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Isolation of Methanogenic Bacteria from Feces of Man

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Occurrence of methane in gas from the human intestine is evidence that methanogenic organisms grow in the human alimentary tract. Both hydrogen and methane can be detected in the respired air of many humans (1). This note reports the results of experiments to detect methanogenic bacteria in feces and to isolate them in pure culture.

Samples of fresh feces were suspended anaerobically in nine volumes of sterile distilled water and diluted through a series of tubes containing an agar mineral salts medium (2), plus 10% bovine rumen fluid and 10% of an extract of human feces. The feces extract was prepared by diluting fresh feces 10-fold with distilled water, and both the feces extract and the rumen fluid were filtered through Whatman no. 4 filter paper. The culture medium was prepared anaerobically under a mixture of 80% H_2 and 20% CO_2, as previously described by Paynter and Hungate (3), and dispensed in 4.2-ml amounts into butyl rubber-stopper tubes. Before use, 0.05 ml of 3% cysteine hydrochloride, 0.25 ml of 10% sodium bicarbonate and 0.15 ml of H_2S gas were added to each tube. The syringe method (R. E. Hungate, *submitted for publication*) was used in making dilutions and in all subcultures except when colonies were picked. Picking was performed with a drawnout bent Pasteur pipette with air excluded from the tube by gassing with the H_2-CO_2 mixture.

Each 2 or 3 days the tubes were examined for a decrease in pressure that would indicate utilization of hydrogen and carbon dioxide according to the equation, $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$. The procedure was to fill a sterile 10-ml syringe with the H_2-CO_2 mixture, insert the needle through the stopper of the culture tube, and from movement of the plunger measure the amount of gas drawn into the tube. Samples of gas from tubes showing uptake were examined chromatographically for methane. Methane was produced in the dilution cultures from feces of five out of seven individuals tested. Methane could be detected in the lowest dilution tube after 2 days of incubation; but 20 to 30 days were required for it to appear in easily measurable amounts in the highest positive dilution. High dilutions which showed more than 1 ml of methane per tube were examined under the dissecting microscope to identify colonies which increased in size as more methane was produced. These were picked and diluted through agar medium and picked further until pure cultures were isolated. Pure cultures were obtained from four of the five individuals positive for methanogenic organisms. The strains appeared to be identical.

One strain was studied in some detail with characteristics as follows: gram-positive short rods or ovals, 0.6 μm in diameter and 0.8 to 1.2 μm long; colonies flat to convex, circular with entire edges, white to yellowish in color, becoming visible after 5 to 10 days of incubation, reaching a diameter of 3 mm after 3 weeks of incubation; subsurface colonies smaller, lenticular, buff colored; no flagella detected on electron micrographs.

Hydrogen gas and carbon dioxide are used for the production of methane, and formate is used to some extent. Not used are acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, serine, succinate, glucose, pyruvate, methanol, propanol, isopropanol, and butyl alcohol.

These characteristics indicate that the isolated strains are *Methanobacterium ruminantium* (4).

Growth was better in agar media containing rumen fluid with or without feces extract than in media containing fecal extract or sludge liquor only. The strain grew equally well in feces-rumen agar or in 1% yeast extract, 1% Casamino Acids, and 1% Brain Heart Infusion Agar (Difco).

One individual was tested four different times, and from the highest dilution showing methanogenesis the culture counts of methanogenic bacteria were 2×10^6, 2×10^6, 2×10^{10}, and 2×10^6 per gram, respectively. The other four positives showed methane bacteria in numbers of 2×10^7, 2×10^6, 2×10^9, and 2×10^8 organisms per gram, respectively. The sample showing 2×10^9 methanogenic bacteria per gram was obtained from a patient in the Veterans Administration Hospital, Madison, Wis., by E. M. Lapinski. Unusually large

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quantities of methane had been detected in the expired gas of this patient. A fecal sample was sent airmail to Davis, California, sealed with no access of oxygen. Presumably, the lower than body temperature in transit prevented growth, and the value found is an approximation of the viable bacteria in the sample.

The metabolic significance of methanogenesis in humans has not been explored. Retention time in the alimentary tract may be a factor in determining if methanogenic bacteria can maintain themselves. The consistency of methanogenesis in particular individuals and the effects of diet on this process can be examined by analyses of respired gas, but cultural evidence on the nature of the bacteria encountered may also be useful.

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