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Isolation of propionate degrading bacterium in co-culture with a methanogen from a cattle dung biogas plant

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Abstract. Various anaerobic hydrolytic and methanogenic bacteria active in cattle dung biogas plants are reported in the literature. Anaerobic bacteria with ability to use volatile fatty acids constitute a vital bridge between hydrolytic bacteria and methanogenic bacteria. The present paper describes the isolation of *Syntrophobacter wolinii* a propionate degrading bacterium in co-culture with a hydrogen utilizing methanogen *viz., Methanobacterium formicicum* from the fermenting slurry of cattle dung biogas plant. Earlier studies on propionate and butyrate degradation indicated *Methanospirillum hungatei* as the hydrogen utilizing partner of the co-culture whereas in the present studies this was not the case. Temperature 35° C, pH 7·5 and 20 mM of propionate were found optimal for growth and activity of co-culture.

Keywords. Cattle dung; propionate degradation; *Syntrophobacter;* methanogen.

1. Introduction

Anaerobic digestion is a complex microbial process wherein, a variety of bacteria are involved. These bacteria can be broadly classified as fermentative, acetogenic and methanogenic bacteria (Mclnerney and Bryant 1981). Hydrolytic bacteria bring about initial degradation of complex biopolymers such as cellulose, hemicellulose, proteins and lipids into dicarboxylic acids, volatile fatty acids (VFA), ammonia, carbondioxide, hydrogen, etc. Methanogenic bacteria which play a key role in the terminal step of anaerobic digestion use only a few compounds like acetate, methanol, methylamine, hydrogen and carbondioxide. VFA and dicarboxylic acids are thus needed to be converted as much as possible to acetate, hydrogen and carbondioxide for maximum production of methane. This is brought about by hydrogen producing acetogenic bacteria which grow only in syntrophic association with hydrogen scavengers such as sulphate reducing or methanogenic bacteria (Mclnerney *et al* 1979). Propionate degrading bacterium *Syntrophobacter wolinii* in co-culture with *Methanobacterium hungatei* has been reported from anaerobic sewage sludge (Boone and Bryant 1980). Later on a butyrate degrading bacterium *Syntrophomonas wolfei* has also been reported in co-culture with a hydrogen utilizer (Mclnerney *et al* 1981).

Importance of biogas technology as a means to satisfy house hold energy needs is now well established, particularly in India. Efforts are going on to make biogas plants more and more economical. As a part of these efforts, emphasis is also given to understand microorganisms involved in the process and their interactions. Both

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hydrolytic and methanogenic bacteria involved in the process have been reported (Ranade *et al* 1980; Godbole *et al* 1981; Gore *et al* 1985; Kelkar *et al* 1990). However isolation of acetogenic bacteria from the cattle dung has not been reported. The present paper describes isolation of a propionate degrading bacterium in co-culture with a hydrogen utilizing methanogen from the fermenting slurry of cattle dung biogas plant.

2. Materials and methods

2.1 *Enrichment and isolation of co-culture*

Enrichment culture for propionate degrading bacteria was initiated in 65 ml serum vials with fermenting slurry of the 3 M^3 biogas plant running on cattle dung. Anaerobic techniques of Miller and Wolin (1974) were used. Basal carbonate yeast extract tripticase (BCYT) medium (Touzel and Albagnac 1983) with pH 7·0 was used. The gas phase in the anaerobic bottle was N_2: CO_2 (80:20). Filter sterilized solution of sodium propionate was used as energy source in final concentration of 20 mM. In all the experiments incubation temperature was 35° C unless otherwise stated. Propionate degrading co-culture was isolated from serially diluted enrichment using roll tube technique.

2.2 *Methanogenic bacteria*

Isolation of methanogenic bacteria from the propionate degrading co-culture was attempted using roll tube technique, with BCYT medium containing H_2:CO_2 (80:20) as well as with acetate as substrate and nitrogen as head space gas. Identification of methanogen was done according to Boone and Mah (1989).

2.3 *Effect of environmental factors*

Experiments on the effect of pH, temperature, and VFA were conducted in 125 ml anaerobic vials containing 50 ml medium at pH 7·0 except in pH experiment. Fifty per cent actively growing co-culture was used as the inoculum. Experiments were conducted for a period of 10 days at 35° C except in the temperature experiment. The pH range studied was from 4·5 to 9·0 at an interval of 0·5 unit. The temperature range studied was from 25 to 60° C at an interval of 5°C. VFA (propionate) concentration, from 10 mM and 20 to 200 mM at an interval of 20 mM was studied.

2.4 *Analysis*

Methane in gas samples was analysed on gas Chromatograph Chemito 3800 equipped with Poropak Q column (SS, 80/100, 6' × 1/8") and FID (110°C). Carrier gas was nitrogen (30 ml/min). VFA were analysed on gas Chromatograph equipped with Chromosorb W (HP) column (80/100, SS, 6'× 1/8" saturated with 10% FFAP). Injector, oven and detector were maintained at 210, 150 and 210° C respectively. Carrier gas (nitrogen), hydrogen and air were used at 40, 30 and 300 ml/min respectively.

3. Results

3.1 *Enrichment*

A stabilized enrichment culture degrading propionate to methane was obtained after eight successive transfers for about 4 months. Microscopic observations showed consistently two types of bacteria *viz.* rod-shaped non-methanogenic bacteria and rod like methanogens. Differentiation between methanogenic and non-methanogenic bacteria was made based on microscopic observation under UV excitation filter at 365 nm.

3.2 *Co-culture isolation*

One co-culture designated as P-l was obtained by roll tube method from propionate enrichment culture. It showed the presence of two kinds of rod shaped bacteria. Some of these bacteria showed blue-green fluorescence when observed under fluorescence microscope and hence were methanogens. The second type of cells did not fluoresce when exposed to UV. These were non-motile, non-spore forming rods (figure 1) and were responsible for VFA degradation. A methanogen isolated from co-culture P-l showed Gram positive cells, non-motile, straight or irregularly curved rods, occurring singly and sometimes in clusters, utilized hydrogen and formate as substrate but not acetate, propionate or butyrate for growth. Optimum pH and temperature were found to be 7·0 and 35° C respectively.

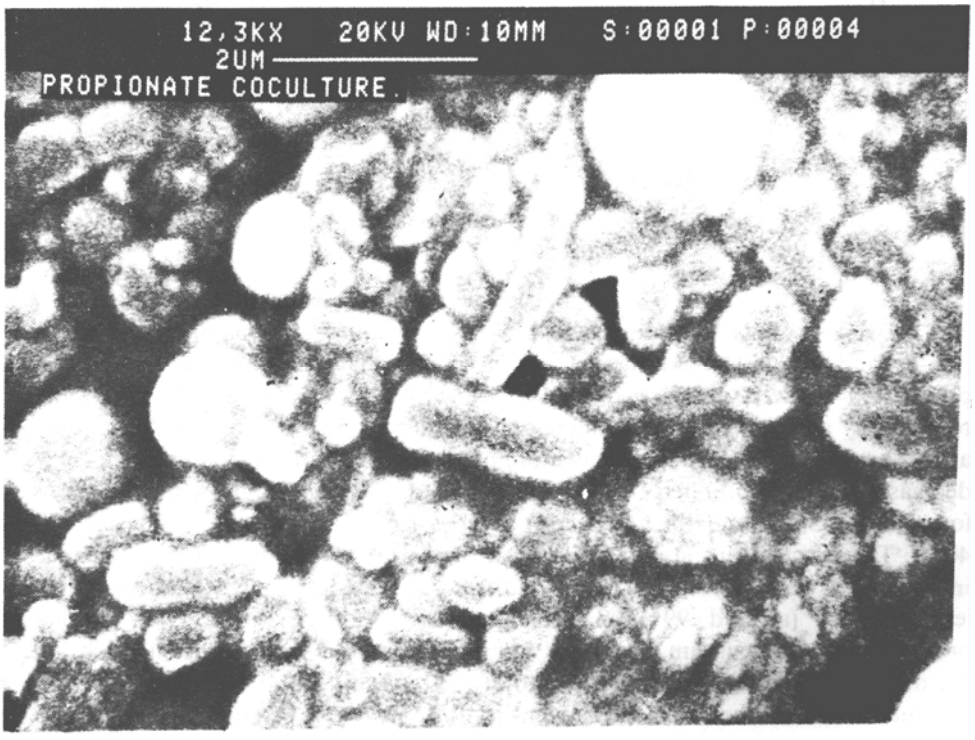

Figure 1. Propionate degrading co-culture.

3.3 *Effect of environmental factors*

Co-culture P-l showed doubling time of 140 h based on its methane producing ability. Doubling time was estimated by the formula:

$$\text{Doubling time} = \log 2 \div \left[\frac{\log_{10} z - \log_{10} z_0}{t - t_0} \right] \times 2{\cdot}303,$$

where, t_0 and t are time at the beginning and end of exponential growth, whereas z_0 and z represent methane produced (μmol) at time t_0 and t respectively (D R Boone, personal communication).

Catabolic activity of co-culture at different pH is shown in figure 2. Maximum propionate degradation was observed at pH 7·5. It was less than 59% at pH 8·5

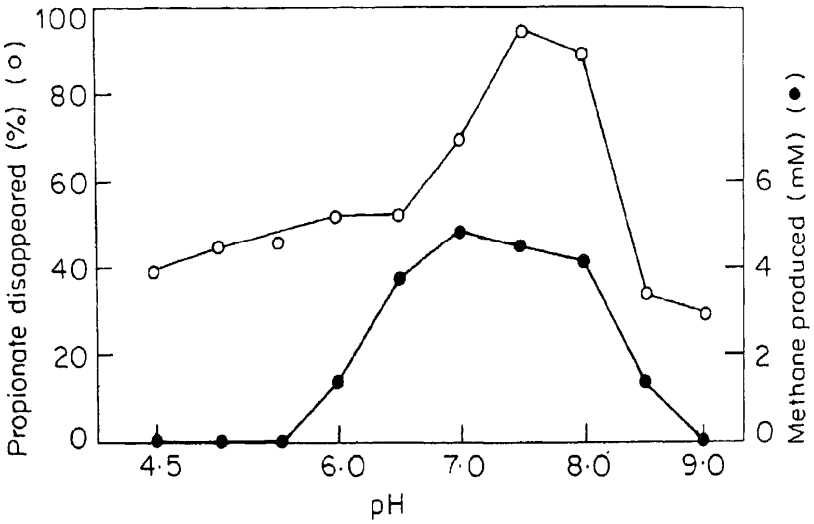

Figure 2. Effect of pH on co-culture P-l.

and above as well as at 6·0 and below. Methane production was observed between pH 5·5 and 8·5 with maximum at pH 7·0. Propionate degradation and methane production showed increase from 10·3 to 17·4 mM and from 1·9 to 5·38 mM respectively as temperature was increased from 25 to 35° C (figure 3). At 45° C and above, propionate degradation was more or less the same. However methanogenesis decreased and got inhibited between 50 and 65° C. Thus optimum temperature was found to be 35° C and the range for the co-culture activity was seen from 25 to 40° C. The effect of propionate concentration on the co-culture activity is depicted in figure 4. It is seen from the figure that disappearance of propionate was more or less constant (around 99%) up to 60 mM concentration. However methane production was maximum at 60 mM propionate. Beyond this concentration both the disappearance of propionate and total methane produced decreased. This may be due to inhibition of methanogen by intermediate products of propionate degradation.

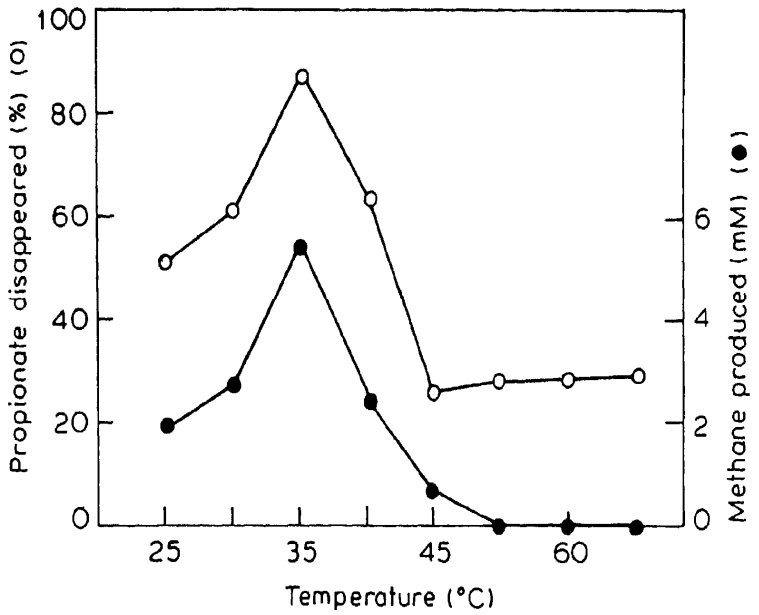

Figure 3. Effect of temperature on culture P-l.

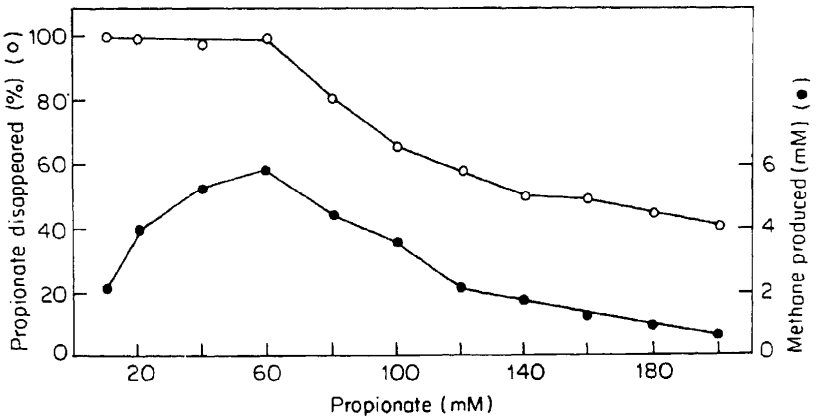

Figure 4. Effect of propionate concentration on culture P-l.

4. Discussion

VFA are the most important extracellular organic intermediates in the anaerobic fermentation of organic matter to methane. Among these propionate is quantitatively the most important fatty acid (Kasper and Wuhrmann 1978; Mccarty 1971; Zehnder 1978), and accounts for maximum methane than any other VFA except acetic acid (Boone and Luying Xun 1987). Though propionate concentration in digester is low its turnout is rapid (Boone 1984). As a result, any inhibition in propionate degradation may result in a sudden increase in its concentration, which

is detrimental to the anaerobic digestion. The P-l co-culture could degrade propionate to methane with acetate as one of the intermediates. Based on morphological and physiological characters, the co-culture P-l was found to be identical to *S. wolinii* (Boone and Bryant 1980). The hydrogen utilizing partner of co-culture was identified as *Methanobacterium formicicum.* In India, cattle dung biogas plants work at ambient temperatures and neutral pH. Co-culture isolated in the present studies has shown 7·0 to 7·5 pH requirement for its optimal growth and 35° C for its maximal activity. In our country such temperature conditions are available only in hot seasons, when most of the cattle dung biogas plants produce high biogas yields. This may be due to the availability of optimum conditions for the VFA degrading and methanogenic bacteria present in the cattle dung biogas plants. In addition to pH and temperature, for obtaining maximum methane yield by propionate degrading co-culture such as P-l, concentration of propionate should be around 60 mM.

The methanogenic partner in the co-culture isolated by Boone and Bryant (1980) was *M. hungatei.* In the present studies such spiral shaped methanogen was not observed in enrichment. However only rod shaped methanogens were present and only *M. formicicum* was isolated from the co-culture. This type of methanogen is common in the cattle dung biogas plants (Ranade *et al* 1980). The coculture isolated herein indicates the importance of *M. formicicum* in cattle dung biogas plant. It will be interesting to carry out comparative studies on both the co-cultures *i.e. S. wolinii* with *M. formicicum* and *M. hungatei.*

Acknowledgement

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