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Detection of Bifidobacteria by Using Propionic Acid as a Selective Agent

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This study introduces the use of the filtration membrane technique and the enrichment method to detect and enumerate bifidobacteria from various animal species.

A previous study (1) reported the development of an elective and selective culture medium for *Bifidobacterium* spp. that contains 5 or 10 ml of propionic acid per liter and has been adjusted to pH 5.0. The purpose of this work was to introduce the use of the filtration membrane technique and the enrichment method for the detection and enumeration of bifidobacteria found in the environment, including those derived from feces of various animals.

The commercial basal media used in this study were Columbia (Pasteur Production) supplemented with glucose (5 g/liter), cysteine hydrochloride (0.5 g/liter), agar (5 g/liter) (final agar concentration, 15 g/liter), pH 7.3, Brain Heart broth (BH) (Biomerieux) supplemented with yeast extract (5 g/liter), and cysteine hydrochloride (0.5 g/liter), pH 7.2. The solid medium was obtained by adding agar at 20 g/liter. TPY medium (2) was also used as solid medium.

Fluid TPY and BH were used as the enrichment media. The medium was placed in tubes.

For the selective medium, liquid propionic acid for synthesis was purchased from Merck (purity, 99%). It was added to either liquid or solid commercial basal medium at a concentration of 5 or 10 ml/liter; the pH was then adjusted to 5.00 by using 1 N NaOH. The medium should not be sterilized. It is recommended that solid and liquid media not be used more than 48 h after preparation. Anaerobic conditions were obtained by following the GasPak procedure or by using plastic bags (Generbag anaer [Biomerieux]) incubated at 37°C.

The detection of bifidobacteria in feces from a number of different animals was obtained after surface inoculation of selective Columbia agar of a dilution (1/10, 1/100) in a modified one-fourth-strength Ringer solution. The results are given in Table 1.

The membrane filtration technique permitted the detection of bifidobacteria in a large volume of liquid and could be used to determine the presence of these organisms as fecal contaminants in sewage or polluted waters. When 0.1, 1, and 5 ml of filtrate from sewage was tested in medium, the control (Columbia) was overgrown with streptococci, whereas there were, respectively, 4 colonies, 45 colonies, and large numbers (overgrowth) of bifidobacteria colonies on the selective medium.

The various species are known to differ in their resistance to adverse conditions, and, because the cells may become injured, they would require resuscitation. The effect of injury was determined by comparing counts of a particular *Bifidobacterium* species in fermented milk with and without resuscitation, using the membrane filtration technique. The membrane supporting the filtrate was incubated on nonsupplemented Columbia agar for 6 h at 37°C under anaerobic conditions before transfer to the selective agar. In one sample, without resuscitation there were 7.3×10^5 colonies; after resuscitation, there were 6.4×10^6 colonies. Liquid enrichment media TPY and BH exhibited identical results. Tests were performed using pure cultures of *Bifidobacterium* spp. and other organisms, as well as fecal samples. With pure cultures, it was found that liquid medium containing 5 or 10 ml of propionic acid per liter at pH 5.0 inhibited the growth of members of the family *Enterobacteriaceae* and other gram-positive bacteria, as well as strains of *Enterococcus*, *Staphylococcus*, and *Micrococcus* spp. Most of the strains of *Bifidobacterium* spp. grew to the same degree in the medium with 5 ml of propionic acid per liter and in the TPY control medium. When 10 ml of propionic acid per liter was used, counts were lower and most of the bifidobacteria appeared to be coccoidal. With fecal samples, the enrichment culture gave very good growth and Gram staining showed the presence of a pure culture of gram-positive bacilli.

The selective medium described here facilitates the isolation and enumeration of all species of *Bifidobacterium* from any habitat. Thus, the use of selective medium will help in the (i) study of the ecology of *Bifidobacterium* spp., (ii)

TABLE 1. Detection of *Bifidobacterium* spp. in fecal samples

Source	No. of animals sampled	No. of samples positive for bifidobacteria
Wild rabbit	6	0
Domestic rabbit	3	3
Roebuck	2	0
Horse	2	1
Cow	2	0
Hen	1	1
Goose	1	0
Swallow	2	2
Magpie	1	0
Crow	6	2
Domestic rat	1	1
Dog	2	2
Pig	8	8
Fly[a]		
Fox	1	1
Turtle	2	0
Fish	2	0
Human	50	50

[a] A mixture of 20 flies was used, and bifidobacteria were present.

detection of specific fecal contamination, and (iii) development of new criteria for classifying and identifying strains. If an unknown species of an anaerobic gram-positive bacteria grows readily under the specified conditions, it may be a good indication that the organism belongs to the genus *Bifidobacterium*. The ability to grow on selective medium may thus be used as a characteristic for taxonomic purposes.

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