Bacteroides succinogenes菌体外膜の血清学的および形態学的観察

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Serological and Morphological Observation on the Outer Membrane of *Bacteroides succinogenes*

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The outer membrane of gram negative bacteria contains immunologically important antigens. Antigenicities of cell wall components such as polysaccharide and lipopolysaccharide (LPS) were established well in *Bacteroidaceae* [6, 7, 9]. Serological techniques distinguished strains of rumen bacteria such as *Butyrivibrio* [4], *Selenomonas* [5], and *Bacteroides* [13, 14]. However, it has not shown the immunological properties of the outer membrane of rumen bacterial species such as *Bacteroides succinogenes*. In the present study, we investigated the serological and morphological characteristics of the outer membrane of ruminal *B. succinogenes*.

A standard strain of *B. succinogenes* S-85 (*Fibrobacter succinogenes*) [10] was used. The organisms were suspended in Yeast Extract-Trypticase-Rumen Fluid (YTR) broth, and incubated in an anaerobic jar (GasPak; BBL, Baltimore, Md.) at 37°C for 48 hrs prior to use. The organisms were then suspended in large amount of YTR broth under anaerobic condition. After incubation at 37°C for 48 hrs, the organisms were washed three times with 0.15 M NaCl by centrifugation at 8,000 g at 4°C. The outer membrane of *B. succinogenes* was prepared by the method of Mansheim and Kasper [8]. Briefly, the pelleted organisms were suspended in buffer solution containing 0.01 M EDTA, and incubated in a water bath at 60°C for 30 min. The suspension was mixed in a Waring blender for 10 sec, and centrifuged at 12,000 g for 20 min. The supernatant was collected and centrifuged at 80,000 g for 2 hrs. The pellet was collected and centrifuged again at 12,000 g for 20 min and 80,000 g for 2 hrs. Purity of the outer membrane preparation was confirmed by electron microscopy. Rabbit antiserum raised against the outer membrane was prepared by the procedure of Mansheim *et al.* [9].

Immunodiffusion in agar was performed with use of the outer membrane antigen of *B. succinogenes*, with homologous and heterologous antisera. The outer membrane antigen showed precipitin line with the homologous antiserum (Fig. 1). No precipitin lines were observed with heterologous antisera of the other bacterial species such as *B. ruminicola* subsp. *ruminicola, B. ruminicola* subsp. *brevis,* and *Selenomonas ruminantisum*.

Indirect immunofluorescent test was performed by using rabbit antiserum raised against the outer membrane antigen and fluoresceinisothiocyanate (FITC) conjugated anti-rabbit immunoglobulin (Ig)G(H+L) goat serum (ICN Immuno Biologicals, Israel). The antiserum of *B. succinogenes* was intensely reactive with homologous organisms fixed on slide glasses. The antiserum was not reactive with any other bacterial species. The results demonstrate the presence of species-specific outer membrane antigens of *B. succinogenes*, as was suggested by Sharpe [14].

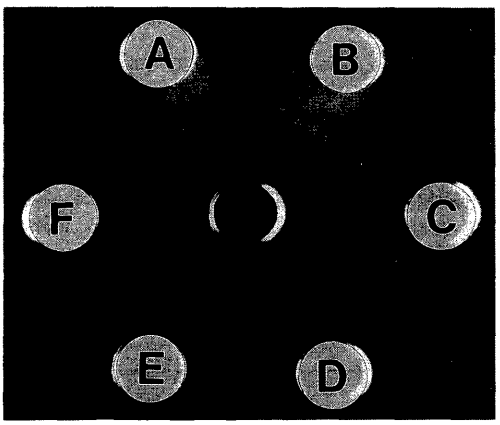

Fig. 1. Immunodiffusion in agar using the outer membrane antigen of *B. succinogenes* (center well) and rabbit antisera raised against the outer membrane antigen of *B. succinogenes* (A, B), *B. ruminicola* subsp. *ruminicola*(C), *B. ruminicola* subsp. *brevis*(D) and*Selenomonas ruminantium*(E, F). The outer membrane antigen showed precipitin line with the homologous antiserum.

B. succinogenes was examined electronmicroscopically by using rabbit antiserum raised against the outer membrane and ferritin-conjugated anti-rabbit IgG goat serum (Miles Lab. Inc., USA). Many ferritin particles were located close to the outer cell wall, and were distributed most randomly over the whole of the cell wall (Fig. 2). No ferritin particles were observed inside the confines of the cell wall. The results resemble to the immunoelectron microscopic localization of LPS in the cell wall of *B. oralis* and *Fusobacterium nucleatum* [3], and of *Salmonella typhimurium* [15]. The outer membrane of *B. succinogenes* showed triple-laminar structure which was typical of gram negative bacteria.

Previously Ogimoto *et al.* [11, 12] reported the agglutinating antibodies in bovine sera against many species of rumen bacteria including *B. succinogenes*. Although the antibodies against indigenous bacteria such as rumen bacteria are considered to be natural antibodies [1, 2], the mechanism of antibody production and the immunological role in host protection have not been revealed. The outer membrane antigen of *B. succinogenes* may be useful for serological techniques not only distinguish the organisms but also investigate the bovine immune response to *B. succinogenes*.

Fig. 2. Electron micrograph of *B. succinogenes* treated with rabbit antiserum raised against the outer membrane and ferritin-conjugate. Ferritin particles (arrows) are located on the outer cell wall. Bar=0.1μm.

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要　約

Bacteroides succinogenes 菌体外膜の血清学的および形態学的観察（短報）：佐藤　繁・扇元敬司・中井　裕[1)]（東北大学農学部家畜衛生学教室，[1)]茨城大学農学部家畜衛生学教室）——ルーメン・セルロース分解菌の *B. succinogenes* から作製した菌体外膜は，抗外膜ウサギ血清を用いた免疫拡散法および螢光抗体法によって，種特異的抗原性を示すこと，また免疫電顕法によって，抗原は菌体外膜に存在することが証明された．