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Inhibition of methanogenesis by interaction of aluminium ion with co-factor, F-420, in *Methanosarcina barkeri*

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Methane emission was inhibited by aluminium ion in paddy fields. Addition of Al^{3+} (20 m*M*) to the culture medium containing cells of pure *Methanosarcina barkeri,* inhibited methanogenesis. Methanogenic co-factor, F-420, was isolated and purified from *Methanosarcina barkeri* MS. Spectrophotometric and spectrofluorometric analysis of interaction between co-factor, F-420, and Al^{3+} revealed that they formed a complex compound that might have blocked methanogenesis.

Keywords: Aluminium ion, Methane emission, Methanogenesis, *Methanosarcina barkeri*

Methanogenic bacteria are found in anaerobic environment in natural wetland ecosystem, paddy fields and also in rumen of cattle and guts of termites etc[1]. A substantial amount of methane is emitted from inundated paddy fields[2]. There is a variability of methane emission from such paddy fields depending upon many environmental factors, such as *p*H, temperature, organic and inorganic components etc of the soil[3-5]. Methane emission from paddy fields can be reduced by soil aluminium ions[6]. Biological production of methane is the result of a specific type of energy yielding metabolism known as methanogenesis[7]. In the elucidation of this central pathway, several conventional coenzymes have not been found in methanogenic bacteria but several novel molecules have been found[8]. The enzyme-mediated reactions that involve six new co-enzymes are little understood. Methanogens have unique coenzymes for electron transfer, CoM and F-420 (ref. 4). Most obvious metabolic role of F-420 would be the transfer of electrons from hydrogen to the consecutive intermediates of methane biosynthesis[9]. Baron and Ferry[10] have identified the cytological localization of factor F-420 (8-hydoxy-5-deazaflavin). They have also isolated and purified F-420 reducing dehydrogenase from *Methanobacterium formicicum*. Factor F-420 is so named due to absorbance maximum at 420 nm of its oxidized state[11]. The oxidized state exhibits a bright blue-green fluorescence with a maximum around 470 nm and is a two-electron acceptor under physiological conditions[12].

This fluorescence is a distinguished feature of methanogenic bacteria and has been used for identification purpose[13]. In acidic media, absorbance maximum at 420 nm shifts to around 380 nm. The reduced form of F-420 loses its absorbance at 420 nm and becomes nonfluorescent[14]. Fluorometry has been successfully used to measure factor F-420 as indicator of total and methanogenic activities[13]. F-420 was extracted from *Methanosarcina barkeri* cells and separated from other factors and flavins by column chromatography. In this paper, we have spectroscopically studied the interaction between aluminium ions with isolated and purified F-420.

Pure strain of *Methanosarcina barkeri* MS was obtained from Professor J Reeve, Ohio State University, USA, and cultured in a modified growth medium[15]. Fine chemicals were purchased from Sigma Chemical Co.(USA) and Difco Laboratories. Different concentrations of aluminium ions in the form of aluminium chloride (0 to 100 m*M*), aluminium sulphate (0 to 100 m*M*) or aluminium nitrate (0 to 100 m*M*) were added to growth medium separately, prior to inoculation. Anaerobic condition of culture vials was maintained by using CO_2 and H_2 gas mixture, and at *p*H 7 of the medium. Inoculum (5%) was taken from late exponential culture and inoculated in growth medium (20 ml) in 100 ml sealed gas pressure vials and the vials were kept at 37°C. Each culture was done at least in 4 vials and all data were averaged.

To isolate and purify co-factor, F-420, wet cells (10g) of *Methanosarcina barkeri* MS were taken in a test tube and suspended in de-ionized water (10 ml).

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The tube was then placed in a boiling water bath for 15 min. After cooling, the mixture was centrifuged at $10,000 \times g$ for 10 min. A clear greenish-yellow solution was obtained as supernatant. The supernatant was checked for co-factor, F-420, leached out from the cells, by observing absorbance peak at 420 nm and fluorescence emission peak at 470 nm when excited at 420 nm. The supernatant was transferred in a dialysis bag and reduced its volume 20 times approximately by keeping the dialysis bag inside sucrose powder (200g). The dialysis bag was taken out and dense coloured solution was transferred to a fresh dialysis bag and dialyzed against de-ionized water for 24 hr. The dialyzed solution was then eluted through Sephadex G-100 column (column volume was 18 ml and eluting agent was de-ionized water). Fractions containing co-factor, F-420, were collected and again concentrated up to 0.5 ml using dust sucrose and dialyzed similarly against de-ionized water. Dialyzed fraction (100 μl) was then loaded in a DEAE agarose spun column (column volume 5 ml) and eluted with a short spin (500 g). This second step purification gave an excellent result of purified co-factor, F-420, which was confirmed by checking its absorbance spectra in presence of 0.1 N NaOH and 0.1*M* of HCl. Purified and concentrated co-factor, F-420, was taken in an eppendorf tube and kept at 4°C for further studies.

Concentrated F-420 (10 μl) was taken in a quartz tube containing de-ionised double distilled water (490 μl) and absorbance spectra was observed in presence of different Al^{3+} concentration (0 to 5 m*M*) by Shimadzu spectrophotometer and fluorescence spectra were similarly observed by Perkin-Elmer spectrofluorometer.

The binding constant and its nature between purified co-factor, F-420, and aluminium ions was studied by Binding- isotherm equation[16], as follows:

$$1/\Delta F_c = 1/\Delta F_{max} + 1/(\Delta F_{max}.K) \times 1/C$$

where F is fluorescence intensity of the fluorosphore, and C is the concentration of quencher. The binding constant K is determined from the value of the intercept $1/\Delta F_{max}$ and slope $1/\Delta F_{max}.K$ of the plot ($1/\Delta F_c$ vs $1/C$).

Biomethanogenesis is sensitive to several groups of inhibitors, including alternate electron acceptors, heavy metals, halogenated hydrocarbons and cations etc.[17,18]. Because methane is a significant and increasing greenhouse gas, its source fluxes and their potential reduction are of concern[19,20].

In our previous report we have shown that methanogenesis in paddy fields is inhibited by soil aluminium ions to some extent[6]. In the present study it was observed that a linear relationship existed between methane production and fluorescent emission intensity at 470 nm when excited at 420 nm with the methanogens cell concentration. Aluminium ions inhibited methane production when added to the growth medium. Methane emission could be blocked totally by addition of 20 m*M* or more of Al^{3+} to growth medium (Fig. 1). Since co-factor, F-420, is the major electron carrier in the methanogenic pathway of methanogens, so any alterations in F-420 will certainly affect methanogenesis. We isolated and purified co-factor, F-420, from pure strain of *Methanosarcina barkeri*. Spectral analysis of F-420 confirmed its purification (Fig. 2).

A clear isosbestic point was observed at 420 nm of the absorbance spectra of co-factor, F-420, when titrated with different concentrations of Al^{3+} (Fig. 3). This confirmed that there was a complex formation between F-420 and Al^{3+}.

In the present study it was observed that fluorescence emission intensity at 470 nm when excited at 420 nm of co-factor, F-420, was quenched on addition of Al^{3+}. Only aluminium nitrate ion (0.4 m*M*) was

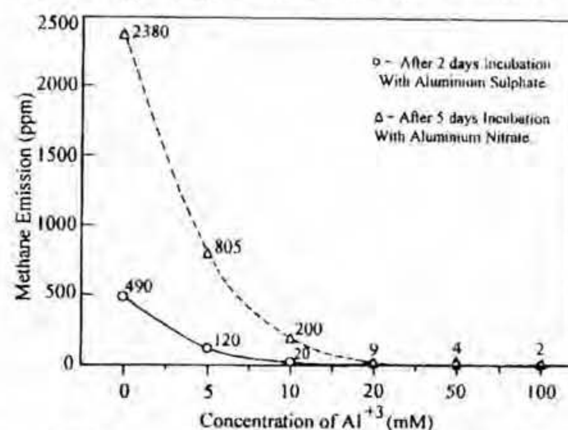

Fig. 1 — Methane emission by *Methanosarcina barkeri* in culture vials in presence of different concentrations of Al^{3+}.

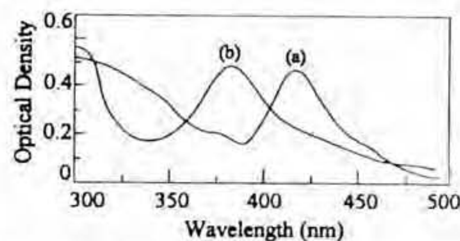

Fig. 2 — Absorption spectra of purified F-420 in presence of (a) NaOH (0.1 *M*) and (b) HCl (0.1 *M*).

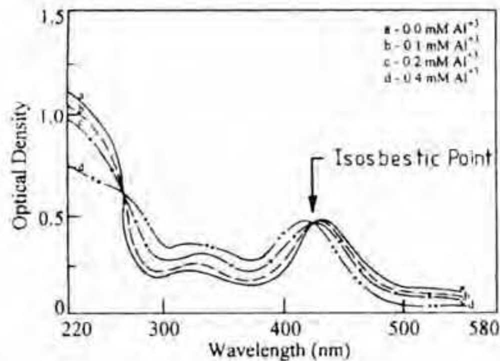

Fig. 3 — Absorption spectra of purified F-420 in presence of different concentrations of Al^{3+}.

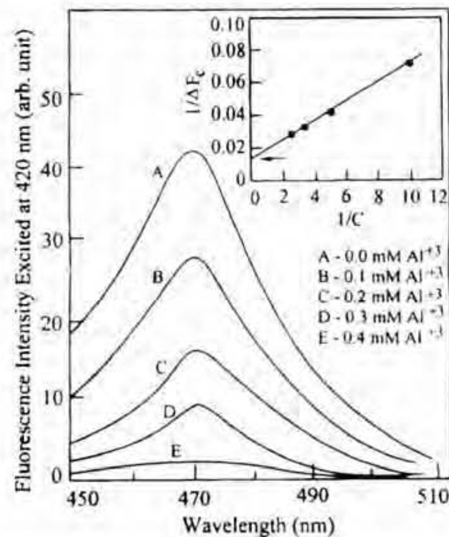

Fig. 4 — Fluorescence spectra of purified F-420 in presence of different concentrations of Al^{3+}. Binding-isotherm plot was shown in the inset.

sufficient to suppress completely the emission peak at 470 nm (inset of Fig. 4). We also used aluminium chloride and aluminium sulphate in the above experiments. In every case, we found that aluminium ion could act as a fluorescence quencher for F-420 that could suppress the emission peak at a concentration of 4 m*M* or above. In this paper, methane emission was drastically reduced by addition of aluminium ions (20 m*M*) to the culture medium of *Methanosarcina barkeri*. Binding constant between purified F-420 and Al^{3+} was estimated as 12.4×10^3 M^{-1}, using Binding-isotherm plot (inset Fig. 4). It is expected that Al^{3+} may bind to the ring structure of co-factor, F-420, which is the chromophoric group of F-420. This binding may introduce modification in the chromophoric ring structure and thereby reduce the possibility of energy trapping or release, known to take place during methanogenesis.

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References

1 Balch W E, Fox G E, Magnum L J, Woese C R & Wolfe R S, Methanogen: Re-evaluation of a unique biological group, *Microbiol Rev*, 43 (1979) 260.
2 Neue H U, IRRI studies: Role of rice field methane in global warming, *IRRI News Release* (Nov), 1991.
3 Oremland R S, Marsh L & DesMarais D J, Methanogenesis in Big Soda Lake, Nevada: An alkaline, moderately hypersaline desert lake, *Appl Environ Microbiol*, 43 (1982) 462.
4 Zinder S H, Methanogenesis: Ecology, physiology, biochemistry and genetics, edited by Ferry J G (Chapman and Hall, New York) 1993, 128.
5 Kayhanian M, Performance of a high-solids anaerobic digestion process under various ammonia concentrations, *J Chem Tech Biotechnol*, 59 (1994) 349.
6 Chattopadhyay B D, Thakur A R, Mangal P C, Purkait N N & Saha A K, Inhibitory role of aluminium in methane emission in rice fields, *Indian J Exp Biol*, *32* (1994) 495.
7 Zeikus J G, The biology of methanogenic bacteria, *Bacterial Rev*, 41(2) 1977, 514.
8 Rouviere P E & Wolfe R S, Novel biochemistry of methanogenesis, *J Biol Chem*, 262 (1988), 7913.
9 Pol A, ven der Drift C, Vogels G D, Cupper J H M & Laarhoven W H, Comparison of co-enzyme F $_{420}$ from *Methanobacterium bryantii* with 7- & 8-hydroxy-10-methyl-5 deazaisoalloxazine, *Biochem Biophys Res Commn*, 92 (1980) 250.
10 Baron S F & Ferry J G, Archae: a laboratory manual, edited by Robb F T, Place A R, *et al.* (Cold Spring Harbour Laboratory, NY) 1995, 217.
11 Danson M J, Hough D W & Lunt G G, The Archaebacteria: Biochemistry and biotechnology (Portland Press, London , UK) 1992, 41.
12 Kates M, Kushner D J & Matheson A T, The Biochemistry of archaea (Archaebacteria) (Elsevier Amsterdam, The Netherlands) 1993, 41.
13 Peck M W & Chynoweth D P, On-line monitoring of the methanogenic fermentation, *Biotech Bioeng*, 39 (1992) 151.
14 DiMarco A A, Bobik T A & Wolfe R S, Unusual co-enzymes of methanogenesis, *Annu Rev Biochem*, 59 (1990) 355.
15 Chattopadhyay B D, Thakur A R, Poddar R K & Dasgupta D, Effect of calcium ion on *Methanosarcina barkeri* MS, *Indian J Exp Biol*, 31 (1993) 738.
16 Lakowicz J, Principles of fluorescence spectroscopy (Plenum Press, NY) 1983, 261.
17 McCarty P L, One hundred years of anaerobic treatment, In: Anaerobic Digestion, edited by Hughes D E *et al.* (Elsevier Biomedical Press, Amsterdam), 1991, 3.
18 Muller R F & Steiner A, Inhibition of anaerobic digestion caused by heavy metals, *Wat Sci Tech*, 26 (1992) 834.
19 Chynoweth D P, Global significance of biomethanogenesis, in Global envir chemistry, edited by Dunnette D and Brian R O (ACS Symposium Series) 1992, 483.
20 U S EPA, Options for Reducing Methane Internationally, in *Technical options for reducing methane emission*, Vol. I, edited by Hogan K B (Report to Congress) 1993.