

AN ELECTRON MICROSCOPIC STUDY ON *BIFIDOBACTERIUM PSEUDOLONGUM* SS-24 WITH EXTRACELLULAR MATERIAL AND NAKED *BIFIDOBACTERIUM THERMOPHILUM* SS-19

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Introduction

Bifidobacterium has been used for viable bacterial preparations (VBP). These VBP have been used for prevention of diarrhea as well as for stimulation of growth of weaned puppies, early weaned bull calves, sucking piglets and poultry. *Bifidobacterium* has been used also clinically, especially in the pediatric field. VBP have been used successfully, however, the efficacy of VBP is still controversial and its mechanism of action as well as the morphology of *Bifidobacterium* are not clearly understood. Therefore, we conducted an examination of morphology via electron microscopy, of two species of *Bifidobacterium*, *B. pseudolongum* SS-24 and *B. thermophilum* SS-19.

Materials and Methods

The organisms used were *Bifidobacterium pseudolongum* SS-24 type b preparation (Korolac D, Nisshin Flour Milling Co., Ltd, Saitama, Japan) isolated from dog and *B. thermophilum* SS-19 type d and its preparation (Korolac B) isolated from swine by Mitsuoka (1969). Samples of 8-h bacterial cultures were prepared for examination by scanning electron microscope (SEM) and transmission electron microscope (TEM) as previously described (Patterson et al., 1975).

Results

SEM showed that *B. pseudolongum* SS-24 was a short rod with coryneform appearance approximately 0.5 to 0.7 μm wide and 0.7 to 1.4 μm long (figure 1A), and showing extensive secretion of extracellular material. Cells of *B. thermophilum* SS-19 were slender rods, approximately 0.5 to 0.6 μm wide and 0.9 to 2.0 μm long (figure 1B),

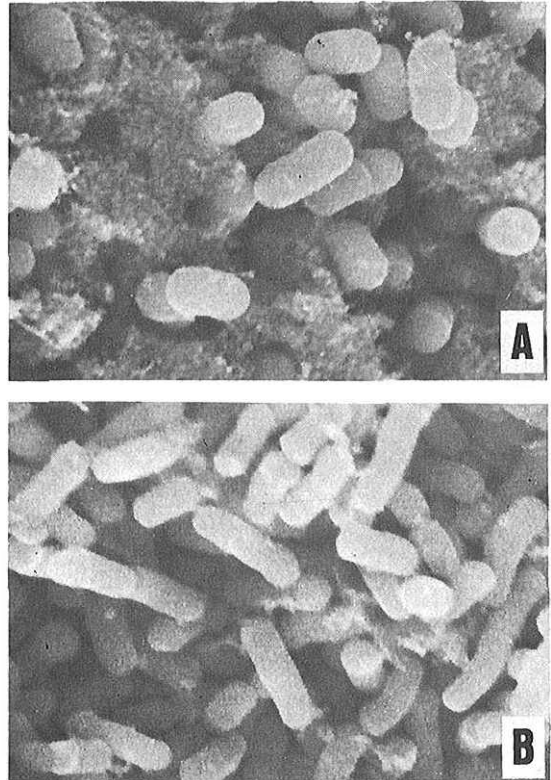


Figure 1. SEM micrographs of *B. pseudolongum* SS-24 (A) and *B. thermophilum* SS-19 (B). Note that the cells of *B. pseudolongum* are covered with a large amount of extracellular material and some of this glycocalyx fibers have coagulated and are seen as masses of branching foamy fibers.

although short plump rods were occasionally observed. The secretion of extracellular material was not evident in *B. thermophilum*.

TEM revealed that the cell envelope of *B. pseudolongum* clearly showed thick (ca. 0.2 μm)

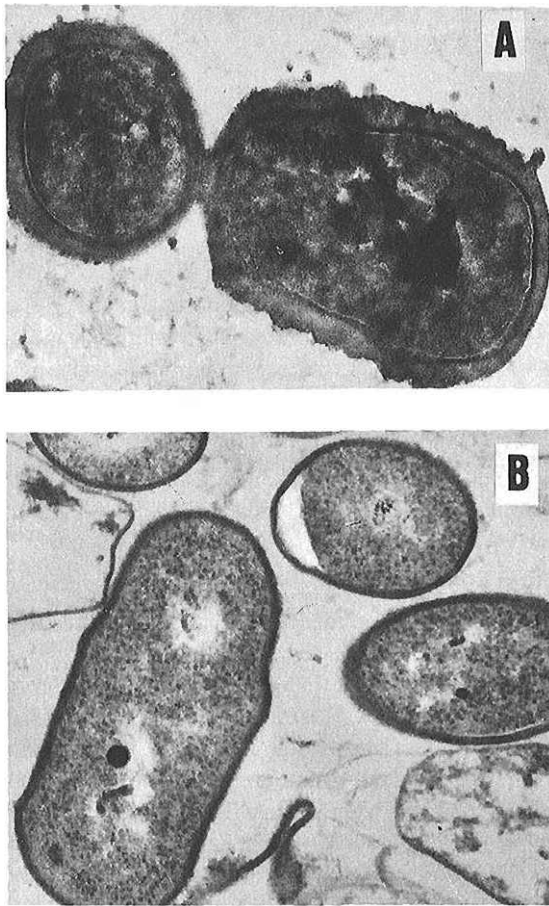


Figure.2. TEM of ruthenium red-stained preparation of *B. pseudolongum* SS-24 (A). Note the very thick irregular Gram-positive cell wall ($+ 0.2 \mu\text{m}$). *B. thermophilum* SS-19 (B). Note the thin regular Gram-positive cell wall ($\pm 0.05 \mu\text{m}$).

Gram-positive cell wall (figure 2A). On the other hand, the cell envelope of *B. thermophilum* showed only a poorly resolved cytoplasmic membrane and thin ($0.05 \mu\text{m}$) electron-dense and

relatively homogeneous Gram-positive structure type. Thus, the cell walls of *B. pseudolongum* and *B. thermophilum* were totally different. This naked bacterial strain of *B. thermophilum* is probably a mutant strain produced and adjusted in laboratory conditions. Bacteria in the digestive tract have a variety of surfaces to which they may attach by means of extracellular material, and distinct population of bacteria adheres to each different surface. From an ecological viewpoint, bacteria with the ability to attach to feed particles and walls of digestive tract have a great advantage over nonattaching bacteria, which would be removed more quickly from the environment by flow (Kudo et al., 1987). Therefore, the naked bacterial strain of *B. thermophilum* SS-19 investigated have an advantage in laboratory condition as an energy saver but not in natural conditions.

A part of this work was conducted under the joint project on the "Improvement of the Productivity of Tropical Ruminants" of Tropical Agriculture Research Center and Universiti Pertanian Malaysia.

(Key Words: *Bifidobacterium Pseudolongum*, *B. thermophilum*, Electron Microscopy)

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