Features of a *Clostridium*, strain CV-AA1, an obligatory anaerobic bacterium producing acetic acid from methanol

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Isolation and characterization of a new, obligatory, anaerobic, methylotrophic, homoacetogenic bacterium is described. This bacterium is a mesophilic, motile, slightly curved rod that demonstrated a negative Gram reaction, formed spherical, (sub)terminal spores and performed a homoacetic fermentation with methanol, a CO₂-2H₂-gas mixture, glucose or fructose, respectively, as the substrate. The methanol fermentation proceeded only when a suitable amount of NaHCO₃ was available in the nutrient solution supplied.

INTRODUCTION

Part of the methanol supplied to a laboratory scale anaerobic upflow reactor, treating a methanolic waste, was converted into volatile fatty acids, instead of being directly fermented to CH₄ and CO₂ (Lettinga et al., 1979, 1981). This formation of acid intermediate was clearly related to the concentration of bicarbonate present and to the availability of sufficient trace elements.

The main volatile fatty acids formed were acetic and butyric acids. Except when the acid production gave rise to pH values lower than 4.5, acetic acid formation was predominant.

The present study was initiated in order to obtain some insight as to the type of bacteria responsible for the production of volatile fatty acids from methanol.

The ability of obligatory anaerobic bacteria to grow at the expense of methanol is still restricted to a limited number of known bacterial types. The first organism recognized in this respect was likely *Methanosarcina barkeri*, isolated and studied by Schnellen (1947). He also mentioned the enrichment of a thermophilic methanol-utilizing, rod-shaped and spore-forming methanogen with a
very great oxygen tolerance, but he did not succeed in isolating it into pure culture. In 1969 Pantskhava and Pchyolkina claimed the isolation of Methanobacillus kuzneceovi, a thermophilic organism that converted methanol into methane and acetate. Recently another methanogen able to grow on methanol, Methanococcus mazei, was isolated and described by Mah (1980).

A non-methanogenic bacterium able to grow on methanol was shown to be Clostridium formicoaceticum by Braun et al. (1981), whereas Sharak Genthner et al. (1981) described Eubacterium limosum, isolated from the rumen of sheep, being the most numerous methanol-utilizing bacterium under certain conditions in this environment. Zeikus et al. (1980) introduced Butyribacterium methylo trophicum, first isolated in Marburg, West-Germany. This so-called Marburg strain was able to grow on methanol and on a number of other substrates, and it performed homoacetonic, homobutyric or heteroacidic fermentations dependent on the kind of substrate available.

The present paper describes the enrichment of methanol-utilizing, fatty acid-producing bacteria and the isolation and brief characterization of an acetic acid-producing strain.

**MATERIALS AND METHODS**

*Enrichment procedure*

About 50 g of methanol-adapted anaerobic sludge was transmitted into a serum bottle and supplied with 1 liter of a nutrient solution. The serum bottle was flushed with oxygen-free carbon dioxide before being connected to a Mariotte flask for gas collection. Gas production was found by measuring the volume of acidified water displaced from the Mariotte flask.

The nutrient solution, used in maintaining this enrichment culture, was composed of: NH₄Cl, 1 g; NaHCO₃, 1 g; KH₂PO₄, 0.4 g; K₂HPO₄·3H₂O, 0.4 g; MgSO₄·7H₂O, 0.1 g; NaCl-free sea salts (as a source of trace elements), 0.1 g; FeSO₄·7H₂O, 0.01 g; demineralized water, 1 liter; methanol 99.5%, 10 ml.

The incubation temperature was 30°C.

*Isolation procedure*

Isolation of bacteria producing volatile fatty acids from methanol was performed by making dilution series in anaerobic roll tubes, filled with nutrient agar and methanol, 0.02 ml per 5 ml of nutrient agar. Colonies of methanogenic bacteria were identified with a Leitz Dialux 20 EB epifluorescence microscope. The non-fluorescent colonies were tested for production of volatile fatty acids in a liquid nutrient medium with methanol as the substrate.

The composition of the nutrient medium, used for isolation and study of methylotrophic acetogenic bacteria was: NaHCO₃, 10 g; Merck yeast extract, 1 g; NH₄Cl, 0.5 g; KH₂PO₄, 0.4 g; K₂HPO₄·3H₂O, 0.4 g; MgSO₄·7H₂O, 0.1 g; CaCl₂