

A Stable Variant of *Simonsiella muelleri* with Unusual Colonial and Cellular Morphology

R. L. S. WHITEHOUSE,^{1*} H. MERRILL,¹ M. C. JACKSON,² AND H. JACKSON³

Department of Medical Microbiology and Infectious Diseases, Faculty of Medicine, University of Alberta, Edmonton, Alberta T6G 2H7,¹ The Charles Cammell Hospital, Edmonton, Alberta T5M 3A4,² and Department of Food Science, Faculty of Agriculture and Forestry, University of Alberta, Edmonton, Alberta T6G 2P5,³ Canada

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The unusual morphology and cellular arrangement of a member of the genus *Simonsiella* is described. The organism is characterized by the formation of very long trichomes, which can be greater than 1,000 μm in length.

The genus *Simonsiella* is characterized by a unique multicellular morphology; the organisms are commonly described as filamentous with gliding motility (3). The filamentous designation is based upon observations of Gram-stained preparations viewed by oil immersion light microscopy. More detailed examination by scanning electron microscopy reveals that the “filaments” are in fact made up of many curved rodlike cells whose cellular width is greater than the length. Starr and Skerman (7) and Starr and Schmidt (6) have suggested that this type of structure be termed a trichome—a chain of closely apposed bacterial cells—as found, for example, in certain genera of the cyanobacteria (5), the genus *Toxothrix* (2), and the genus *Caryophanon* (8). The term trichome will be used in this sense in this paper.

A strain of *Simonsiella muelleri* was isolated from a human neonate (9). In this isolate the trichome appeared constricted at regular intervals into subunits of 10 to 12 cells by the formation of smaller cells. These subunits were seen to separate and were commonly found in pairs. During subsequent studies of this isolate, an atypical colony was observed on blood agar plates (BAP). The colony had a “fried-egg” appearance: a raised central portion about 2 mm in diameter surrounded by a flatter, rougher region extending up to 8 mm in diameter. This was in contrast to the original isolate, which produced smooth, moist colonies less than 4 mm in diameter on BAP. The colony was picked and subcultured on BAP. Subcultures were of consistent morphology upon repeated transfer, indicating this to be a stable phenotypic variant.

The strain of *S. muelleri* isolated by Whitehouse et al. (9) and the variant, designated SMV, were compared. The organisms were tested for hemolysis; oxidase; catalase; urease; utilization of citrate; motility; aerobic or anaerobic growth; oxidation or fermentation of glucose, sucrose, maltose, and lactose; and susceptibilities to clindamycin, ampicillin, co-trimoxazole, amikacin, tobramycin, chloramphenicol, tetracycline, gentamicin, vancomycin, erythromycin, penicillin, cephalosporin, and methicillin. The methods used were those reported by Whitehouse et al. (9). The results of all of the biochemical and cultural tests performed, as well as the results of the tests of susceptibility to the antimicrobial agents, were identical for the two organisms. The only observed difference was in colonial morphology.

Cultures were examined by both light and scanning elec-

tron microscopy. Examination by light microscopy of Gram-stained smears of SMV from colonies on BAP revealed extremely long trichomes compared with the original isolate. The maximum length was estimated to be at least 1,000 μm . These trichomes were apparent even at a $\times 100$ magnification (Fig. 1), although at this magnification the individual cells themselves could not be seen. With a $\times 1,000$ magnification the striated nature of the trichomes could be observed (Fig. 2). This striation was due to the arrangement of the curved rodlike cells perpendicular to the long axis of the trichome.

Colonies of SMV on BAP, which were 6 to 8 mm in diameter after a 3-day incubation at 37°C, were examined by scanning electron microscopy. Selected colonies were removed along with a block of agar on which they were growing and added to a solution of 1% osmium tetroxide in cacodylate buffer (pH 6.8). These colonies were left overnight at room temperature, washed twice in cacodylate buffer for 15 min each time, and then dehydrated by passage, for 15 min at each concentration, in 25, 50, 75, and 90% ethanol. They were then placed in 100% ethanol and left for several hours. The treatment in 100% ethanol was repeated three times, and the colonies were stored in 100% ethanol at

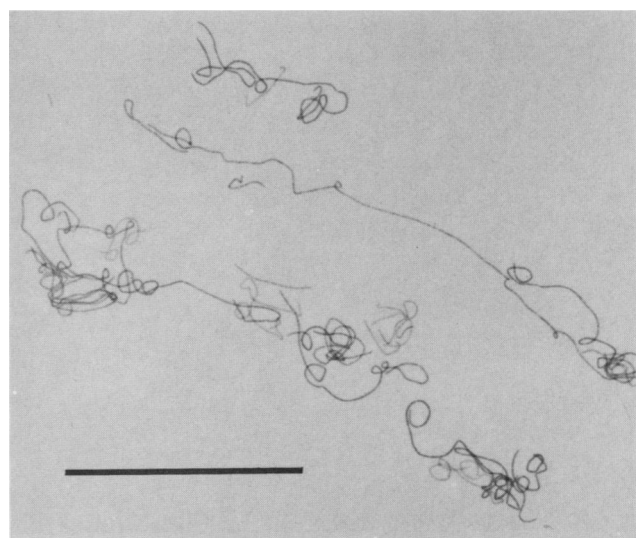


FIG. 1. Light micrograph of a Gram-stained preparation of SMV. Bar = 250 μm .

* Corresponding author.

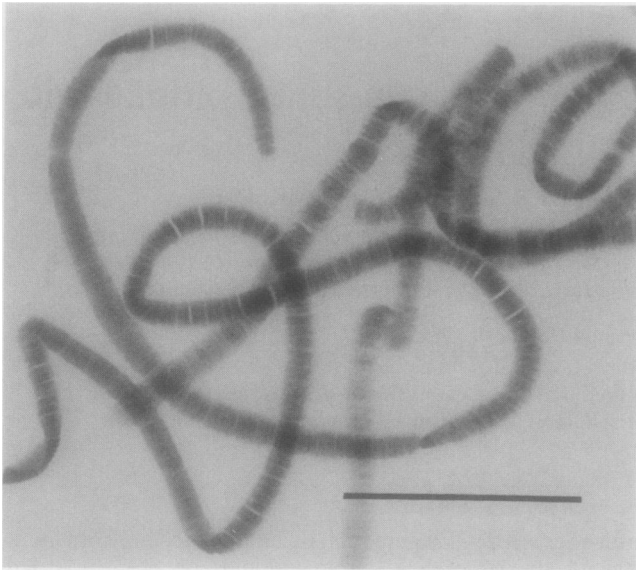


FIG. 2. Light micrograph of a Gram-stained preparation of SMV. The striations are due to individual bacterial cells attached to each other by their long sides. Bar = 25 μ m.

4°C for several days. The colonies with agar were then processed for scanning electron microscopy by standard techniques, namely, critical-point drying, mounting on stubs, and sputter-coating with gold (1). Specimens were examined with a Stereoscan 250 scanning electron microscope (Cambridge Instruments Co., Cambridge, United Kingdom). The original strain of *S. muelleri* breaks up into subunits by the production of shorter cells within the trichome when the unit has reached a certain size (9). Although the variant SMV also produces shorter cells within the trichome, they are not as small as those found in the original strain, and most of them tend to remain attached to adjacent cells. Consequently, the organism develops into very long trichomes made up of hundreds of cells (Fig. 3). Organisms from the edge of a colony showed a tendency to form spectacular spiral forms (Fig. 4).

The stable variant of *S. muelleri* described in this communication is of interest because of its most unusual multicellular structure and arrangement. Although similar complex multicellular bacteria have been isolated from a wide variety of ecological sites, *Simonsiella* spp. would appear unusual in being of strictly animal origin. The normal habitat of *Simonsiella* spp. is the oral cavity of many warm-blooded vertebrates (3). The ability to colonize surfaces in the oral cavity

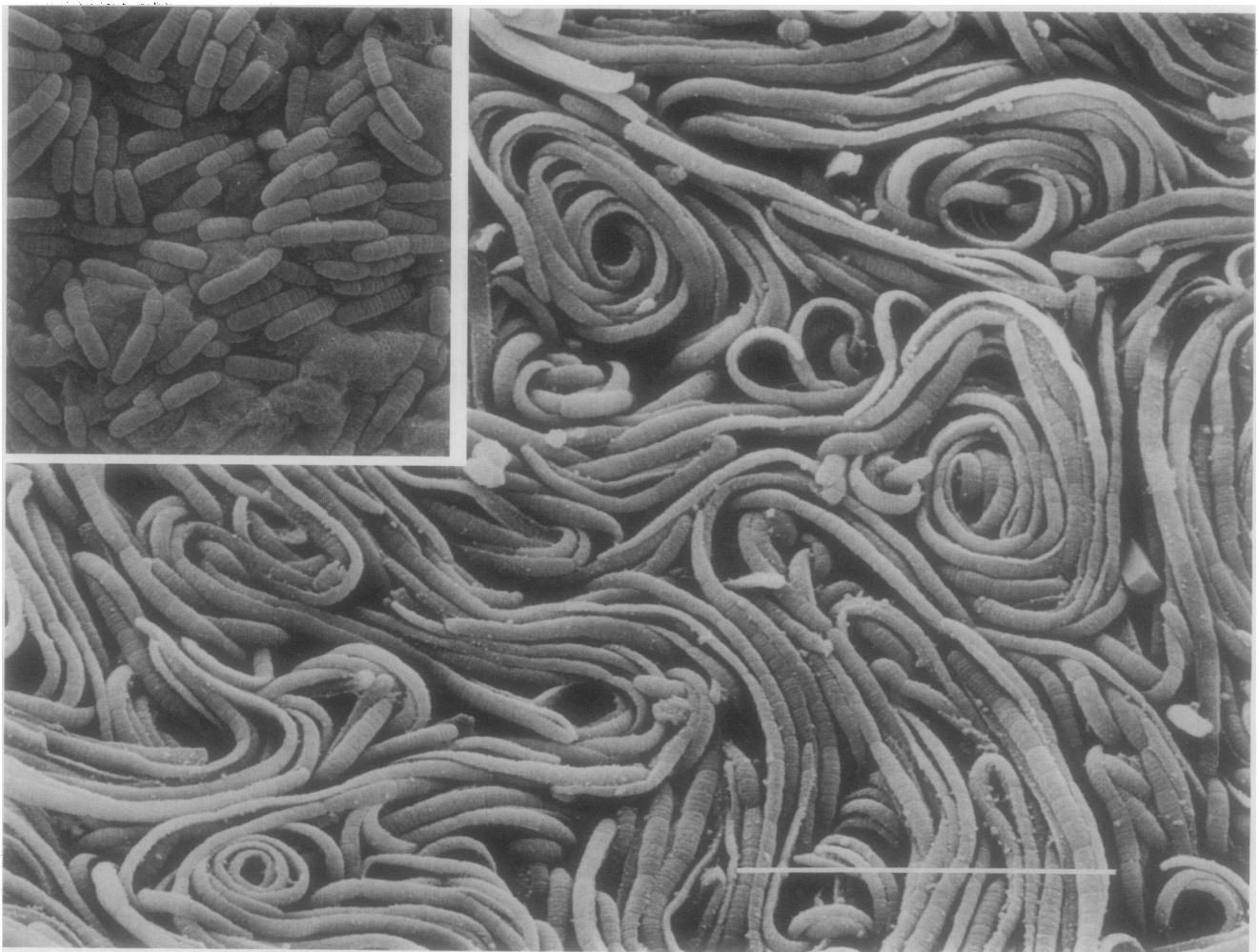


FIG. 3. Scanning electron micrograph of SMV, showing the long trichomes. Individual bacterial cells can be discerned. The insert shows, at the same magnification, the original *S. muelleri* strain from which the variant was derived. Bar = 40 μ m.

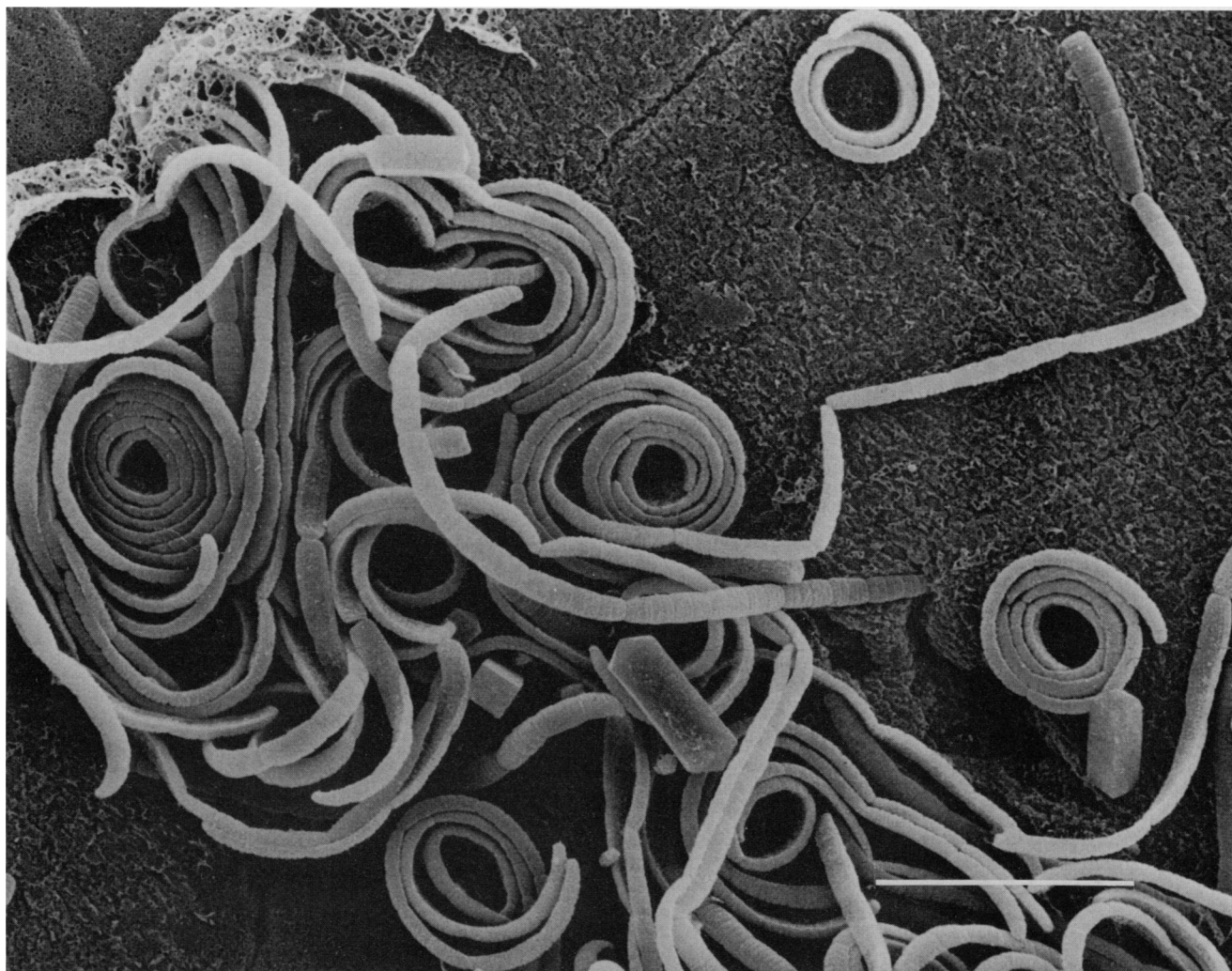


FIG. 4. Scanning electron micrograph of SMV at the edge of a colony on BAP, showing spiral forms. Bar = 20 μ m.

can be related to the peculiar structure of *Simonsiella* spp., i.e., their concave surfaces with fibrillar attachments apparently adapted for this purpose (4). The variant described in this paper, with its extensive elongation and spiral formations, would appear to have little affinity for surfaces.

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