37°. During elongation of the cell, formation of septum was observed in the middle of soma, and fission took place by constriction. After the fission, two adjacent daughter cells slipped and became aligned parallel. Thereafter, microcolony was formed by repetition of such a process. Cultural temperature affected the cell elongation and generation time, but not the pattern of fission and post-fission movement. The same pattern of cell division was also observed in *Bacillus subtilis* IFO 3035 as shown in Fig. 3. Mode of such a cell division is named

**Simple type**
The following bacteria essentially belonged to this type: *Alcaligenes faealii* ATCC 8750, *B. megaterium* IAM 1030, *Enterobacter aerogenes* ATCC 13084, *Erwinia amylovora* CCM 1114, *E. coli* ATCC 11775, *Flavobacterium heparium* ATCC 13125, *Proteus vulgaris* IAM 1025, *Pseudomonas aeruginosa* ATCC 7700, *Ps. fluorescens* ATCC 13525, *Serratia marcescens* IAM 1105 and *Vibrio tyrogenes* ATCC 7085. Cells of *B. megaterium* IAM 1030 showed good septation but not well developed slipping. In such a case long chains were formed and folded at septa. The formation and folding of the long chains may be ascribed to the strong adhesion of daughter cells and not to the incomplete cell division.

Fig. 3. Growth of *Bacillus subtilis* IFO 3033 on nutrient agar at 30°C

A: 1.5 hr, B: 3.0 hr, C: 4.0 hr, D: 4.5 hr, E: 5.0 hr,
F: 5.5 hr, G: 6.0 hr, H: 6.5 hr, I: 7.0 hr.
As shown in Fig. 4, a cell of Coryn. diphtheriae ATCC 11913 elongated within 90 min at 37° but the length did not exceed twice that of the starting cell. During elongation formation of a septum could be seen in the middle of the soma, and then the cell divided into two daughter cells by snapping within a few seconds. Such a quick break could be seen more clearly on the movie film as shown in Figs. 5 and 6. During the division of this type of bacteria, the constrictive form like a segment, which was found in E. coli ATCC 9637, was not observed. The two daughter cells resulting from snap-
Fig. 5. Snapping division of *Corynebacterium diphtheriae* ATCC 11913 on brain-heart infusion agar at 37°. Film taken every 15 sec.

Fig. 6. Snapping division of *Micrococcus glutamicus* ATCC 13032 on nutrient agar at 30°. Film taken every 15 sec.
ping tended to remain attached and the angle between the cells was usually 90° to 120°. The characteristic V-form was developed in this manner. After the snapping, the angle of the cells reduced and the cells became arranged in parallel. The second snapping of the two daughter cells gave the zigzag or irregular arrangement. Septation at the middle or subterminal of the cell took place at random and the daughter cells did not always divide simultaneously. Further, larger cells tended to divide more frequently than smaller ones, and consequently the cells of this bacterium became smaller in size. From these facts, the diversity of cell shape in this group of bacteria was...
understood. Mode of such a cell division is named a “snapping type.” The same pattern of cell division was also observed in *Brev. lactofermentum* ATCC 13869 as shown in Fig. 7. The snapping division could be widely recognized in the group of coryneform bacteria employed in this work, and the following strains were capable of reproducing mainly by the snapping division: *Brev. ammoniagenes* ATCC 6872, *Brev. stationis* ATCC 14403, *Coryn. xerosis* ATCC 7711 and *Microb. flavum* ATCC 10340. *Micrococcus glutamicus* ATCC 13032 also grew by snapping division, and showed rod-shaped, not spherical, as shown in Fig. 6.
As shown in Fig. 8, cells of *Arthr. ureafaciens* ATCC 7562 elongated straight or curving, and septa formed at the middle or subterminal of the somata, and constricted forms like a segment were not observed. The cells bent slowly at the septa and often became V-form. Connection between the two daughter cells detached easily by elongation. Mode of such a cell division is named a "bending type." The same pattern of cell division was also observed in *Brev. linens* ATCC 8377 as shown in Fig. 9. Cells of *Coryn. hydrocarboeclastus* IAM 1484 were rather larger than those of other coryneform
bacteria, and developed by bending and slipping as shown in Fig. 10. Microb. lacticum ATCC 8180 elongated very slowly, and secondary branched cells formed from the primary cell. Two or more branched cells could often be seen and the cells did not detach easily like transient branching. Microcolony like a filamentous network formed by further development as shown in Fig. 11. The following strains exhibited the mode of cell division similar to those of Arthr. ureafaciens ATCC 7562 and Brev. linens ATCC 8377: Arthr. atrocyaneus CCM 1645, Arthr. citreus CCM 1647, Arthr. globiformis ATCC 8010, Arthr. ramosus ATCC 13727, Arthr. simplex ATCC 6946, Arthr. tumescens ATCC 6947, Arthr...
tumescens IAM 1447, Cellul. biazotea ATCC 486, Cellul. fimic ATCC 8183, Coryn. aquaticum ATCC 1665, Coryn. hydrocarboclastus IAM 1484 and Microb. lacticum ATCC 8180.

DISCUSSION

GRAHAM-SMITH (6) reported the four bacterial groups on the basis of the bacterial growth, i.e., snapping organisms, loop-forming organisms, slipping organisms, and folding organisms, but this grouping mainly depended on the post-fission movement which seems to be affected by various factors. The present authors were interested in observing the mode of cell division which is not easily affected by environmental conditions, and found that the pattern of growth of bacterial cells consists of two fundamental characters, i.e., elongation and fission. From the viewpoint of classification, it was concluded that rod-shaped bacteria belonging to the so-called eubacteria and coryneform bacteria could be differentiated by the three modes of cell division, i.e., simple type, snapping type, and bending type, as shown in Fig. 12 and Table 2. The bacteria belonging to the simple type elongate regularly at the first period and form septa at the middle of somata, followed by fission with constriction. The bacteria belonging to this type were restricted to the so-called
Fig. 12. Schematic mode of cell division of rod-shaped bacteria.

Table 2. Grouping of bacteria on the basis of the mode of cell division.

<table>
<thead>
<tr>
<th>Mode of Cell Division</th>
<th>Simple Type</th>
<th>Snapping Type</th>
<th>Bending Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple type</td>
<td><img src="example.com" alt="Simple type schematic" /></td>
<td><img src="example.com" alt="Snapping type schematic" /></td>
<td><img src="example.com" alt="Bending type schematic" /></td>
</tr>
<tr>
<td><strong>Table 2</strong></td>
<td><em>Alcaligenes faecalis</em> ATCC 8750</td>
<td><em>Flavobacterium heparinum</em> ATCC 13125</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus megaterium</em> IAM 1030</td>
<td><em>Proteus vulgaris</em> IAM 1025</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> IFO 3035</td>
<td><em>Pseudomonas aeruginosa</em> ATCC 7700</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em> ATCC 13048</td>
<td><em>Pseudomonas fluorescens</em> ATCC 13525</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Erwinia amylovora</em> CCM 1114</td>
<td><em>Serratia marcescens</em> IAM 1105</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 9637</td>
<td><em>Vibrio tyrogenes</em> ATCC 7085</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 11775</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Snapping Type**

*Brevibacterium ammoniagenes* ATCC 6872    | *Corynebacterium xerosis* ATCC 7711
*Brevibacterium lactofermentum* ATCC 13869 | *Microbacterium flavum* ATCC 10340
*Brevibacterium stationis* ATCC 14403       | *Micrococcus glutamicus* ATCC 13032

**Bending Type**

*Arthrobacter atrocyaneus* CCM 1645      | *Brevibacterium linens* ATCC 8377
*Arthrobacter citreus* CCM 1647           | *Cellulomonas biazotea* ATCC 486
*Arthrobacter globiformis* ATCC 8010      | *Cellulomonas fimii* ATCC 8183
*Arthrobacter ramosus* ATCC 13727         | *Corynebacterium aquaticum* ATCC 14665
*Arthrobacter simplex* ATCC 6946          | *Corynebacterium fascians* ATCC 12974
*Arthrobacter tumescens* ATCC 6947        | *Corynebacterium hydrocarboclastus* IAM 1484
*Arthrobacter tumescens* IAM 1447         | *Microbacterium lactium* ATCC 8180
*Arthrobacter ureafaciens* ATCC 7562      |
eubacteria, such as *Pseudomonas, Alcaligenes, Escherichia, Bacillus, etc.*, and no coryneform bacteria were included in this type. Since the work of HILL (7) and of GRAHAM-SMITH (6), the term "snapping" has been employed in describing the characteristic V-form found in coryneform bacteria but its taxonomic significance has remained obscure. BISSET (8) stated that snapping is merely due to mechanical restraint in the solid culture medium. However, the present authors found that snapping takes place in the liquid medium in which mechanical restraint is negligible, and concluded that the division of snapping type is considered to be unique in some of coryneform bacteria. In connection with the nature of snapping, the present authors attempted to examine the cell division of irregular forms induced by sodium citrate as reported by ITAGAKI et al. (9). In the presence of 2 to 4% of sodium citrate, *Corynebacterium diphtheriae* ATCC 11913 demonstrated various irregular forms, branched cells and multicellularity, but the characteristic V-form caused by snapping was still found. In the case of higher concentration of sodium citrate, bacterial growth was strikingly inhibited and snapping tended to disappear. When a cell having two or three septa in the middle of the soma induced by sodium citrate was transferred to a fresh medium without sodium citrate, the cell division took place at both terminal parts of the soma but not in the

---

**Fig. 13.** Growth of citrate-induced multiseptated cells of *Corynebacterium diphtheriae* ATCC 11913 on brain–heart infusion agar without citrate at 37°.

A: 0 hr, B: 3.5 hr, C: 4.0 hr, D: 5.0 hr, E: 6.0 hr, F: 7.0 hr.
middle part which lysed later as shown in Fig. 13. Further, the branched cells of *Corynebacterium diphtheriae* ATCC 11913 induced by sodium citrate tended to become the normal cell form after 4 to 5 snappings when they were transferred to a fresh medium without sodium citrate, as shown in Fig. 14. From these facts, it is evident that snapping does not take place under unfavorable conditions. Therefore, snapping is considered to be one of the fundamental modes of bacterial cell division.

*Nocardia minima* IAM 374 elongated very slowly and formed complicated-
ly branched mycelia. Long branched mycelia cultured for 72 hr broke into a bacillary form by fragmentation after 120 hr, as shown in Fig. 15. The true branching found in *Nocardia* accompanying the life cycle is clearly distinguished from the branched cells found in coryneform bacteria which has been termed “rudimentary” or “transient” branching. Therefore, the branching found in the coryneform bacteria capable of growing by snapping is not considered to be fundamental mode of bacterial growth.

Fig. 15. Fragmentation of *Nocardia minima* IAM 374 on nutrient agar at 30°.
A: 72 hr, B: 120 hr.
STARR and KUHN (10) observed in Arthr. atrocyaneus that snapping is one of the modes of formation of V-form. However, the present authors did not find snapping in Arthr. atrocyaneus CCM 1645 (same as the strain employed by STARR and KUHN), and this bacterium grew by a cell division of the bending type and not the snapping type.

In the case of spherical bacteria, the mode of cell division somewhat contrasts to that of rod-shaped bacteria. A cell of Micrococcus luteus ATCC 398 swelled and slightly elongated in the first stage of growth, and followed by septation. In this period, it looks like an insect cocoon. After a complete constriction, the cell divided into two daughter cells. During the first division, another septum for the second division could be seen at right angle to the first septum in some of Micrococcus. The post-fission movement such as slipping and folding was not clear in the case of cocci and they contrasted to those of rod-shaped bacteria.

From such facts, non-sporeforming strains of Bacillus can be distinguished from coryneform bacteria, and coccoid form bacteria also separated from spherical bacteria. The mode of cell division is considered to be useful for bacterial determination and classification.

REFERENCES

4) H. Kurth, Z. Hyg., 28, 409 (1898).
6) G.S. Graham-Smith, Parasitology, 3, 17 (1916).