Methanobacterium thermoautotrophicum composition

ASSIGN BUSTER

INTRODUCTION

Methanobacterium are methane-producing archaebacteria. They are generally known as methanogens. A genus of firmly anaerobic bacteria that reduce CO using molecular hydrogen, H2, to give methane. They demonstrate a number of features that distinguish them from other bacteria, and are now classified as a separate cluster, the Archaebacteria. Methanobacteria are found in the anaerobic sediment at the underneath of ponds and marshes (hence marsh gas is the common name for methane) and as fraction of the microflora of the rumen in cattle and anyother herbivorous mammals (Brock et al.).

The species within the genus Methanobacterium differ widely in length and filaments are common. Cell walls emerge to be Gram positive, but are composed of pseudomurein rather than peptidoglycan. They are non-motile and flagella are missing. Metabolism is strictly anaerobic and H2 or formates are used as an electron donor. All species develop with H2 and CO2 as a substrate for methanogenesis. Cells are mesophillic or thermophillic. All species not succeed to grow under aerobic conditions and mainly are acid tolerant (will grow at pH less than 5). There are 12 species of genus Methanobacterium and they are isolated from aneraobic digestors, sewage sludge, manure, groundwater, and development water of oil-bearing rocks (Prescott et al.).

Methanobacterium thermoautotrophicum is a methane producing micro-organism (methanogen) that was usually isolated from the municipal waste-

treatment facility in Champaign, Illinois, USA. It grows readily and quickly under laboratory conditions (Can Karyn, 1997).

In vitro methanogenic extracts and enzymes have been purified and studied for more than 20 years. It is an ‘ absolute’ autotroph, requiring only CO2, H2 and salts for development. It is a representative of the methanogens that occupy all biodegradation facilities.

DOE (Department of Energy) supports research determined on biochemistry and molecular biology of methane production with the goals of maximising the utilize of biotechnology to change waste materials into methane as an alternative to fossil fuels, and determining how to control methane generation and methane discharge to the atmosphere (Can Karyn, 1997).

Methanobacterium thermoautotrophicum is an Archaeon that grow highly at ~650C, and its genome sequence reveals the structure of many enzymes that catalyze reactions at this elevated temperature.

There are several media accessible for the isolation of methanobacteria. Most of the media consist of yeast extort in combination with compounds such as xylose, tryptone, and glucose. Also, antibiotics in conjunction with enrichment techniques can be beneficial as selective agents in separating methanobacteria. However, methanobacteria must be present in the soil in order for successful isolation to happen.

CLASSIFICATION

Scientific classification

Domain: Archaea

Kingdom: Euryarchaeota

Phylum: Euryarchaeota

Class: Methanobacteria

Order: Methanobacteriales

Family: Methanobacteriaceae

Genus: Methanobacterium

Species: thermoautotrophicum

IDENTIFYING CHARACTERISTICS OF M. thermoautotrophicum

0. 2-1. 0 mm – 1. 2 -120mm in size.

Optimum temperature ranges from 35 – 700 C (moderate thermophile among an optimum growth temperature of 650 C).

Optimum pH range from 6. 0-8. 5.

Strictly anaerobic.

Cell stains Gram positive.

Nonmotiles.

Endospores are not present.

Cell envelope composition is mostly pseudomurein.

Chemoautotroph.

Requires only CO2, H2 and salt for growth.

All utilize ammonium, sulphide and elemental sulfur.

Habitats contain low NaCl concentrations.

Positioned close to the ‘ center’ of the methanogen evolutionary tree.

ISOLATION

The isolation and characterization of an enormously thermophilic, methanogenic bacterium isolated from sewage sludge. This nonmotile, strict anaerobe is gram-positive, display autotrophic nutritional requirements, and morphologically similar to the hydrogen utilizing, methane-producing mesophile Methanobacterium sp. strain M. O. H. isolated from cultures of Methanobacillus omelianskii. This recently described organism is named Methanobacterium thermoautotrophicum (Zeikus and Wolfe, 1971).

The isolation of methanogenic bacterium requisite a medium containing inorganic salts, an atmosphere having ofan 80: 20 mixture of hydrogen – carbon dioxide, and incubation temperature of 65 to 700C. Isolates of M. thermoautotrophicum be gram- positive, on motile, unevenly curved rods which frequently formed long filaments. The organism was found to be an autotroph and a firm anaerobe, and to have a pH optimum of 7. 2 to 7. 6. The optimum temperature for development was 65 to 700 C, the maximum being 750 C and the minimum about 400 C. The generation period at the optimum was about 5 hr. The deoxyribonucleic acid of M.

thermoautotrophicum had a guanine plus cytosine(GC) contented of 38%. When excited, intact ribosomes of Methanobacterium sp. were stable up to 550 C and had a Tm of 73 C. In contrast, riobosomes of strain M. O. H. were steady up to 550 C and had a Tm of 730 C. In contrast, ribosomes of M. thermoautotrophicum were steady up to 750 C and had a Tm of 820 C. Upon total thermal denaturation, ribosomes of strain M. O. H. underwent a 59% hyperchromic shift, whereas those of the thermophile show only a 20% enhance in hyperchromicity. Methane development in cell-free extract of M. thermoautotrophicum was temperature-dependent and necessary hydrogen and carbon dioxide; methyl cobalamin served as a methyl donor and accumulation of coenzyme M stimulated methanogenesis (Farell et al., 1969).

M. thermoautotrophicus is a exceptional organism. The ability of this bacterium to proliferate at temperatures above 700 C clearly differentiated it as an extreme thermophile. The fact that exceedingly thermophilic anaerobes have not been reported in the past may reflect a lack of sternly anaerobic conditions coupled with enrichment temperatures below 650 C (Pace et al., 1967). These problems are overcome by use of the Hungate technique customized for high temperatures. By use of this process, it may be possible to provide additional evidence that microbial diversity is not necessarily restricted by temperature extremes.

M. thermoautotrophicus, can be grown in a medium having ammonia as nitrogen source, sulfide as sulfur source, hydrogen, carbon dioxide like energy and carbon source, and inorganic salts (Farell et al., 1969). The organism’s ability to utilize simply hydrogen for reducing power and carbon

dioxide as sole carbon source evidently establishes this bacterium as a hydrogen-oxidizing autotroph. In addition, doubling times beneath these conditions were less than 5 hr. These short generation times, relation to methane bacteria and not Escherichia coli, distinguish this organism as one of the greatest growing methane bacteria known.

M. thermoautotrophicus display that methane formation is a temperature-dependent reaction requiring hydrogen and carbon dioxide, that methyl cobalamin can supply as a methyl donor, and that coenzyme M appears to be involved in methane formation in this novel methane bacterium (Pace et al. 1967). M. thermoautotrophicus may confirm to be the “ organism of choice” for mass culturing of methane bacteria, as the appearance of contaminants in huge fermentors is not a difficulty when inorganic media and high temperatures are used.

The name Methanobacterium thermoautotrophicus is planned, the species name being derived from the organism’s ability to multiply autotrophically at high temperatures.

MORPHOLOGY AND CELL WALL COMPOSITION

The fine structure of Methanobacterium thermoautotrophicum which was mature at the optimal temperature, 650 C, as well as at the temperature limits for growth is described. The most distinguishing feature of this organism is the existence of intracytoplasmic membranes. The internal membrane system have triplet membranes which are stacked closely together, frequently appearing as concentric circles without parting by cytoplasm. M. thermoautotrophicum proliferates as unevenly curved rods at

650 C and has a fine structure similar to many other gram-positive bacteria. Both low (450 C) and high (750 C) growth temperatures persuade structural modifications. These structural changes include rod to spheroidal morphological changes, cell wall abberations, distortion of separation septa, misdivisions, and interior membrane deterioration (Zeikus and Wolfe, 1973).

Morphology

The morphology of M. thermoautotrophicum is greatly prejudiced by the growth temperature. The maximum temperature for growth is between 650 and 700 C; no growth occurs below 400 or above 760 C. At 650 C this bacterium grows as elongated, irregularly curved rods which can appear filamentous. Filaments consist of chains of correlated cells. This organism undergoes a drastic morphological change at the great temperatures for growth. At 450 C the cells and filaments curl up tightly in a “ cork screw”-like manner; similarly, at 750 C the cells begin to knot up, but to the similar degree (Zeikus and Wolfe, 1973).

Composite internal membrane systems, in contrast to mesosomes, are restricted to a few members of bacteria such as the photosynthetic, nitrifying, and methane-oxidizing bacteria, all of which are gram negative. The intracytoplasmic membranes observed in thin sections of this organism are made of triplet membrane structures which consist of closely stacked parallel pairs of individual membranes. These membranous structures emerge to be formed by invagination of the cytoplasmic membrane. In this respects, the internal membranes of M. thermoautotrophicum be similar to the internal membrane systems present in species of nitrifying and methane-utilizing bacteria However, intracytoplasmic membranes made up of triplet

membranes stacked nearly together, frequently appearing in sections as concentric circles without seperation by cytoplasm, have not been earlier reported in chemolithotrophic bacteria. The occupation of the internal membranes present in M. thermoautotrophicum remains an interesting but unexplained problem. The function of the internal membranes in the photosynthetic, nitrifying, and obligate methane oxidizing bacteria has been supposed to be associated with specific electron transport and phosphorylation. The energy-yielding metabolism in M. thermoautotrophicum, as well as previous species of methane bacteria, involves the oxidation of hydrogen with the concomitant decrease of carbon dioxide to methane.

High and low temperatures can persuade structural in M. thermoautotrophicum. These alterations include: rod to spheroidal morphological variations, cell wall aberrations, distortion of septa, misdivisions, and internal membrane deterioration. Somewhat alike structural alterations have been shown to happen when B. subtilis is grown at low temperature and when a temperature-sensitive mutant of B. subtilis is developed at high temperature. The structural abnormalities which arise at the temperature extremes for growth of M. thermoautotrophicum may possibly be accredited to a lesion in the cytoplasmic membrane affecting whichever cell wall synthesis, DNA synthesis, or both.

Ultrastructure at the optimal temperature for growth

The general appearance of M. thermoautotrophicum is shown in Fig. 2. Cells which seen long or filamentous, when observed by phase-contrast microscopy, in fact are chains of closely linked cells delineated by cross

walls. Individual cells are irregularly curved and show a gram-positive cell envelope structure. The cell division method in M. thermoautotrophicum resembles that of distinctive gram-positive bacteria such as Bacillus subtilis (Glauert et al., 1961) . Figure 3 illustrates the general ultrastructural character of M. thermoautotrophicum at high magnification. This organism has a usual gram-positive cell wall (average thickness of 16. 5 nm), followed by a cytoplasmic membrane (6. 5-7 nm), an scatter, granular ribosome region, centrally situated nuclear material with deoxyribonucleic acid (DNA) fibrils, and the appearance of internal membranous structure. Intracytoplasmic membranes happen in all cells and are observable in both longitudinal and cross section. The internal membranes seen in thin sections of M. thermoautotrophicum may be created by invagination of the cytoplasmic membrane as was earlier postulated for membranous organelles present in the photosynthetic and nitrifying bacteria (Murry et al., 1965). It can be seen that the interior membranes are not necessarily restricted to one area of localization but can occur throughout the cell.

The spatial arrangement of internal membranes of M. thermoautotrophicum is distinct in cross section at high magnification. The membranes are oriented nearly in stacks membrane structures of this organism actually consist of directly stacked triplet membranes. Two triplet membranes are visible; a triplet membrane is made up of two individual membranes. Individual membranes are about 6. 5 nm thick and contain two outer electron-dense layers and an inner electron-transparent layer (Neale et al., 1970). They are alike in size and appearance to the cytoplasmic membrane. The parallel, close stacking of the individual membranes in pairs gives

increase to the triplet structure. The average width of a triplet membrane is 13 nm.

GENOME STRUCTURE

The total 1, 751, 377-bp sequence of the genome of the thermophilic archaeon Methanobacterium thermoautotrophicum deltaH has been resolute by a whole-genome shotgun sequencing approach. A whole of 1, 855 open reading frames (ORFs) have been identified that appear to encode polypeptides, 844 (46%) of which have been done putative functions based on their similarities to database sequences with assigned activities. A total of 514 (28%) of the ORF-encoded polypeptides are connected to sequences with unknown functions, and 496 (27%) have few or no homology to sequences in public databases. Comparisons with Eucarya-, Bacteria-, and Archaea-specific databases says that 1, 013 of the putative gene products (54%) are most similar to polypeptide sequences discussed previously for previous organisms in the domain Archaea (Kandler and Konig, 1993). Comparisons with the Methanococcus jannaschii genome data underline the extensive divergence that has formed between these two methanogens; only 352 (19%) of M. thermoautotrophicum ORFs determine sequences that are > 50% identical to M. jannaschii polypeptides, and there is slight conservation in the relative place of orthologous genes. When the M. thermoautotrophicum ORFs are compared to sequences from only the eucaryal and bacterial domains, 786 (42%) are more alike to bacterial sequences and 241 (13%) are more alike to eucaryal sequences(Brown et al., 1995). The bacterial domain-like gene products include the majority of those predicted to be concerned in cofactor and little molecule biosyntheses,

intermediary metabolism, transport, nitrogen fixation, regulatory functions, and connections with the environment. Most proteins predicted to be involved in DNA metabolism, transcription, and translations are more alike to eucaryal sequences. Gene structure and organization have quality that are typical of the Bacteria, including genes that encode polypeptides closely connected to eucaryal proteins. There are 24 polypeptides that might form two-component sensor kinase-response regulator systems and homologs of the bacterial Hsp70-response proteins DnaK and DnaJ, which are remarkably absent in M. jannaschii. DNA replication initiation and chromosome covering in M. thermoautotrophicum are predicted to have eucaryal features, based on the occurrence of two Cdc6 homologs and three histones; however, the existence of an ftsZ gene indicates a bacterial type of cell division initiation. The DNA polymerases comprise an X-family repair type and an unusual archaeal B type formed by two split polypeptides(Belfort et al., 1995). The DNA-dependent RNA polymerase (RNAP) subunits A', A", B', B" and H are encoded in a distinctive archaeal RNAP operon, although a second A' subunit-encoding gene is there at a remote location. There are two rRNA operons, and 39 tRNA genes are diffuse around the genome, although most of these happen in clusters. Three of the tRNA genes have introns, including the tRNAPro (GGG) gene, which contains a second intron at an unprecedented place (Brown et al., 1995). There is no selenocysteinyl-tRNA genen or evidence for classically ordered IS elements, prophages, or plasmids. The genome have one intein and two extended repeats (3. 6 and 8. 6 kb) that are members of a family with 18 senate in the M. jannaschii genome.

METABOLISM

Methane is metabolized mainly by methanotrophs and the methanogens in the global carbon cycle. Methanotrophs consume methane as the simply source of carbon, while methanogens create methane as a metabolic byproduct. Methylotrophs, which are microorganisms that can find energy for growth by oxidizing one-carbon compounds, such as methanol and methane, are located between methanotrophs and methanogens.

The creation of deuterated methane by Methanobacterium thermoautotrophicum in H20-20 mixtures was determine by high-resolution mass spectrometry. The hydrogen in the methane arose exclusively from water and not from hydrogen gas. Hydrogen gas served only as an electron source in methanogenesis. A whole cell product isotope inequity of 1. 5 favoring hydrogen over deuterium was seen in methane production in 81 atom% deuterated water. The distribution of deuterated methane species is describe by a plain model of the overall reaction.

The bacterium Methanobacterium thermoautotrophicum use carbon dioxide as together sole carbon source and catabolic electron acceptor. Some 95% of its sum carbon flux is catabolic in the formal reaction of equation 1, the overall reduction of carbon dioxide to methane by four equivalents of hydrogen gas (Thauer et al, and Zeikus, 1977.)

4H2 + CO2 -*CH4 + 2H20 (1)

Although this carbon must surpass through the oxidation states equivalent to formate, formaldehyde, and methanol, only the methanol level intermediate has been recognized, as methyl coenzyme M, (McBride et al., 1971., Taylor

et al., 1974. Zeikus and J. G. 1977). To begin our studies of the sequence of enzymatic reactions that must contain equation 1, we wish to establish the origin of the hydrogen in the creation methane as either water or hydrogen gas, or both. (“ Protium” and “ deuterium” and H and D are used to demote to specific isotopes of hydrogen; “ hydrogen” refers to H or D in spite of of molecular connection, “ hydrogen gas” and “ water” refer to H2, HD, or D2, and H20 or D20 and any mixtures thereof.) Both origins have been projected. (Pine et al., 1956) reported (no data shown) that a mixed culture grown on ethanol in D20 created CD4. Pine and Vishniac (Pine et al., 1957) showed that enrichment cultures from San Francisco Bay mud formed CHD3 from CD3COOH, signifying that one hydrogen on acetate-derived methane comes from water. Neither of the above experiments excludes direct hydrogen integration from hydrogen gas. Penley and Wood (Penley and Wood, 1972) have exposed that in methane production from methylcobalamin by extract of M. bryantii (Balch et al., 1979), the hydrogens on the methyl ligand of methylcobalamin are reserved and the fourth hydrogen from water. More recently, (Sauer et al., 1979) have suggested that hydrogen gas slightly than water is the source of hydrogen in methane formed by M. ruminantium based on experiments in which 3H2 and 3H20 were employed. (Fuchs et al., 1979) have calculated the deuterium enrichment of M. thermoautotrophicum whole cell material compared with natural abundance in water and hydrogen gas and concluded that most cellular hydrogen is imitative from water, deuterium enrichment in methane was not calculated.

The reactions comprising equation 1 are likely to start with one or more hydrogenases which, by analogy to the hydrogenases in other genera, (Rose

and L. A., 1970) are possible to catalyze the swap to H2 and D20 to produce HD and D2. If a hydrogenase catalyzed the heterolytic scission of hydrogen gas to a proton and enzymebound hydride (Hoberman et al. 1943, Krasna et al. 1954, Rose and L. A., 1970) and then transferred the hydride openly to a nonexchangeable redox coenzyme such as the 8-hydroxy-5-deazaflavin factor 420 (F420) (Ashton et al. 1979, Eirich et al., 1978, Zeikus et al., 1977) or nicotinamides, it is realistic that that hydrogen might be again transferred straightly in one or more of the reductive steps of methanogenesis. Hydrogens in methane clearly arise eventually from water and not hydrogen gas. A model predicting the distribution of deuterated methane species as a purpose of the isotope discrimination and deuterium enrichment in water is there.

Methane, also known as natural gas, is formed biologically from carbon dioxide by a series of 2-electron reductions in a process known as methanogenesis. It is approximate that 1015g methane is produced yearly by methanogenic bacteria, or methanogens, worldwide. Methanogens are strictly anaerobic and create methane in environments such as sediments, rice paddies and the guts of ruminant microorganisms or other organism.

In some environments, such as lakes, it has been seen that 90% of biogenic methane is oxidized before going the atmosphere. Methane is biologically oxidized in aerobic environments, for example the upper regions of lakes, by a series of 2-electron oxidation reactions known together as methanotrophic metabolism, or methanotrophy. The environmental coupling of methanogenesis and methanotrophy leads to a elevated turnover global cycle of C1 compounds. C1 wreckage from many sources are metabolized by

disparate bacteria and ultimately feed into the global C1 cycle shown beneath. UM-BBD pathways frequently produce C1 compounds. Links to many of them are establish at the bottom of C1 compound pages.

APPLICATIONS

Methane, also known as natural gas or biogas, is generated in the last step of the anaerobic biodegradation of industrial, urban and agricultural waste supplies and, as such, represents a major, renewable energy source. Though, methane is also a greenhouse gas and methane formation contributes substantially to global warming.

USES OF METHANOGENS IN BIOREMEDIATION

Biodegredation is being use by all industrialized and non-industrialized countries to decrease and detoxify municipal, commercial and agricultural wastes, in industrial and municipal sewage action facilities, in septic tanks and in landfills. The process works empirically, but opportunities may well be present to improve and control this biotechnology, to change more waste to methane and to decrease methane suuply to global warming.

SUMMARY AND CONCLUSION

The methanogenic bacteria as a group propose the unique opportunity to study trophic interrelationships in anaerobic ecosystems. In anaerobic habitats where decay of organic matter is happening, methanogens are the terminal organisms in the microbial food chain. The outstanding feature of this decomposition procedure is that its successful operation depends on the interaction of metabolically dissimilar bacteria.

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