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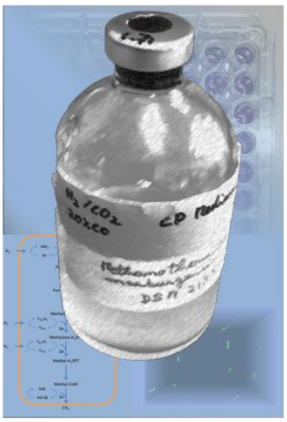

Adaptation Studies and Proteomic Analysis of Carboxidotrophic Methanogens

Ricardo Afonso Gonçalves Pereira

Thesis to obtain the Master of Science Degree in

Biological Engineering

Supervisors: Prof. Diana Zita Machado de Sousa

Prof. Miguel Nobre Parreira Cacho Teixeira

Examination Committee

Chairperson: Prof. Arsénio do Carmo Sales Mendes Fialho

Supervisor: Prof. Miguel Nobre Parreira Cacho Teixeira

Members of the Committee: Prof. Nuno Gonçalo Pereira Mira

December 2014


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Abstract

Carbon monoxide (CO) is generated by a variety of natural and anthropogenic processes. Nevertheless, CO is only present at trace amounts in the atmosphere, which is partly due to its utilisation by a wide range of diverse prokaryotes, both aerobic and anaerobic. Organisms able to autotrophically grow on CO, i.e. to use CO as carbon and electron source, are designated carboxydotrophs. Anaerobic carboxydotrophs are particularly interesting for biotechnological applications because they can produce added-value compounds, such as hydrogen, methane, fatty-acids and alcohols. CO is one of the main compounds of synthesis gas (or syngas) - product resulting from the gasification of carbonaceous sources (e.g. lignocellulosic biomass and wastes). In this way, syngas can be used by carboxydotrophs as a sustainable source for the production of chemicals and/or biofuels.

The present thesis focused on the study of methanogens for the production of methane from CO and CO/H_2 mixtures as sole substrate/electron donor. Several methanogenic species were tested for their ability to metabolise CO and/or CO/H_2 and the mechanisms and requirements for CO tolerance and oxidation were studied. The physiological response of these archaea to the presence of CO was detailed and the previously unreported carboxidotrophic ability for *Methanothermobacter marburgensis* was demonstrated. Furthermore, proteomic analysis of the CO-metabolizing capability of this organism was performed.

Keywords: Carbon Monoxide; Synthesis Gas; Methanogenesis; Adaptation; Proteomics; *Methanothermobacter marburgensis*

Resumo

O monóxido de carbono (CO) é produzido por uma variedade de processos naturais e antropogénicos, estando, no entanto, presente em quantidades vestigiais na atmosfera, o que é em parte devido à sua utilização por uma ampla gama de diversos procariotas, tanto aeróbios como anaeróbicos. Os organismos capazes de crescer autotroficamente em CO, *i.e.* de usá-lo como fonte de carbono e electrões, são designados carboxidotróficos. Os carboxidotróficos anaeróbios possuem especial interesse em aplicações biotecnológicas devido ao seu potencial de produção de compostos com valor acrescentado, tais como hidrogénio, metano, ácidos gordos e álcoois. O CO é um dos principais compostos do gás de síntese (ou syngas) - produto resultante da gasificação de fontes carboníferas (e.g. biomassa lignocelulósica e resíduos) e, deste modo, pode ser usado por carboxidotróficos como uma fonte sustentável para a produção de químicos e/ou biocombustíveis.

O foco da presente tese é o estudo de metanogénicos para a produção de metano a partir de CO e misturas de CO/H_2 como único substrato/dador de electrões. Várias espécies metanogénicas foram testadas quanto à sua capacidade de metabolizar CO e/ou CO/H_2 e os mecanismos e requisitos necessários à tolerância e oxidação de CO foram também estudados. A resposta fisiológica destes archaea à presença de CO foi detalhada e foi pela primeira vez demonstrada a capacidade carboxidotrófica de *Methanothermobacter marburgensis*. Além disso, foi efetuada a análise proteómica da capacidade metabolizante de CO deste organismo.

Palavras-Chave: Monóxido de Carbono; Gas de Síntese; Metanogenese; Adaptação; Proteómica; *Methanothermobacter marburgensis*

Aknowledgements

First and foremost I'd like to offer my gratitude to Prof. Diana Sousa and Prof. Fons Stans for giving me the opportunity to perform this work at the Microbial Physiology group in the Microbiology Department of Wageningen University. Their invaluable guidance tempered by the willingness to allow me to venture out on my own, made for a great learning experience. I'd particularly like to thank Diana for her kind words and incentive in the rougher moments of my stay.

I'd also like to give a special aknowledgement to Ana Luísa Pereira who patiently taught me the basics of anaerobic culturing and shared a lot of great moments in the lab in the first few months.

Other special thanks go to the ever present technitians, particularly Ton van Gelder, whose teachings and concern for my safety at all times I will not forget.

In my office I had a wonderful environment of support and friendship in Ahmad Khadem, Anna Florentino, Lara Paulo and Vicente Nunez. Thank you for all for your sage advice and friendship. As we would say: "thanks for listening".

I also cannot forget the wonderful talks (scientific and otherwise) with my co-workers. Particularly Irene Sanchez-Andrea, Peer Timmers, Michael Visser and Martijn Diender were always available to discuss my scientific troubles which I appreciated.

Finally I'd like to thank the entire MicFys group for making me feel welcomed and a part of the family.

This study has been funded by FEDER, through the Operational Programme Thematic Factors of Competitiveness - COMPETE, and by Portuguese funds, through the Portuguese Foundation for Science and Technology (FCT), in the frame of the project FCOMP-01-0124-FEDER-027894 - "SYN2value - Syngas bio-upgrading to fuels and chemicals".

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Compounds

CO_2	Carbon Dioxide
CO	Carbon Monoxide
H_2	Hydrogen
H_2S	Hydrogen Sulphide
CH_4	Methane
N_2	Nitrogen
O_2	Oxygen
CH_3COO^-	Acetate
AQDS	Anthraquinone-2,6-disulfonate

Enzymes

ACS	Acetyl-CoA Synthase
CODH	Carbon Monoxide Dehydrogenesa
Eha	Energy converting [Ni,Fe]-hydrogenase
Fd	Ferredoxin
Ftr	Formylmethanofuran: H_4MPT formyltransferase
Fwd	Formyl-MFR dehydrogenase
H_4MPT	Tetrahydromethanopterin
Hdr	Heterodisulfide reductase
Mch	Methenyl- H_4MPT cyclohydrolase
Mcr	Methyl-coenzyme M reductase isoenzyme I
Mfr	Methanofuran
Mvh	F_{420}-non reducing [Ni,Fe]-hydrogenase

1 Introduction

1.1 Research motivation and background

Rapid growth of world population, coupled with the emergence of developing countries, results in an ever increasing need for energy and fuel sources. Furthermore, limited availability and price fluctuations of fossil fuels have driven the energy market towards the search for renewable alternatives. In this context, biofuels offer an appealing opportunity for quenching these requirements in the near future.

Biofuels can be divided into first-generation and second-generation sources. Whilst first generation biofuels have recently received ample criticism for their often direct competition with food stocks placing their sustainability into question, second-generation biofuels are mostly attained from non-food lignocellulosic biomass and have no such drawbacks, making them a far more attractive solution for biofuel production (Mohammadi *et al.* 2011).

Lignocellulosic biomass is one of the most abundant sources for biofuels. It consists mainly of cellulose, hemicellulose and lignin and is a general term for describing the non-starch based fibrous plant material. However, the high availability is counterbalanced by the poor degradability of lignin by biological processes. Consequently, microbial conversion of materials where lignin is abundant, such as straw and wood, to fuels is challenging. Pre-treatment of the biomass to separate lignin can be achieved through steam-explosion and acid hydrolysis but such methods are not economically feasible (Carere *et al.* 2008).

A cost-effective and promising alternative to circumvent this hurdle is gasification of the biomass in order to generate syngas, a gas mixture composed predominantly of carbon monoxide (CO), hydrogen (H_2) and carbon dioxide (CO_2). Syngas can be further converted, both by chemical or microbial processes, to several added-value compounds. Bioconversion processes have several advantages over catalytic processes: i) milder temperatures and pressures can be used, ii) no requisite of a fixed CO/H_2 ratio, iii) less susceptibility to impurities in the feed gas, and iv) no need for costly pre-treatment of the feed gas or expensive metal catalysts (Henstra *et al.* 2007, Abubackar *et al.* 2011) .

1.2 Syngas and microbial routes for syngas/CO conversion to fuels and chemicals

Gasification consists of a partial oxidation of the carbonaceous materials at elevated temperatures (700-1000 °C) with a controlled amount of oxygen and/or steam. Substrates for gasification include a wide array of carbon containing sources, such coal, cokes, sewage sludge, municipal wastes, lignocellulosic biomass, and reformed natural gas. Syngas is mainly composed by CO, H_2 and CO_2, but relative amounts of these compounds depend greatly both on the carbonaceous source used during gasification (vide Figure 1), as well as in the process parameters (*e.g.* gasifying agent, type of gasifier, etc). Syngas often contains minor amounts of other gases such as methane (CH_4), nitrogen (N_2) and hydrogen sulphide (H_2S) (Henstra *et al.* 2007).

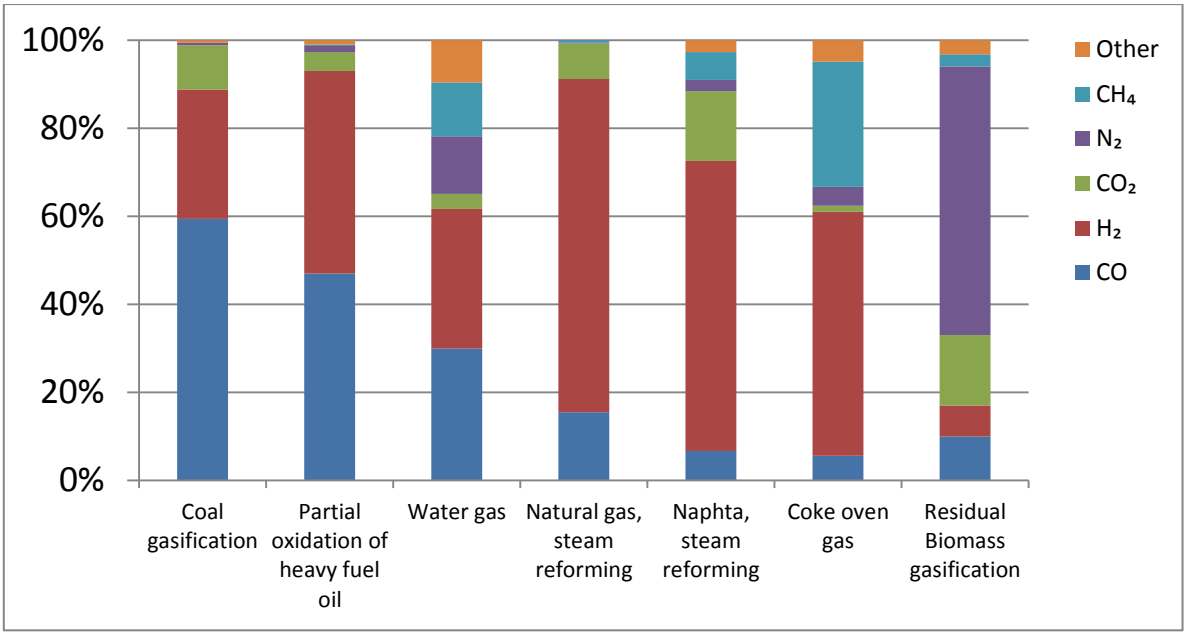

Figure 1 - Composition of syngas mixture depending on the source used for gasification (Sipma *et al.* 2006).

As can be expected, these variations in the composition of syngas are of major importance to determine its subsequent use. The presence of contaminants, such as H_2S can be hazardous to the chemical processes used in the downstreaming of gasification, even at trace amounts. Carbon monoxide, in particular, provides a technological obstacle due to its toxicity towards biological reductive processes and poisoning of chemical catalysts (Sipma *et al.* 2006).

While chemical methods to reduce CO levels in the syngas mixture have been developed (*e.g.* the water-gas-shift reaction), these typically occur at extremely high temperatures and require a relatively homogenous feed, thwarting the economic feasibility and therefore the attractiveness of the entire process.

Microbiological conversion of CO by carboxidotrophic organisms could thus offer a potential alternative route to circumvent these problems. Fermentation of syngas/CO into several of the same

compounds attained by chemical processes have been reported (*vide* Figure 2). Fermentative processes are less sensitive to the contaminants often present in syngas and to variations in its composition. This allows for the use of low grade synthesis gas coming from a wider array of feed sources.

Methane in particular offers an interesting route as a final end product of biological conversion of syngas. Methanogenesis can be performed with a variety of substrates including some of the products from other biological convertions of syngas (such as acetate, ethanol and hydrogen). This ability could be useful to metabolize remaining compounds that could not be extracted from previous convertions of syngas mixture. Consequently, biogas, a denser and less toxic gas mixture, can be easily derived from syngas. This biomethanation process can thus be particularly useful in aiding the transportation and usability of syngas (Techtmann *et al.* 2009).

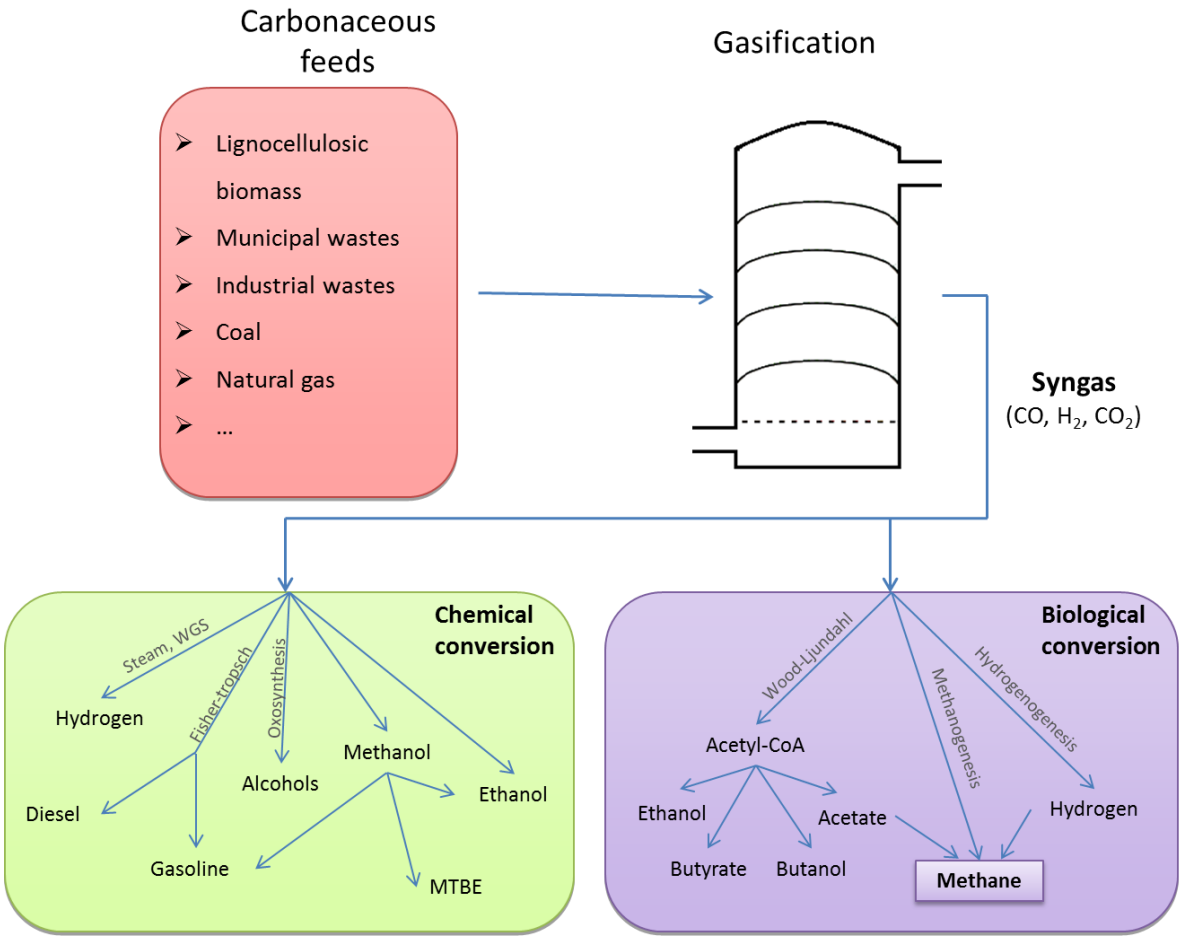

Figure 2 - Possible routes for the chemical and microbial conversion of syngas to fuels and chemicals.

Table 1 – Potential products obtained from CO or H_2/CO mixture

Product	Reaction	$\Delta G^{o\prime}$
		kJ mol CO^{-1}
Reactions from CO		
Methane	**$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$**	**−53**
Acetate	$4CO + 2H_2O \rightarrow CH_3COO^- + H^+ + 2CO_2$	−44
Butyrate	$10CO + 4H_2O \rightarrow CH_3(CH_2)2COO^- + H^+ + 6CO_2$	−44
Ethanol	$6CO + 3H_2O \rightarrow CH_3CH_2OH + 4CO_2$	−37
Formate	$CO + H_2O \rightarrow HCOO^- + H^+$	−16
Hydrogen	$CO + H_2O \rightarrow H_2 + CO_2$	−20
n-Butanol	$12CO + 5H_2O \rightarrow CH_3(CH_2)_3OH + 8CO_2$	−40
Reactions from H_2/CO		
Methane	**$CO + 3H_2 \rightarrow CH_4 + H_2O$**	**−151**
Acetate	$2CO + 2H_2 \rightarrow CH_3COO^- + H+$	−67
Butyrate	$4CO + 6H_2 \rightarrow CH_3(CH_2)2COO^- + H^+ + 2H_2O$	−80
Ethanol	$2CO + 4H_2 \rightarrow CH_3CH_2OH + H_2O$	−72
Methanol	$CO + 2H_2 \rightarrow CH_3OH$	−39
n-Butanol	$4CO + 8H_2 \rightarrow CH_3(CH_2)_3OH + 3H_2O$	−81

An emerging biotechnological solution for the production of electricity from biomass is the microbial fuel cell (MFC). This device harnesses the electrons and protons generated during oxidation of an electron donor (*e.g.* CO) in order to produce electrical current. Coupling of this technology to feeds of syngas and CO has been demonstrated in several studies (Kim and Chang 2009, Mehta *et al.* 2010, Hussain *et al.* 2011).

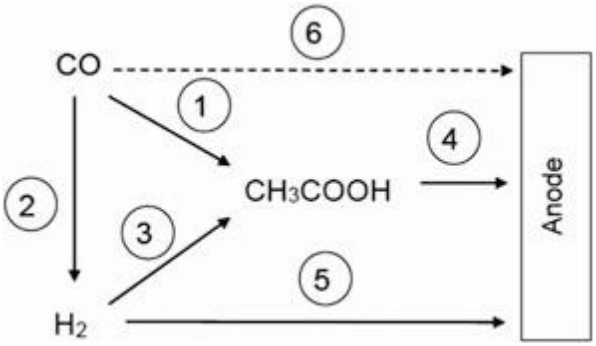

Figure 3 - Proposed pathways of electricity production from CO and syngas in an MFC (Mehta *et al.* 2010). (1) CO conversion to acetate by acetogenic carboxydotrophs; (2) CO conversion to H_2 by hydrogenogenic carboxydotrophs; (3) H_2 conversion to acetate by homoacetogens; (4, 5) acetate and H_2 consumption by electricigenic microorganisms; and (6) CO consumption by electricigenic carboxydotrophs (hypothesized).

1.3 Other applications of CO-oxidizing microorganisms

While the previously mentioned applications in the processing of gasification products towards biofuel and organic acids are one of the most promising and the driving force for the development of this dissertation, it should be mentioned that CO-utilizing microorganisms have also raised interest in other fields.

A promising prospect is the potential for bioremediation by other means than the aforementioned route of conversion of waste products through gasification. In theory, purified CO-converting enzymes could be used in bio-filters for reducing CO levels in heavily polluted areas. This would be particularly useful in enclosed areas such as underground car parks. Furthermore, due to the close relation of CO to CO_2, the methods of CO removal could, in theory be applied for removal of the CO_2 from the atmosphere where it acts as a greenhouse gas that is relatively inert (Ragsdale 2004). CO conversion mechanisms have also been associated to reductive dehalogenation and dechlorination being a potential basis for degradation of toxic compounds (Tiquia-Arashiro 2014).

Development of gas biosensors is another field of study where these organisms can play a pivotal role. Considering the overall toxicity of the gas towards several targets (*vide* section 1.5), reliable and efficient methods for its detection have considerable commercial interest. Currently, many CO detectors lack the specificity/accuracy required often resulting in false alarms or readings. Potential uses for CO biosensors include detection in confined spaces where CO is generated (mines, underground car parks, road tunnels, and industrial settings), as a control system for combustion (either in industrial setting or general fire alarms). (Colby *et al.* 1985).

1.4 Microbial metabolism of carbon monoxide

The first indications of microbial capability of metabolizing CO into cell material goes as far back as 1903 when thin films of bacteria were documented as growing in mineral media with coal gas mixture (mainly H_2 and CO) as potential carbon source. (Beijerinck and van Delden 1903)

In the following years, some studies were conducted that showed aerobic bacterial growth of isolated strains (*Actinobacillus oligocarbophilus*, *Actinobacillus paulotrophus* and *Actinomyces oligocarbophilus*) in the presence of CO but a rigorous confirmation for CO oxidation was lacking until 1953 in the work of Kistner with *Hydrogenomonas carboxydovorans* (Beijerinck and van Delden 1914, Lantzsch 1922, Mörsdorf *et al.* 1992).

While the number of organisms documented as capable of growth on CO as a sole carbon and electron source has grown substantially since, it is likely that the extent of carboxydotrophs is still grossly underestimated in literature. Use of this substrate often goes untested in growth studies and, due to initial inhibition imparted by CO, carboxidotrophic potential can be easily overlooked (Alves 2013).

The term carboxydotroph was first used in 1983 to describe utilization of CO as sole carbon and electron source during the study of chemolithoautothrophic aerobic respiratory organisms (Meyer and Schlegel 1983). Aerobic carboxydotrophy uses O_2 as a terminal electron acceptor. This type of CO-metabolism merely yields CO_2 and biomass which makes it less interesting from a biotechnological standpoint. Nevertheless, CO-utilizing microorganisms are taxonomically diverse, including also anaerobic bacteria and archaea (Figure 5 and Figure 6). Anaerobic CO conversion, can be coupled to a wider variety of acceptors (CO_2, sulphate, sulphur, thiosulphate and protons). These anaerobic respiration processes can thus be divided into hydrogenogenesis, desulfurication, acetogenesis, iron-reduction and methanogenesis (Figure 4) (Oelgeschlager and Rother 2008).

For carboxydotrophy to be accomplished, the organisms have to be able to perform 3 necessary steps: oxidation of CO into CO_2, utilization of the electrons derived from the previous oxidation for growth, incorporation of CO_2 into cell carbon. In addition, the microbe has to have the capacity for withstanding inhibition by CO.

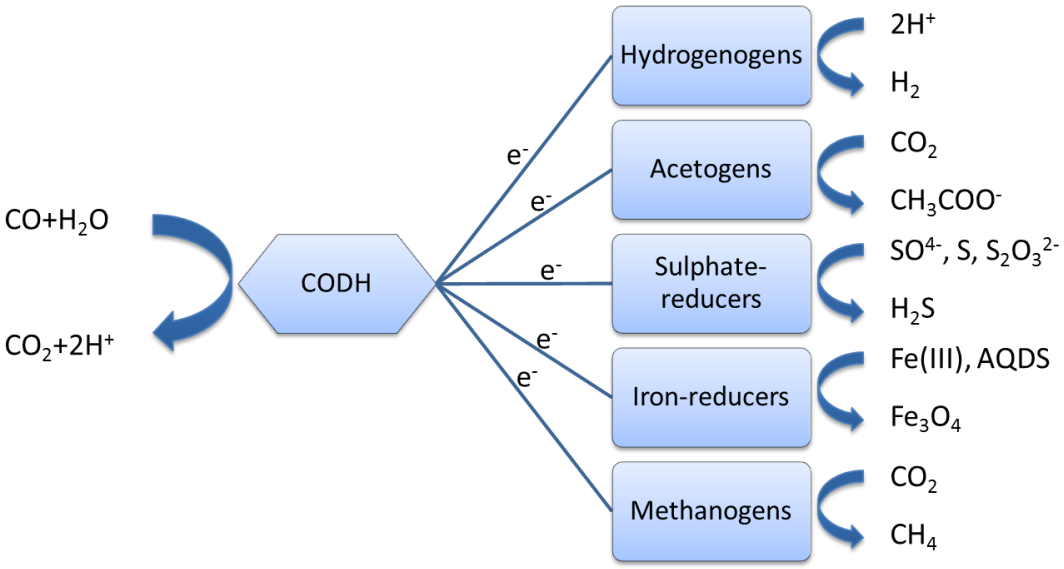

Figure 4 – Terminal electron acceptors in anaerobic carboxydotrophy.

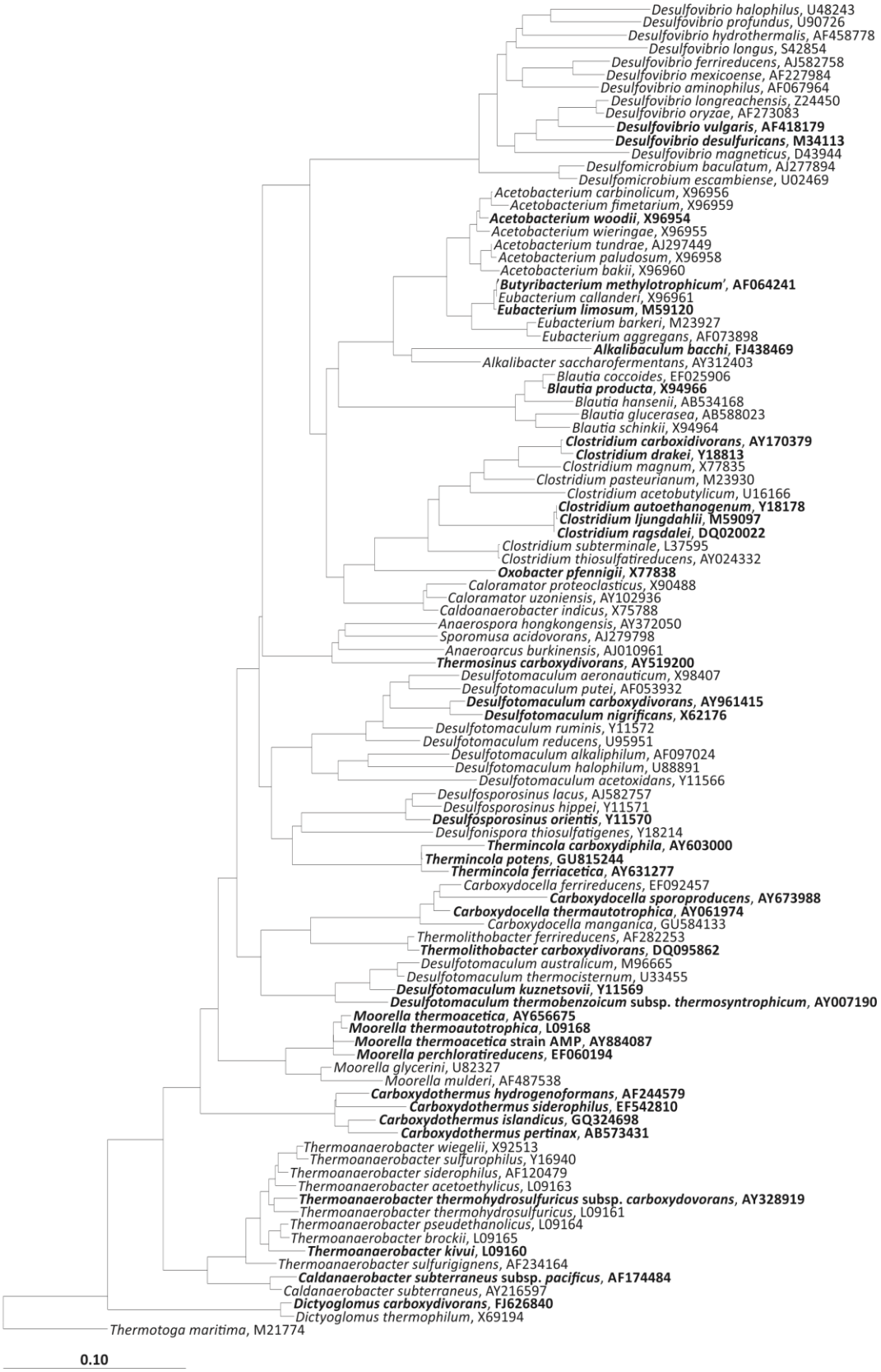

Figure 5 – Phylogenetic tree of anaerobic carboxidotrophic bacterial species. 16S rRNA gene sequenced-based tree was constructed using neighbor-joining methods. Branch length with 10% dissimilarity is represented in the scale bar. Known carboxydotrophs are indicated in bold.

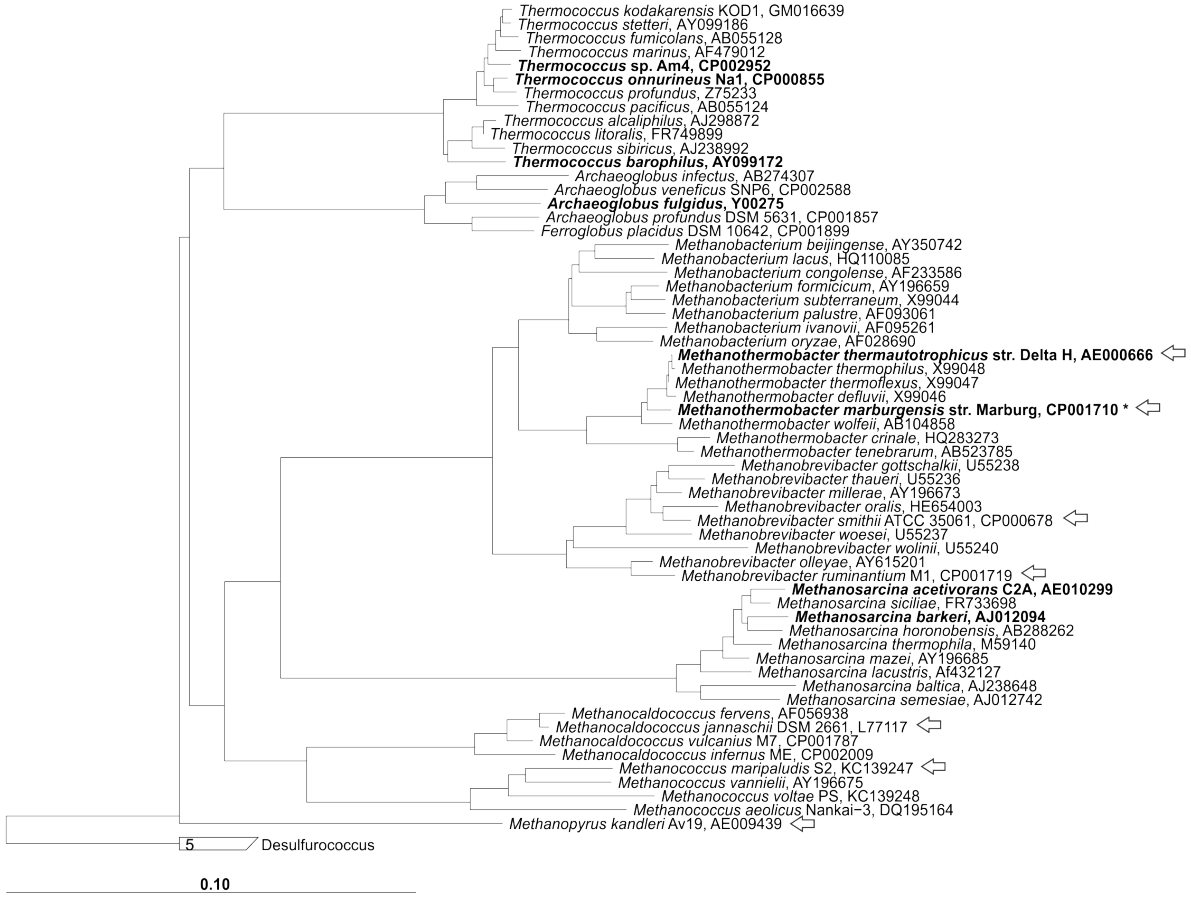

Figure 6 – Phylogenetic tree of anaerobic carboxidotrophic archeal species. 16S rRNA gene sequenced-based tree was constructed using neighbor-joining methods. Branch length with 10% dissimilarity is represented in the scale bar. Known carboxydotrophs are indicated in bold. * indicates information obtained in this thesis. Arrows evidence strains tested during this work.

1.4.1 Hydrogenogenic CO-oxidizers

The term hydrogenogenic was proposed by Svetlitchnyi to denominate organisms capable of producing H_2 from CO (Svetlitchnyi *et al.* 2001). This process occurs by oxidation of CO with protons derived from H_2O to form H_2 and CO_2 according to the following stoichiometry:

Equation 1 $$CO + H_2O \rightarrow CO_2 + H_2 \qquad \Delta G^0 = -20\ kJ/mol\ CO$$

Such reaction is the biological analogue of the water-gas-shift reaction used to chemically lower CO percentages in gas mixtures. The CO_2 generated is then partly processed into cell carbon by way of the Calvin Cycle (Dashekvicz and Uffen 1979).

This type of CO metabolism is shared by a wide array of phylogenetically diverse prokaryotes. However, analysis of the genes coding for CODH revealed a close similarity amongst the hydrogenogens suggesting that the ability for this type of carboxydotrophy was propagated through lateral gene transfer (Techtmann *et al.* 2009, Techtmann *et al.* 2012).

A prominent hydrogenogen is *Carboxydothermus hydrogenoformans*. This hydrogenogenic bacterium was first described in 1991 as an obligate carboxydotroph (Svetlichny *et al.* 1991) but has since been shown to be capable of utilizing a number of electron donors and acceptors (Henstra and Stams 2004). It can grow in the presence of 100% (v/v) CO and the robust growth presented by this organism in the gaseous substrate has made it one of the most well studied carboxydotrophs (Gonzalez and Robb 2000, Henstra and Stams 2004, Zhao *et al.* 2011, Wang *et al.* 2013).

A particular subset of hydrogenogens is composed by phototrophic bacteria. Members of this group of facultative anaerobes have been shown as capable to utilize CO in the dark. While phototrophic bacteria's tolerance for CO was known (Hirsch 1968) it was not until the work of Uffen that their use of CO as a sole carbon and energy source in the dark was detailed. *Rubrivivax* (formely *Rhodocyclus*) *gelatinosus* was shown as capable of growth in 100% CO (v/v) (Uffen 1976). Later, *Rhodospirillum rubrum* was also identified as capable of metabolizing CO into cellular material and energy. (Uffen 1981). Typically exhibiting low growth rates on CO, this group requires light to achieve optimum cell growth. For some strains, such as *Rhodopseudomonas palustris*, growth ceases in the dark even though hydrogenogenic CO-oxidation is occurring. This was interpreted as the cells only receiving maintenance energy in these conditions (Jung *et al.* 1999).

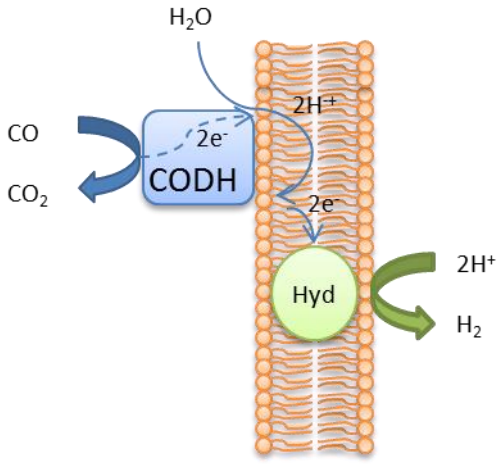

Figure 7 – Example of CO uptake in phototrophic bacteria as determined for *R. rubrum* (Ensign and Ludden 1991)

1.4.2 Acetogenic bacteria

Acetogenic bacteria are obligate anaerobes capable of producing acetate through a variety of sources, including CO_2 through the Wood-Ljungdahl pathway. In this well-studied pathway, CO_2 molecules are combined with Co-enzyme A to form acetyl Co-A by the CODH/ACS enzyme complex. It has also been observed that some acetogens can use CO directly in this pathway (Kerby *et al.* 1983, Ljungdahl 1986, Wood 1991).

Equation 2 $$4CO + 2H_2O \rightarrow CH_3COO^- + 2CO_2 + H^+ \Delta G^0 = -43.6\ kJ/mol\ CO$$

The first acetogens shown to oxidise CO were *Clostridium thermoaceticum* and *Clostridium formicoaceticum* (Diekert and Thauer 1978). The more recently studied *Clostridium carboxidovorans* can also produce substantial amounts of ethanol, butyrate and butanol from CO (Liou *et al.* 2005).

Other acetogenic bacteria include *Moorella thermoacetica* and *Moorella thermoautotrophica* that can grow on CO as sole energy source (Savage et al 1987; Daniel et al 1990).

The ability of acetogens to produce added-value compounds has made it one of the most promising for chemical and fuel production from syngas/CO.

Besides these *Clostridium* strains, *Acetogenium kivui* was discovered as being unable to utilise CO as electron donor but could use it as carbon source under the presence of H_2 (Daniel *et al.* 1990, Yang and Drake 1990).

However the record for highest tolerance and fastest growth belongs to *Peptostreptococcus productus strains* that have been reported as tolerating CO concentrations (v/v) of 90% (Lorowitz and Bryant 1984).

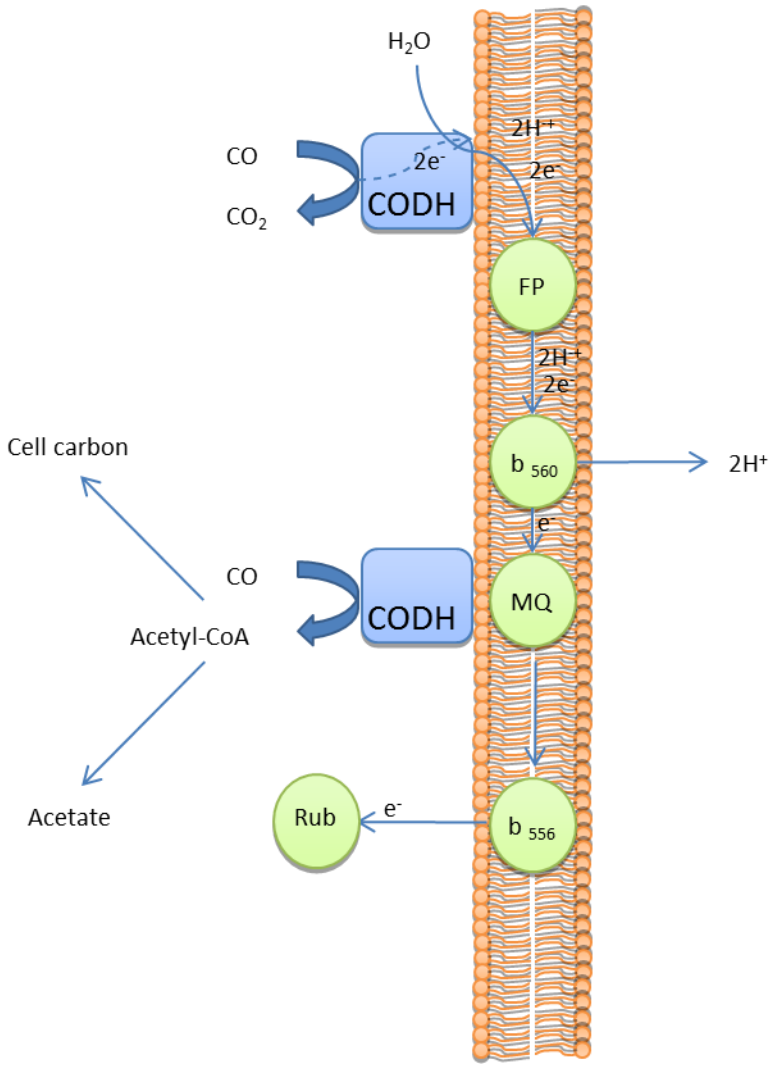

Figure 8 – Metabolism of CO oxidation in acetogenic bacteria.

1.4.3 Sulphate-reducing bacteria

Also counted within the obligate anaerobic bacteria are sulphate reducing bactéria (SRB). They can utilize this compound as an electron acceptor and that ability is spread thorough the SRB in phylogenetically diverse members.

Use of CO as a carbon source for this group was first recounted in 1958 by Yagi with the reaction being accomplished through the following stoichiometry (Yagi 1958, Yagi 1959, Yagi and Tamiya 1962, Thauer *et al.* 1977):

Equation 3 $$4\,CO + SO_4^{2-} + H^+ \rightarrow 4\,CO_2 + HS^- \; \Delta G^0 = -57.8\,kJ/mol\,CO$$

Later, in 1985, CODH from *Desulfovibrio desulfuricans* was characterized and a pathway for acetate degradation that involved CODH/acetyl-CoA synthase, which was demonstrated in

Desulfobacterium autotrophicum, was proposed shortly afterwards (Meyer and Fiebig 1985, Schauder *et al.* 1989).

Desulfovibrio barsii grew on formate and CO_2 in the presence of 1.5% (v/v) CO with removal of 15% of CO towards amino acid production (Jansen *et al.* 1984, Techtmann *et al.* 2009).

Furthermore, *Desulfovibrio vulgaris* (strain Madison) was shown to be capable of utilizing CO as electron donor when this gas was present in concentrations of up to 4% (v/v). This consumption of CO was followed by production of H_2 which was then consumed. However, concentrations above 4.5% were inhibitory (Lupton *et al.* 1984).

Similarly, growth on CO as only energy source at concentrations ranging between 5-20% was observed in *Desulfotomaculum orientis* and *D. nigrificans* with higher concentrations resulting in inhibition (Klemps *et al.* 1985).

Amongst the sulphate reducin microorganisms, *Desulfotomaculum carboxydivorans* is of particular importance since it can not only grow with a gas phase of 100% CO, but is also capable of growth without sulfate, essentially acting as a hydrogenogen (Parshina *et al.* 2005).

Other sulfate reducers, such as *Desulfotomaculum thermobenzoicum* and *Desulfotomaculum kuznetsovii*, not only produce H_2 (as an intermediary) but also acetate (Parshina *et al.* 2005).

Besides sulphate, elemental sulphur, dimethylsulfoxide and thiosulfate were shown to be reduced with CO in *Sulfospirillum carboxydovorans* (Jensen and Finster 2005).

1.4.4 Methanogens

Study on the CO as a substrate for this archaea group has not only trailed behind other substrates for methane production but also the current knowledge on other non-methanogenic species (Ferry 2010).

For methanogens, the first indication of their ability to metabolize CO can be found in the work of Fischer with sewage sludge that described the formation of methane from carbon monoxide with carbon dioxide being proposed as an intermediary. However the co-culture nature of the experiment meant that a syntrophic mechanism could not be discarded with other anaerobes producing methanogenic substrates from CO (Fischer *et al.* 1931, Fischer *et al.* 1932).

Stephenson and Stickland reported in 1933 reduction of CO to CH_4 by a pure culture in the presence of hydrogen according to Equation 4. This work was confirmed in 1947 with cell suspensions converting CO to CH_4 with H_2 as intermediary (Stephenson and Stickland 1933, Kluyver and Schnellen 1947).

Equation 4 $$CO + 3H_2 \rightarrow CH_4 + H_2O \;\; \Delta G^0 = -151\ kJ/mol\ CO$$

Decades later, in 1977, several methanogens were tested for their ability to remove CO from the headspace with the first report of a methanogen capable of using the gas as a sole carbon and energy source. In this study, a hydrogenotrophic methanogen (*Methanothermobacter*

thermautotrophicus strain ΔH) was described as converting CO to CO_2 and CH_4 as well as being capable of acting as electron donor to reduce F420. It was also noted that, despite its ability to grow on the gas, high concentrations of CO were inhibitory (with growth ceasing at concentrations above 60% (v/v)). Furthermore, even at the optimum concentrations of CO tested (30%), growth occurred at 100 times lower rates than with H_2. H_2-dependent growth was also highly affected at 40% CO suggesting inhibition of hydrogenases (Daniels *et al.* 1977).

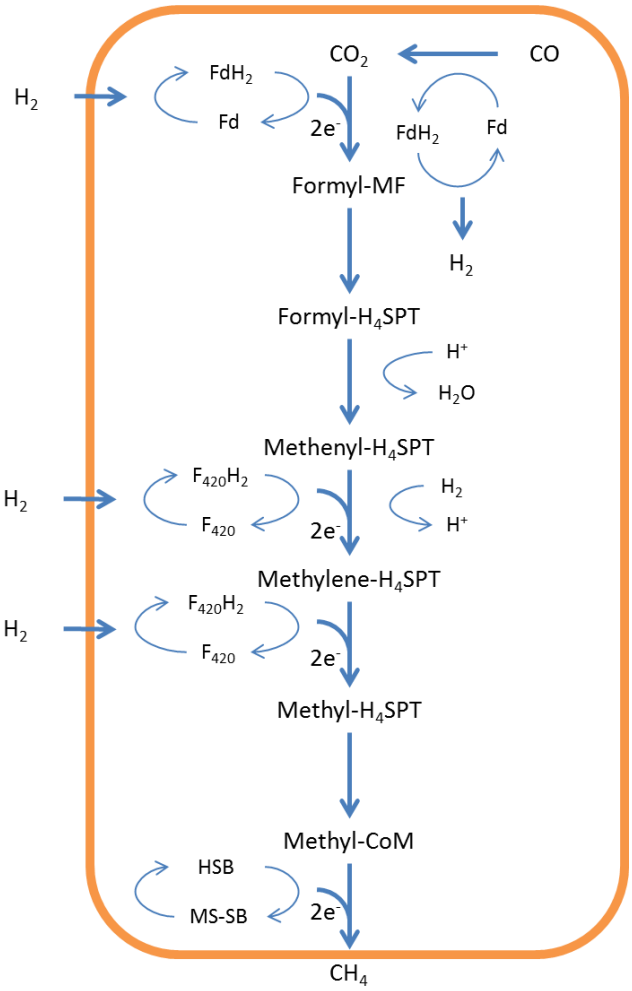

Figure 9 – Methanogenesis pathway in *M. thermautotrophicus* for growth with H_2 as electron donor. Putative pathway changes for growth on CO are indicated.

The proposed stoichiometry of the process followed equation Equation 5 (Daniels *et al.* 1977):

Equation 5 $$4\,CO + 2\,H_2O \rightarrow 3CO_2 + CH_4 \;\Delta G^0 = -52.6\;kJ/mol\;CO$$

In 1984, *Methanosarcina barkeri* strain MS, a methanogen isolated in 1966, was shown to be able to grow on an atmosphere of 50% CO using the gas as a source of energy and carbon. It was observed that H_2 began being detectable when the CO percentage was above 20% supporting the

idea of H_2 being formed as an intermediary in the reduction of CO to CH_4 with the hydrogenases being inhibited by high CO partial pressures in the headspace (Bryant and Boone 1987).

For this acetoclastic species, CO oxidation to CO_2 and H_2 is coupled with production of ATP with ferredoxin mediating electron flow from CODH to membrane bound hydrogenases. O'Brien et al. also detailed that *M. barkeri* was inhibited by the presence of high concentrations of CO (O'Brien *et al.* 1984, Bott *et al.* 1986).

It was, however, fairly recently that a *Methanosarcina* species was observed to grow on CO under conditions suitable for proliferation in the natural environment. The marine isolate, *M. acetivorans* was sequestered from sediments rich in decaying kelp in an environment containing up to 10% CO. The generation time is lower than in other methanogens and growth occurs at CO partial pressures above 1 atm. These attributes have made this *Methanosarcina*, the most well studied methanogen for carboxidotrophic growth (Sowers *et al.* 1984, Lessner *et al.* 2006, Rother *et al.* 2007).

Extensive proteomic analysis of CO metabolism in *M. acetivorans*, indicated a previously unknown pathway for CO-dependent methanogenesis. In the CO reduction pathway evidenced, H_2 does not function as an intermediate in the methanogenesis process which falls in line we the notion of the intolerance to CO observed being connected to the inhibition of hydrogenases (O'Brien *et al.* 1984, Lessner *et al.* 2006).

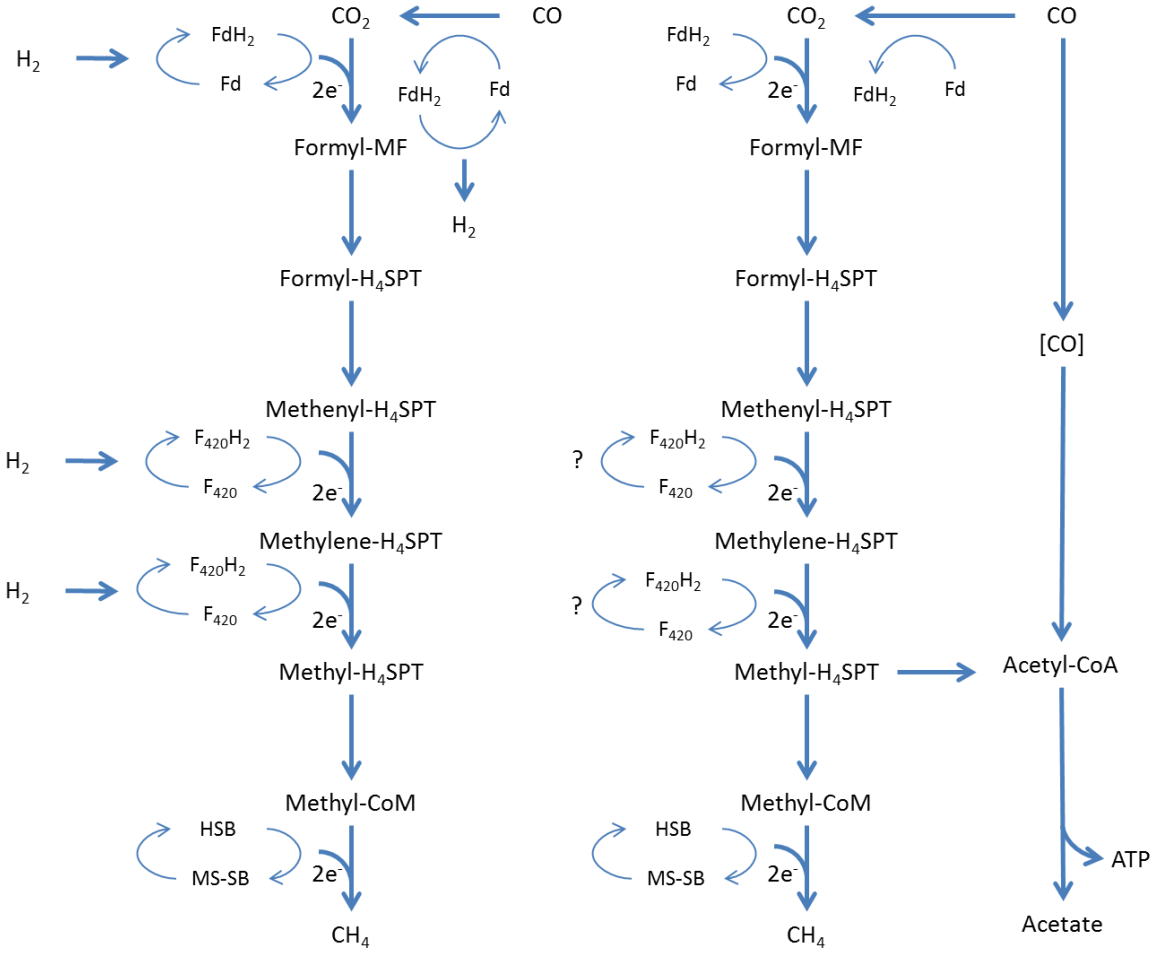

Figure 10 – Proposed pathway for CO-dependent methanogenesis in *M. barkeri* (left) and *M. acetivorans* (right) according to (Ferry and Lessner 2008).

1.5 CO toxicity towards microorganisms

Although CO functions as a metabolite in all three domains of life, it also possesses inherent toxic characteristics. As mentioned previously, CO can form stable complexes with the low valent states of the metal sites in metalloproteins. It is this property that makes it such a strong inhibitor of metalloenzymes such as hydrogenases and hemeproteins and explains its toxicity towards such a wide range of organisms (Ragsdale 2004).

In humans, it is produced as a neural signalling molecule during heme metabolism (Verma *et al.* 1993). It was also found that it has a role as a transcriptional regulator that controls human circadian rhythms (Dioum *et al.* 2002). However, by binding to hemeproteins, it can severally affect oxygen transport, causing several detrimental effects.

For bacteria, it is often present since it is produced in a number of metabolisms, such as amino acid synthesis (Ragsdale 2004).

In the Wood-Ljungdahl pathway, which can occur in both archaea and bacteria, CO functions as an intermediate metabolite (Ragsdale 2004).

According to a prominent hypothesis, at the time of the origin of life, the CO presence in the Earth atmosphere was quite significant. This might help explain the diverse range of organisms found that possess CO metabolizing capabilities.

1.6 Biochemistry of CO oxidation

As previously discussed, several phylogenetically diverse organisms developed the ability to use CO as a metabolic building block.

Common to all different pathways mentioned is the presence of a specific enzyme, CO dehydrogenase (CODH). CODH allows for the interconversion of CO and CO_2, accompanied by the generation of high energy electrons according to Equation 6. CODH is capable of using several different electron acceptors (e.g ferredoxin, flavodoxin, cytochromes)

Equation 6 $$CO + H_2O \leftrightarrow 2\,e^- + 2\,H^+ + CO_2$$

Such an enzyme was first proposed by Yagi with it being identified in acetogenic bacteria and purified later to homogeneity (Yagi 1959, Diekert and Thauer 1978, Ragsdale *et al.* 1983).

These metalloenzymes are the basis of CO metabolism focused in this study. The reaction allows the CO to be used as a carbon source by means of one of the CO_2 fixing pathways.

With regards to the metal present in the active site of CODH, two types can be distinguished, molybdenum containing CODHs (Mo-CODH) and nickel-iron containing CODHs ([Ni,Fe]-CODH).

Mo-CODHs are not oxygen sensitive and are characteristic of aerobic carboxydotrophy. They are characterized by having high Michaelis constant (K_m) values but low turnover rates when compared to the CODH found in anaerobes. This makes them quite capable of uptake of CO for detoxification purposes, even at trace levels, but not extremely efficient at growth in the substrate (Ragsdale 2004).

On the other hand, [Ni,Fe]-CODHs are the backbone of anaerobic CO-oxidation focused in this dissertation. Analysis of currently available genomic sequence databases reveals their presence in a substantial number of bacterial and archaeal genomes (upwards of 6% percent). Furthermore, several carboxydotrophs display multiple CODH coding genes with *Carboxydothermus hydrogenoformans* and *Methanosarcina acetivorans* topping the list with 5 occurrences (Techtmann *et al.* 2009, Techtmann *et al.* 2012).

Often coupled to CODH, acetyl-CoA synthase (ACS) also plays a key role by combining either free or molecular bound CO with a methyl group and Co-enzyme A to form acetyl CoA, a noteworthy base molecule in cell metabolism originating both cell material and ATP. The association of CODH with ACS in one structure, allows for the generation and consumption of CO in active sites that are in close proximity (70 Å) thus potentially reducing the effects of CO toxicity. This multi-enzyme complex organisation is commonly found in certain groups of prokaryotes including methanogens (Ragsdale 2004, Techtmann *et al.* 2012).

1.7 Genomic and proteomic insights

With the expansion of the knowledge base regarding molecular phenomena behind cell life of microorganisms, increasing amounts of information can be derived from sources beyond physiological observations of cultured organisms.

Molecular biology of microorganisms starts at the DNA level which encompasses the sum of all genetic directives for cell function. These molecules can be transcribed into messenger RNA, that in turn is translated into proteins. The latter are then responsible for most cellular functions including the synthesis of base components for the aforementioned biomolecules (Figure 11).

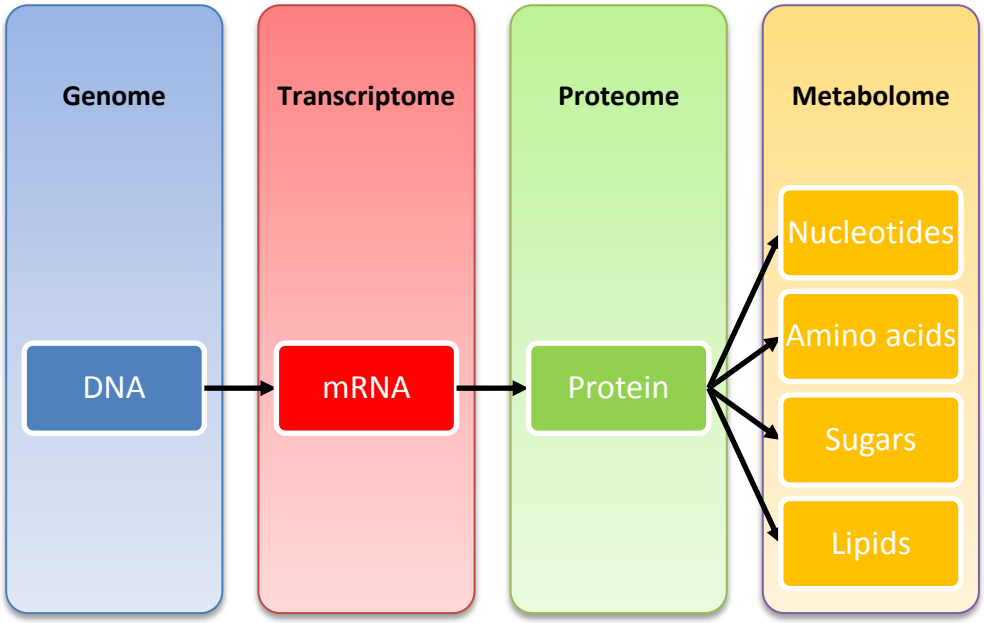

Figure 11 – Genomic, transcriptomic, proteomic and metabolomic approaches that can be used for the study of microbial metabolism.

1.7.1 Genomics

Genomic approaches to the study of microorganisms can be a valuable tool for acquiring a full overview of their functional potential. A significant amount of information can be derived from the detailed analysis of the genome of specific organisms such as the ability to metabolize previously untested substrates. Furthermore, comparison of gene sequences are the basis of the construction of phylogenetic trees.

The first archaeon with a fully sequenced genome was *M. jannaschii* in 1996 (Bult *et al.* 1996). Since then, a considerable number of archaeal species have had their genome sequenced.

Analysis of microbial genomes have demonstrated that CODH is ubiquitous amongst both bacterial and archaeal species. Extensive comparative genomic examination of the CODH coding sequences and their neighbourhood has allowed for some interesting insights into this protein complex

phylogeny bringing evidence of evolution of CODH by both vertical transmission and horizontal gene transfer (Techtmann *et al.* 2012).

1.7.2 Transcriptomics

Transcriptomic analyses consist in the observation of the expression level of genes in a given environment. It can provide crucial insight into the response mechanisms of a given organism since only expressed genes are being analysed.

Studies conducted for the transcriptome of several species subjected to the presence of CO have given information on gene clusters induced by this substrate. Unsurprisingly, these include the cox and cdh operons coding for Mo-CODH and Ni,Fe-CODH respectfully (Santiago *et al.* 1999, Matschiavelli *et al.* 2012). In addition, transcriptomic data performed on methanogens have shown differences of the induction of genes related to methanogenesis (Lessner *et al.* 2006, Zhou *et al.* 2013).

Furthermore, comparative observation of the transcriptome under high and low levels of H_2, such as the study conducted for *M. thermautotrophicus*, provide clues about alternative pathways that can be used under H_2-independent growth (de Poorter *et al.* 2007).

1.7.3 Proteomics

Despite all the data that can be derived from the transcriptome, mRNA molecules are relatively unstable when compared to proteins and not all are necessarily transcribed. Furthermore, post-translational modifications can occur and protein abundance levels are not necessarily strictly paralleled by the amount of corresponding mRNA present. These characteristics limit the usefulness of this approach for accurate determination of the current functions displayed by the cell in response to specific stimuli. Within that context, proteomic analysis can be of paramount importance to further expand the current understanding of cell biology.

Traditionally, proteomic analysis is conducted through protein separation by two-dimensional gel electrophoresis, a technique first demonstrated in 1975 (O'Farrell 1975). High-efficiency chromatographic peptide separation, together with mass spectrometry has advanced significantly this field of study allowing for better protein identification. Together with the development of robust bioinformatics tools, this method allows for a more reliable, less labour intensive and higher throughput analysis (Maron *et al.* 2007).

In relation to the known carboxidotrophic methanogens, adequate proteomic analysis comparing growth on CO to other substrates has only been performed for *M. acetivorans*. These studies evidence a novel pathway for methanogenesis in CO-grown cells (Rother *et al.* 2007).

Molecular biology techniques are being developed at an accelerated rate. These methods combined with potent bioinformatics tools provide previously unworkable approaches to understand cell life. Consequently, analysis of the genome and proteome are now routinely used to provide further insights in functions and interactions within microbial communities and to predict environmental responses. Moreover, the combination of the different approaches have the potential for further elucidating complex biological phenomena and reveal previously unidentified connections between biochemical processes and biological functions.

2 Materials and methods

2.1 Comparative genomic analysys for selection of candidates

Comparative genomic analysis for selection of candidates was performed using the tools available at the IMG/JGI website (Markowitz *et al.* 2014)

2.2 Source of microorganisms

Microorganisms used in this work (Table 1) were obtained from the Leibniz Institute DSMZ – German collection of Microorganisms and Cell Cultures.

Table 2 - Methanogenic microorganisms used in this work

Code	Name	DSM Id	pH_{opt}	t_d (h)	T_{opt} (°C)
mja	*Methanocaldococcus jannaschii*	DSM2661	6.0	0.433	85
mka	*Methanopyrus kandleri* AV19	DSM6324	6.5	0.83	98
mmg	*Methanothermobacter marburgensis*	DSM2133	6.8-7.4	----	65
mth	*Methanothermobacter thermautotrophicus* ΔH	DSM1053	7.2-7.6	1.486	65
mmp	*Methanococcus maripaludis* S2	DSM14266	6.8	0.667	37
msi	*Methanobravibacter smithii PS*	DSM861	6.9-7.4	-----	38
mru	*Methanobrevibacter ruminantium*	DSM1093	6.3-6.8	-----	33

Cultures were grow in 120 mL pressurized serum bottles with a liquid phase of 50 mL (unless otherwise stated) under strictly anaerobic conditions based on the principles established by Hungate and improved by Bach and Wolf (Hungate 1950, Hungate and Macy 1973, Balch and Wolfe 1976)

Methanothermobacter thermautotrophicus and *Methanothermobacter marburgensis* were incubated at 65 °C with and without shaking (70 rpm) as specified. *Methanocaldococcus jannaschii* was grown at 85°C with 100rpm shaking and *Methanococcus maripaludis* were grown at 85 °C, 120 rpm and 37 °C, 100 rpm respectively.

2.3 Medium composition and cultivation

Methanothermobacter thermautotrophicus, *Methanothermobacter marburgensis, Methanobrevibacter smithii* and *Methanobrevibacter ruminantium*, were grown in 50 mL of bicarbonate buffered mineral salts medium containing the following per litre: 0.408g KH_2PO_4, 0.534g $Na_2HPO_4.2H_2O$, 0.3g NH_4Cl, 0.3g NaCl, 0.1g $MgCl_2.6H_2O$, 0.11g $CaCl_2.2H_2O$, 4g $NaHCO_3$, 0.24g

$Na_2S.9\ H_2O$ and 0.5g of yeast extract, 0.0005g of resazurin, Trace elements H^+ trace elements OH^- and vitamins. The pH of finished media was around 7. The headspace was composed either of H_2/CO_2 (80:20) or N_2/CO_2 (80:20) and increasing amounts of CO as specified.

Medium for *Methanocaldococcus jannaschii* was composed of: 0.14g K_2HPO_4, 0.534g $Na_2HPO_4.2H_2O$, 0.25g NH_4Cl, 30g NaCl, 4.1g $MgCl_2.6H_2O$, 0.14g $CaCl_2.2H_2O$, 1g $NaHCO_3$, 0.24g $Na_2S.9\ H_2O$ and 0.5g of Yeast Extract, 0.0005g of Resazurin, 1ml of trace elements H+, 1ml trace elements OH- and vitamins. The pH of finished media was around 7. The headspace was composed either of H_2/CO_2 (80:20) or N_2/CO_2 (80:20) and increasing amounts of CO as specified.

Methanococcus maripaludis was inoculated in medium with the following components per litre: 0.34g KCl, 0.14g K_2HPO_4, 0.25g NH_4Cl, 18g NaCl, 4g $MgCl_2.6H_2O$, 3.45g $MgSO_4.7H_2O$, 2g $Fe(NH_4)_2(SO_4)_2.6H_2O$, 1g $NaCH_3CO_2$, 0.11g $CaCl_2.2H_2O$, 5g $NaHCO_3$, 0.50g $Na_2S.9\ H_2O$ and 0.5g of yeast extract, 0.0005g of resazurin, Trace elements H+ trace elements OH- and vitamins. The pH of finished media was around 7. The headspace was composed either of H_2/CO_2 (80:20) or $N2/CO_2$ (80:20) and increasing amounts of CO as specified at a total pressure of 1.7 bar.

Methanopyrus kandleri medium has the following components per litre: 0.07g K_2HPO_4, 0.534g $Na_2HPO_4.2H_2O$, 0.25g NH_4Cl, 11.8g NaCl, 4.5g $MgCl_2.6H_2O$, 0.78g $CaCl_2.2H_2O$, 1g $NaHCO_3$, 1g $Na_2S.9\ H_2O$ and 0.0005g of Resazurin, 1ml of trace elements H^+, 1ml trace elements OH^- and vitamins. The pH of finished media was around 6.5. The headspace was composed either of H_2/CO_2 (80:20) or N_2/CO_2 (80:20) and increasing amounts of CO as specified.

The acid trace elements solution used for all three media contained 1.8g HCl, 61.8 mg H_3BO_3, 61.25 mg $MnCl_2$, 943.5 mg $FeCl_2$, 64.5 mg $CoCl_2$, 12.86 mg $NiCl_2$, 67.7 mg $ZnCl_2$ per litre. Alkaline trace elements solution was composed of 400 mg NaOH, 17.3 mg Na_2SeO_3, 29.4 mg Na_2WO_4, 20.5 mg Na_2MoO_4 per litre. Vitamins used were 20 mg Biotin, 200 mg Nicotinamid, 100 mg p-Aminobenzoic acid, 200 mg Thiamin (Vit B1), 100 mg Panthotenic acid, 500 mg Pyridoxamine, 100 mg Cyanocobalamine (Vit B12) and 100 mg Riboflavine.

Table 3 – Operational conditions

Code	Shaking (rpm)	pH	T (°C)
mru	100	7.0	37
msi	100	7.0	37
mja	120	6.0	85
mka	--	6.5	98
mmg	70	7.0	65
mth	70	7.0	65
mmp	100	7.0	37

2.4 Adaptation studies

Adaptation studies were performed in 120 ml pressurized serum bottles with increasing amounts of CO in the headspace according to schematic in Figure 12 and aforementioned growth conditions. Cultures were routinely transferred (2% v/v of inoculum/media ratio) whenever all available electron donors were depleted or when CO was no longer being removed from the headspace for a considerable amount of time. Whenever robust growth and/or consumption of CO were detected in a syngas mixture, culture was also transferred into bottles with higher percentages of CO and bottles without H_2 in the headspace. If growth was successful in these conditions, subsequent transfers maintained the gas ratio.

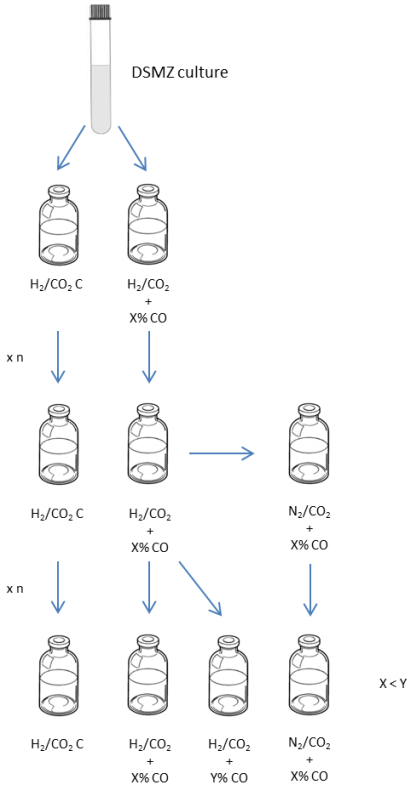

Figure 12 – Schematic of adaptation protocol

2.5 Microscopy

Cultures were regularly observed by phase contrast microscopy using a Leica DM 2000 microscope equipped with 100X magnification objective and a Leica DMC 2900 camera.

For fluorescense microscopy, a Leica DMR microscope equipped with a Leica DFC340FX camera was used. Fluorescence was observed utilizing a LEJ EBQ 100 Isolated Z Ballast Illuminator Mercury lamp for UV excitation.

2.6 Analytical techniques

2.6.1 GC measurements

Variations on the concentration of gaseous compounds (H_2, CH4, CO and CO2) in the cultures' headspace were followed by gas chromatography using a GC-2014 from Shimadzu equipped with a thermal conductivity detector and possessing two separate measuring columns. H_2, CH_4 and CO were measured in a Molsieve 13X column with dimensions of 2m in length and 3mm of inner diameter. Argon and helium were used as carrier gases at a flow rate of 50 ml/min and the injector, column and detector were set to 80, 60 and 130°C, respectively. CO2 was assayed separately in a CP Paraplot column of 25 m x 0.53 mm, with a stationary phase film thickness of 20 µm, employing helium as carrier gas at a flow rate of 15 ml/min. For this column, the temperatures used were of 60 °C for the injector, 34°C for the column and 130°C for the detector.

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2.6.2 HPLC measurements

Soluble compounds were determined using LKB high-performance liquid chromatograph (HPLC) with a Varian Metacarb 67H 300mm column. Mobile phase used was 0.01N of sulfuric acid at a flow rate of 0.8 ml/min and column temperature was set to 60 °C.

2.7 Protein Extraction for LC-MS/MS analysis

For protein extration cells were cultured in 1 litre anaerobic bottles and harvested at late exponential phase. Cultures were centrifuged and pellet was resuspended in TE buffer (10 mM Tris-Cl, pH 7.5; 1 mM EDTA). Protein extraction was then attempted by two methods:

- Centrifugation and resuspension of pellet in SDT lysis buffer (100mM Tris -Cl pH 7.5; 4% sodium dodecyl sulphate ; 0.1M dithiotreitol) followed by sonication cycles on 30s with 30 second intervals;

- Resuspending centrifuged pellet with TE buffer with phenylmethanesulfonylfluoride, and passing cell suspensions through a French pressure cell operated at 138 megapascals.

Proteins were stabelized by addition of 8M of urea in a proportion of 1:1 and samples were concentrated using a 3.5 kDa MWCO filter.

Final protein concentration in samples obtained for LC-MS/MS analysis was assessed using several methods including Qubit® Protein Assay Kit in a Qubit® 2.0 Fluorometer (Life technologies), 2D-Quant kit (Amersham Biosciences) and BCA protein assay kit (Pierce).

2.8 LC-MS/MS analysis

Samples for LC-MS/MS analysis were sent to be analysed by the Proteomics centre in Radboud University of Nijmegen.

Experiments were performed on a 7-tesla Finnigan LTQ-FT mass spectrometer (Thermo Electron) equipped with a nanoelectrospray ion source (Proxeon Biosystems, Odense, Denmark). The LC part of the analytical system consisted of an Agilent Series 1100 nanoflow LC system (Waldbronn, Germany) comprising a solvent degasser, a nanoflow pump, and a thermostated microautosampler. Chromatographic separation of the peptides was performed in a 15-cm fused silica emitter (100-µm inner diameter; New Objective) packed in-house with methanol slurry of reverse-phase ReproSil-Pur C18-AQ 3-µm resin (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) at a constant pressure (20 bars) of helium. Then 5 µl of the tryptic peptide mixtures were autosampled onto the packed emitter with a flow of 600 nl/min for 20 min and then eluted with a 5-min gradient from 3 to 10% followed by a 25-min gradient from 10 to 30% acetonitrile in 0.5% acetic acid at a constant flow of 300 nl/min. The mass spectrometer was operated in the data-dependent mode to automatically switch between MS and MS/MS acquisition. Survey MS spectra (from m/z 350 to 2,000) were acquired in the FTICR with r = 50,000 at m/z 400 (after accumulation to a target value of 1,000,000). The three most intense ions were sequentially isolated for fragmentation in the linear ion trap using collisionally induced dissociation with normalized collision energy of 29% and a target value of 1,000. Former target ions selected for MS/MS were dynamically excluded for 30 s. Total cycle time was ~3 s.

Proteins were identified via automated database searching (Matrix Science, London, UK) of all tandem mass spectra against both NCBInr and an in-house curated M. thermautotrophicus database. Carbamidomethylcysteine was set as fixed modification, and oxidized methionine and protein N-acetylation were searched as variable modifications. Initial mass tolerances for protein identification on MS and MS/MS peaks were 10 ppm and 0.5 Da, respectively. The instrument setting for the MASCOT search was specified as “ESI-Trap.”

3 Results and Discussion

The main objective of this work was to get insight on methane metabolism of carboxidotrophic archaea, combining physiological studies with genomic and proteomic approaches. The work consisted of 3 main stages:

- Genomic screen of methanogens for CO-dehydrogenases (CODH) genes - selection of methanogens containing these genes for further physiological tests

- CO conversion tests and adaptation studies - incubation of the selected methanogens with CO and CO/H2

- Cultivation and protein extraction methods for (improved) LC-LC MS/MS-based proteomics of CO-converting methanogens.
  Note: Proteomics analysis is not performed at our lab; proteins were sent for analysis to an external company but, due to time restrictions, it was not possible to have the results on time to include on this thesis.

3.1 Selection of methanogenic candidates

3.1.1 Genomic comparison of methanogens for potential carboxidotrophic abilities

As previously mentioned, the number of methanogens known to be capable of growth using carbon monoxide as sole carbon and energy source is relatively low, consisting of only 4 so far - *Methanosarcina barkeri, Methanosarcina acetivorans, Methanothermobacter thermautotrophicus* strain ΔH, and *Methanobrevibacter arboriphilicus*. *M. barkeri* and *M. acetivorans* have recently been the subject of several studies, including very detailed proteomic analysis of the mechanisms behind carboxydotrophy for the latter (Lessner *et al.* 2006, Maeder *et al.* 2006, Rother *et al.* 2007). From the other two methanogens, only *M. thermautotrophicus* strain ΔH has a fully annotated genome publically available at present. Even though *M. thermautotrophicus* was the first methanogen identified as capable of growth on CO, a thorough proteomic analysis related to the use of this substract has yet to be performed for this species. Nevertheless, in the context of biological biomethanation of syngas with high CO percentages, this is a promising focus group for the further understanding of carboxydotrophy (Daniels *et al.* 1977, Alves 2013, Markowitz *et al.* 2014)-.

To eventually perform an adequate proteomic analysis for carboxydotrophy, an annotated genome sequence is advantageous. Therefore, the first step of this work was to canvas the JGI database for methanogens that possess a finished version of their genome. This selection resulted in a total of 51 archaeal candidates.

While the full requirements for carboxydotrophy remain unknown, the presence of carbon monoxide dehydrogenase (CODH) is naturally thought of as a key factor due to its known ability to convert CO into CO_2, the initial substrate in hydrogenotrophic methanogenesis. Hence, an initial assessment of the presence of this protein complex was performed. For this purpose, the alpha subunit was chosen since it contains within its structure the catalytic site for this enzyme. Such analysis further reduced the previous list to 37 potential candidates.

To further select for methanogenic candidates for hydrogenotrophic carboxydotrophy, the physiological and genomic data available for *M. thermautotrophicus* was used as a basis for comparison with other hydrogenotrophic methanogens. This archaeon possesses one copy of CODH in its genome (vide Figure 13). For homologue comparison of the putative carboxydotrophs, the alpha subunit of the CODH protein complex was selected. This choice was due to the localization of the catalytic centre within the structure of this subunit. The full results from this alignment can be found on Table 8 in Annex I. A selection is presented in Figure 13 in the form of a phylogenetic tree.

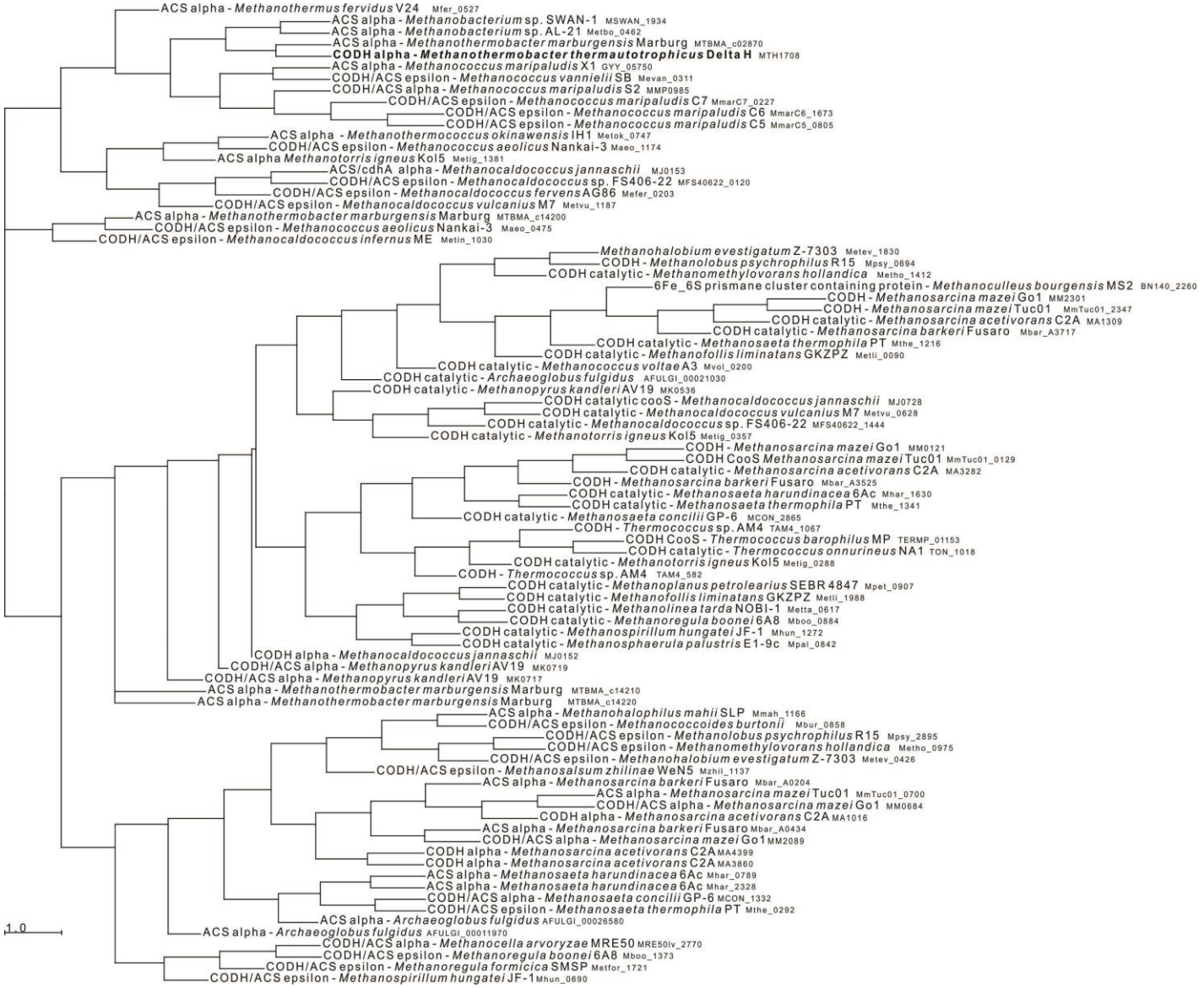


Figure 13 – Tree based on the CODH catalytic subunit in methanogens.

Looking at the sequence alignment results, *M. marburgensis* rises above the rest as the methanogen that possesses a CODH/ACS alpha subunit with the closest similarity to the one from *M. thermautotrophicus* with an identity value of 93.7% (well above the second closest hit for *Methanobacterium* sp. SWAN-1 with 73.5%). Furthermore, the genome of this archaea seems to code for an additional set of catalytic subunits for CODH. This makes it a prime candidate for testing for carboxydotrophy in this study.

This comparison is being performed with a CODH/ACS alpha subunit type enzyme since is the one present in *M. thermautotrophicus*. However there are other types of catalytic CODH subunits that can be present as discussed below.

3.1.2 Further selection by literature search

While the full scale analysis of the relatedness of the genome/CODH genes between the known carboxydotroph (*M. thermautotrophicus*) provides crucial data for the selection, further refinement of the list was needed. With that purpose, an extensive literature search for promising physiological observations was conducted.

For *M. marburgensis,* the species on the top of the previously mentioned list, some studies regarding the metabolism of CO had been conducted but with growth on this substrate alone being unsuccessful. However it was recently noted that methyl-coenzyme M reductase (MCR) synthesis was induced by the presence of CO. MCR is the enzyme involved in the last step of methanogenesis (*vide* Figure 15) and thus one would expect it to be induced in conditions where this pathway could be performed. This observation further supports *M. marburgensis* status as a prime testing candidate (Zhou *et al.* 2013).

Due to its status as the first fully sequenced archaeon, *M. jannaschii* has been the target of several comparative genomic studies, including an in-depth side by side analysis with *M. thermautotrophicus*. This study detailed the key similarities and differences between the methanogenic metabolisms of the two. Looking at the genome of this species, it not only codes for the CODH/ACS alpha subunit that was previously mentioned, but also for a CooS type CODH catalytic subunit which could be favourable towards its carboxidotrophic potential. Furthermore, proteomic analysis of methanogenesis (using H_2 as electron donor) has been conducted (Bult *et al.* 1996, Tersteegen and Hedderich 1999, Zhu *et al.* 2004, Kaster *et al.* 2011).

Methanococcus maripaludis S2 also possesses a relatively high sequence similarity with *M. thermautotrophicus* alpha subunit CODH. A recent study tested for growth with CO and formate but there is no indication of what would happen with only CO as an electron donor. Considering the already identified use of CO as a carbon source and the ability to perform the methanogenesis by an H_2-independent pathway, the potential for growth on this substrate alone is considerable (Costa *et al.* 2013).

Out of all methanogens whose carboxidotrophic ability has yet to be proven, *M. kandleri* AV19 seems to be one with the highest number of diverse CODH coding regions in its genome. The

conducted analysis showed already two dissimilar copies of CO dehydrogenase/acetyl-CoA synthase alpha subunit inside the same operon but another type of CODH can also be found: a cooS type CODH. This totals 3 CODH coding regions which make this hyperthermophile second only to *M. acetivorans*, the carboxidotrophic methanogen that presents the fastest growth on CO with 5 different CODH coding regions (Rother and Metcalf 2004, Techtmann *et al.* 2012).

Finally, in the same study that first described *M. thermautotrophicus* ability to grown on CO as sole carbon and energy source, several other organisms were briefly mentioned as removing CO from the headspace (in the presence of H_2). Amongst them were *Methanobrevibacter smithii* and *Methanobrevibacter ruminantium*. Considering their absence from the above mentioned list, this might seem surprising. Their genome does not seem to code for a CODH/ACS enzyme and therefore, considering the premise used that CODH is a necessary requirement for the conversion of CO, such results seem contradictory. Therefore, these two organisms were added to the already selected candidates as a counterpoint to this assumption (Daniels *et al.* 1977).

3.1.3 Final candidate list for physiological tests

In conclusion, this analysis yielded the candidate list presented in Table 4. As can be easily perceived through that list, there is a wide variety of optimum growth temperatures and the set includes three mesophiles, two thermophiles and two hyperthermophiles.

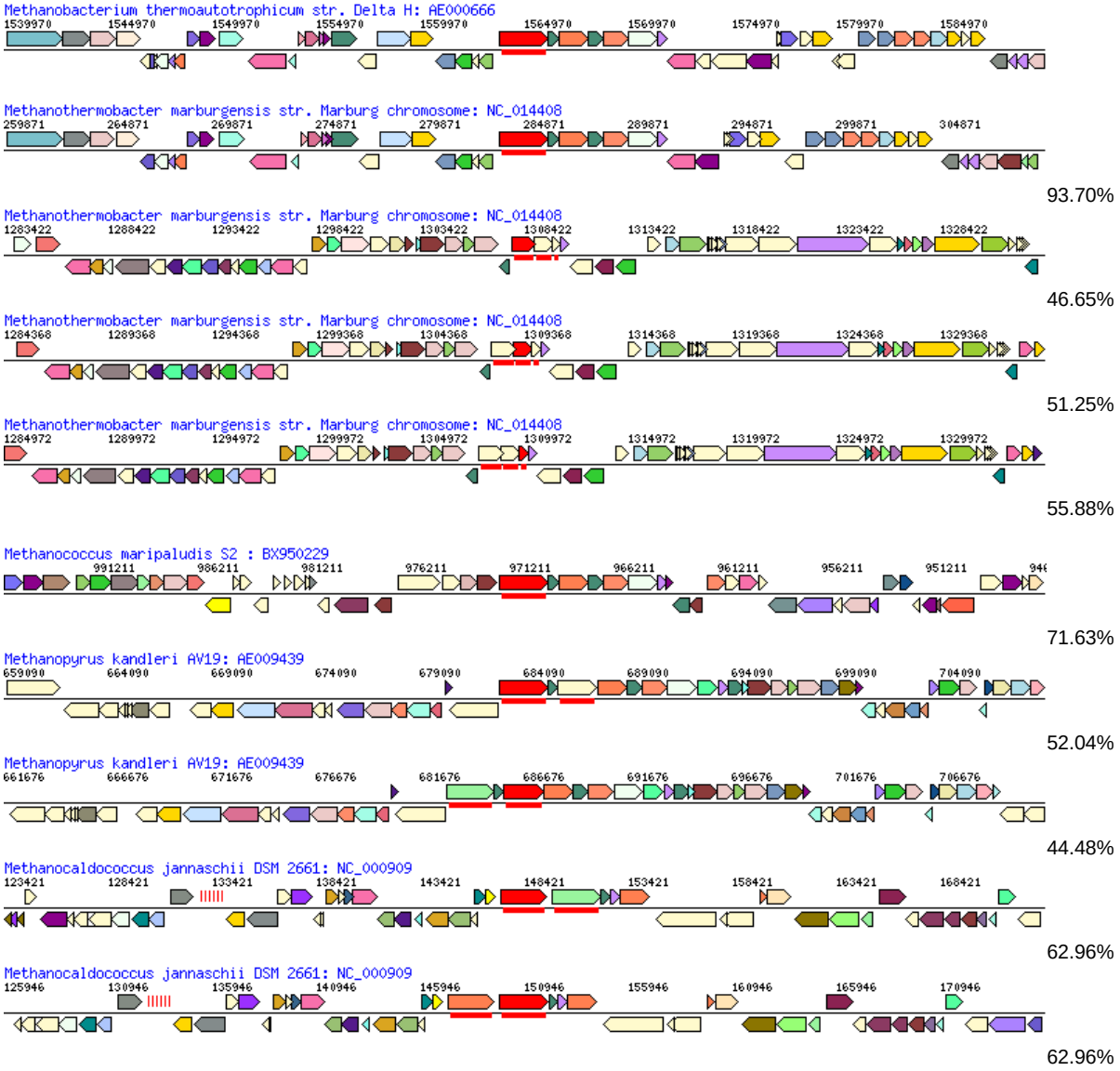
Table 4 – Selected candidates for testing and their physiological characteristics

Code	Name	DSM Id	pH_{min}	pH_{opt}	pH_{max}	t_d (h)	T_{min} (°C)	T_{opt} (°C)	T_{max} (°C)
mru	*Methanobrevibacter ruminantium M1*	DSM1093	----	6.3-6.8	----	----	----	33	----
msi	*Methanobrevibacter smithii PS*	DSM861	----	6.9-7.4	----	----	----	37-39	----
mja	*Methanocaldococcus jannaschii*	DSM2661	5.2	6.0	7.0	0.433	50	85	85
mka	*Methanopyrus kandle AV19*	DSM6324	5.5	6.5	7	0.83	84	98	110
mmg	*Methanothermobacter marburgensis*	DSM2133	5	6.8-7.4	8	----	45	65	70
mth	*Methanothermobacter thermautotrophicus ΔH*	DSM1053	6	7.2-7.6	8.8	1.486	40	65	70
mmp	*Methanococcus maripaludis*	DSM14266	6		8	0.667	37	37	37

As for their CODH coding regions, the basis for the selection study, they are present below in Figure 14.

Cdh type CODH

Sequence similarity



CooS type CODH

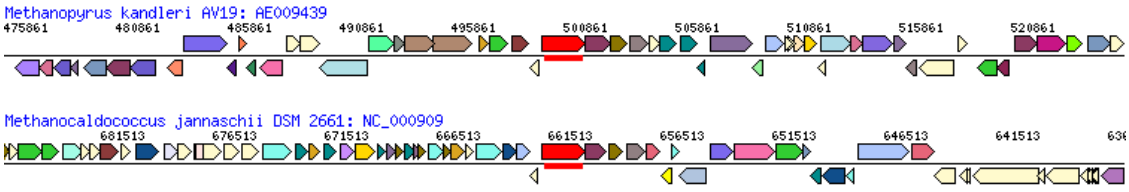
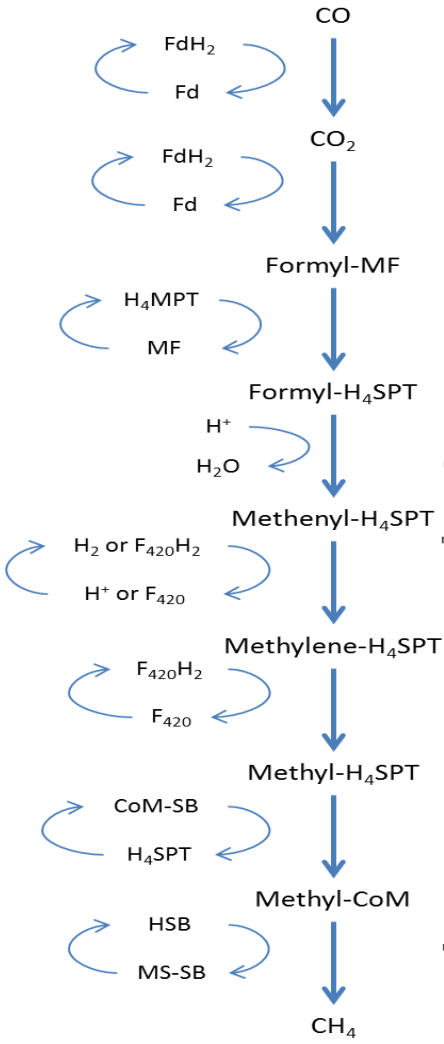


Figure 14 – Gene topology of CODH/ACS alpha subunit neighbourhood for selected strains. Aligned CODH catalytic subunit coding genes are marked in red.

While the selection of candidates was focused on the CODH enzymes due to its primary role in carboxydotrophy, it should be expected that some of the different enzymes involved in the methanogenesis pathway might play a substantial role as well. In particular, reducing steps in this pathway are of primary concern due to their requirement for electrons that need to be provided, directly or indirectly, by CO-oxidation. Figure 15 summarizes the main enzymes (and their respective coding genes) involved during the subsequent steps of methanogenesis:



Enzymes	mth	mmg	mmp	mja	mka	mru	msi
acetyl-CoA decarbonylase/synthase	cdhABCDE (MTH 1708-1713)	cdhABCDE (MTBMA_c 2870-2920)	cdhEDCBA (MMP 980-985)	cdhACABC (MJ 152-156)	cdhABACDE (MK 717-723)		
		cdhBAA (MTBMA_c 14190-14210)		cdhED (MJ 112-113)			
formylmethanofuran dehydrogenase	fwd HFGDACB (MTH 1553-1559) fwd ECBF (MTH 917-926) fwdC (MTH 238) fwdE (MTH 1345)	fwdHFGDACB (MTBMA_c 1390-1450) fwdFECB (MTBMA_c 13050-13130) fwdC (MTBMA_c 6880) fwdE (MTBM_c 17360)	fwdHFGDAC (MMP 1244-1249) fwdEACBB (MMP508-512) fwdC (MMP 70) fwdE (965) fwdE (200) fwdB (1691)	fwdHFGDAC (MJ 1165-1171) fwdB (MJ 1194) fwdC MJ 658)	fwdGDBAC (MK 1525-1530) fwdF I (MK 307) fwdF II (MK 323) fwdB (MK 259) fwdF (MK 1625)	fwdHFGDBAC (mru 339-345) fwdF (mru 830) fwdE (mru 254)	fwdHFGDBAC (Msm 1408-1414) fwdF (Msm 783) fwdE (Msm 1396)
formylmethanofuran-tetrahydromethanopterin N-formyltransferase	ftr I (MTH 1259)	ftr I (MTBMA_c 8020)	ftr (MMP 1609)	ftr (MJ 318)	ftr I (MK 816)	ftr I (mru 1919)	ftr I (Msm 1092)
	ftr II (MTH 403)	ftr II (MTBMA_c 16460)			ftr II (MK 116)	ftr II (mru 441)	ftr II (Msm 308)
methenyltetrahydro-methanopterin cyclohydrolase	mch (MTH 773)	mch (MTBMA_c 11690)	mch (MMP 1191)	mch MJ 1636)	mch (MK 1625)	mch (mru 1619)	mch (Msm 1723)
methylenetetrahydro-methanopterin dehydrogenase	mtd (MTH 1464)	mtd (MTBMA_c 500)	mtd (MMP 372)	mtd (MJ 1035)	mtd (MK 11)	mtd (mru 2142)	mtd (Msm 1204)
5,10-methenyltetrahydro-methanopterin hydrogenase	hmd (MTH 1142) hmd II (MTH 1512)	hmd (MTBMA_c 15260)	hmd (MMP 127)	hmd (MJ 784) hmd II (MJ 1338)	hmd (MK 13)	hmd (mru 526)	hmd (Msm 572)
	hmd III (MTH 504)			hmdIII (MJ 715)			
5,10-methylenetetrahydro-methanopterin reductase	Mer (MTH 1752)	mer (MTBMA_c 3270)	mer (MMP 58)	mer (MJ 1534)	mer (MK 524)	mer (mru 569)	mer (Msm 542)
tetrahydromethanopterin S-methyltransferase	mtrEDCBAFGH (MTH 1163-1156)	mtrHGFABCDE (MTBMA_c 15400-154670)	mtrEDCBAAGH (MMP 1560-1567)	mtrEDCBAFGH (MJ847-854)	mtrEDCBAGH (MK 656-662)	mtrHGFABCDE (mru 1916-1923)	mtrHGFABCDE (Msm 1007-1014)
		mtrA (MTBMA_c 14500)			mtrF (MK 1485)	mtrA II (mru 441)	mtrH (Msm 627)
methyl-coenzyme M reductase	mcrBDCGA (MTH 1168-1164) mrtBDGA (MTH 1132-1129)	mcrAGB (MTBMA_c 15480-15520) mrtAGB (MTBMA_c 15120-15150)	mcr BGA (MMP 1555-1559)	mcrBGA II (MJ 842-846) mrtBGA (MJ 81-83) mrtD (MJ 118)	mcrBGA (MK 651-655)	mcrAGB (mru 1924-1928)	mcrAGB (Msm 1015-1019) mcrAGB (Msm 902-905)
heterodisulfide reductase		hdrCB (MTBMA_c 4490-4500) hdrA (MTBMA_c 17680)	hdrBC (MMP 1053-1054) hdrBC II (MMP 1153-1154) hdrA (MMP 825) hdrA (MMP 1697)	hdrBC (MJ 743-744) hdrBC II (MJ 863-864)	hdrCB (MK 572-573) hdrA I (MK 249) hdrA II (MK 265)	hdrCB (mru 816-817) hdrA (mru 117)	hdrABC (Msm 82-84) hdrA (Msm 1336) hdrBC (Msm 795-796)

Figure 15 – Overview of the presence of possible genes involved in the putative pathway for carboxidotrophic methanogenesis in selective strains

3.2 Growth characteristics of selected methanogens in the presence of CO

All cultures were grown as described previously in the material and methods chapter, in the presence of different concentrations of CO or CO/H_2. Table 5 summarizes the results of the adaptation studies for *Methanothermobacter thermautotrophicus*, *Methanothermobacter marburgensis*, *Methanothermococcus jannaschii* and *Methanococcus maripaludis* – which are the methanogenic species that were shown to use CO/H_2 from the headspace. Furthermore, *Methanothermobacter* strains could also grow on CO as sole carbon and energy source.

Contrastingly, under the experimental conditions available, *M. smithii*, *M. ruminatium* and *M. kandleri* cultures had difficulty growing. Growth of *M. smithii* and *M. ruminantium*, was sluggish even with the addition of growth supplements such as rumen fluid. Due to the short timeframe nature of the work and for the purpose of having a more comparable group in terms of growth requirements, research on these two organisms was discontinued. Regarding *M. kandleri* cultures, grown cultures revealed themselves to be sensitive to the experimental setup defined. Particularly, the cooling down and heating up cycles necessary for batch sampling of the serum bottles by gas chromatography seemed to be a problem. While the first transfers under H_2/CO_2 grew successfully, growth with CO in the headspace was unsuccessful even at low concentrations 5% (0.15 bar of partial pressure). Additionally, further transfers of the control had their growth severally compromised and eventually no discernible growth could be detected either by gas chromatography or by microscopic visualization within a reasonable timeframe (10 times of what it took for the initial inoculation for the control).

Based on growth experiments on CO/H_2, subsequent work was performed with the following strains: *M. thermautotrophicus*, *M. marburgensis*, *M. maripaludis* and *M. jannaschii*.

Table 5 – Overview of growth success with CO in the headspace for the cultures tested

Species	Code	Growth with CO in headspace		Growth with CO as sole carbon and energy source	
		Result	Range tested	Result	Range tested
M. ruminantium	mru	-	0-15%	-	-
M. smithii	msi	-	0-15%	-	-
M. jannaschii	mja	+	0-40%	-	-
M. kandleri	mka	-	0-40%	-	-
M. marburgensis	mmg	+	0-60%	+	10-50%
M. thermautotrophicus	mth	+	0-40%	+	10-30%
M. maripaludis	mmp	+	0-90%	-	-

3.3 Physiological observations/effects of CO

3.3.1 Inhibition by carbon monoxide

For the purpose of testing the inhibition imparted by the presence of CO in the organisms tested, cultures grown in H_2/CO_2 (80/20%, v/v) were transferred to bottles with increasing CO concentrations. If growth was observed, subsequent transfers to higher concentrations were attempted. Table 6 offers an overview of the inhibition observed.

Table 6 - Inhibition by carbon monoxide on the production of methane for tested strains

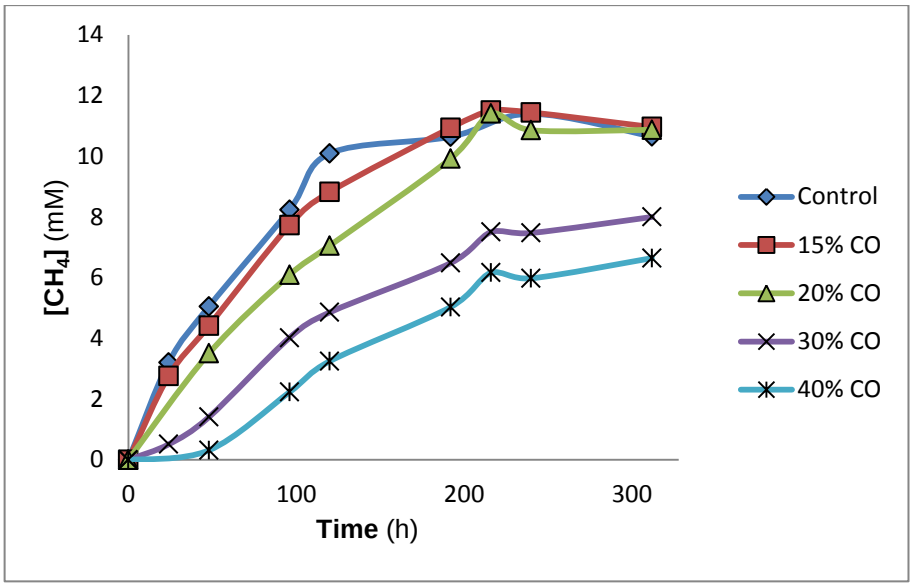
Code	Number of transfers	[CO] tested	CH_4 production rates (mM/h)
mth	6	15-40%	0.06-0.02
mmg	12	5-60%	0.32-0.06
mja	4	5-40%	0.93-0.16
mmp	7	5-90%	0.38-0.0004

All archaea tested demonstrated inhibition by CO in some form. From the studies conducted in this work, it can be observed that inhibition was systematically increased with higher partial pressures of CO (Figure 16).

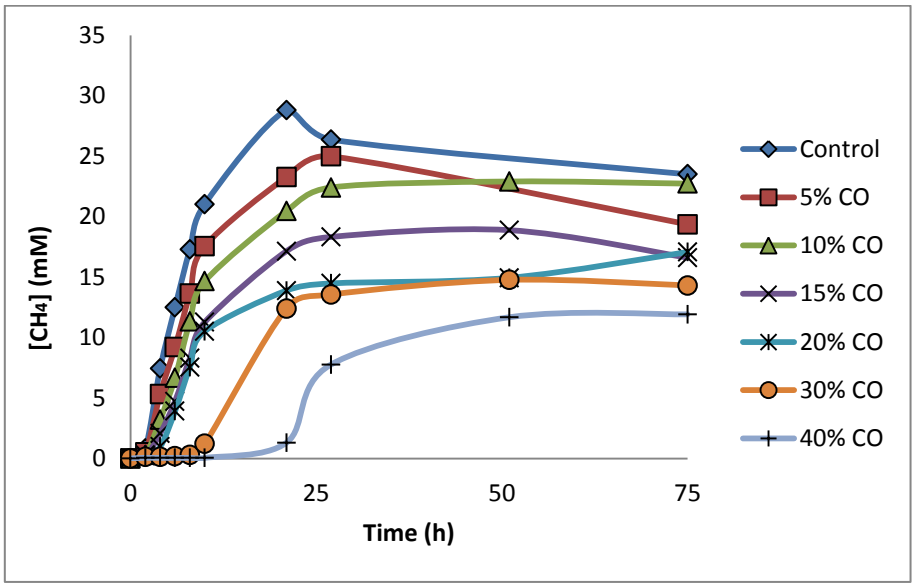
Comparing the methane production profiles of successive transfers for the same concentrations of CO, there was however no significant changes, indicating that these transfers were not strongly effective in attenuating the inhibition observed (Figure 17). The differences observed can be mostly attributed to modifications in the experimental setup factors such as changes in shaking speed and yeast extract concentration (both of which are discussed in more detail in sections 3.4.1.2 and 3.4.1.4 respectively).

It is known that one of the major obstacles for utilisation of carbon monoxide by microorganisms is the inherent toxicity of this gas to their metabolism mostly in the form of the high affinity of this compound to the metal centres of the organometallic enzymes. Of particular importance, in this case, are the hydrogenases which have been known to be inhibited by the presence of CO in other studies. It is therefore feasible to think that some differences in the hydrogenase system could justify differences in adaptability of different strains. Another possibility would be a response that would lower the internal levels of CO inside the cells. Ragsdale has suggested the presence of a gas channel inside the CODH/ACS complex with such purpose (Figure 19). By binding the CO molecules to the protein complex, it reduces its toxicity to other important enzymes such as the hydrogenases. As was assessed in section 3.1.1 during the genomic comparison of the CODHs of these methanogens, all strains tested possess CODH/ACS complexes which can convert CO to other, less toxic metabolites (CO_2 and acetyl-coA).

M. thermautotrophicus
(2nd transfer)



M. jannaschii
(2nd transfer)



M. maripaludis
(2nd transfer)

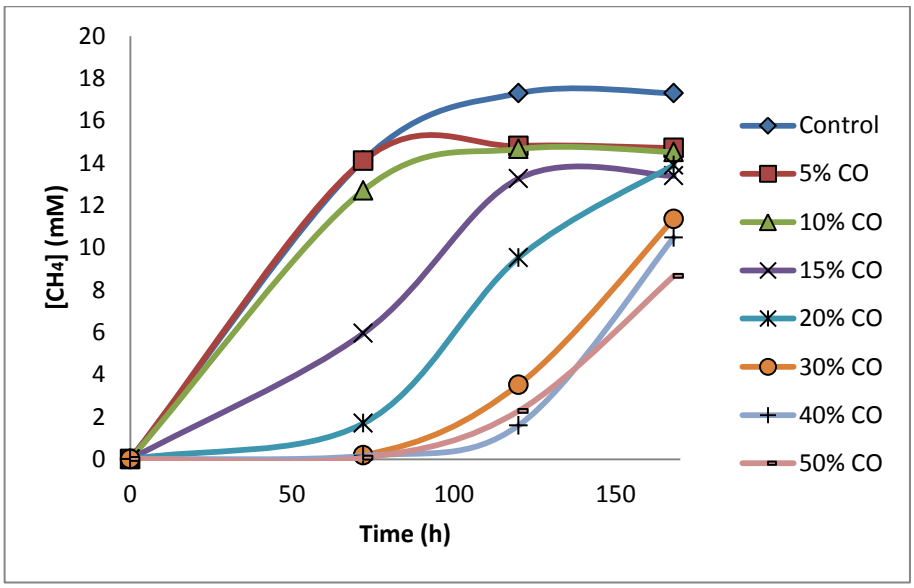


Figure 16 - Effects of inhibition in the production of methane with increasing concentrations of CO for different tested strains.

M. marburgensis

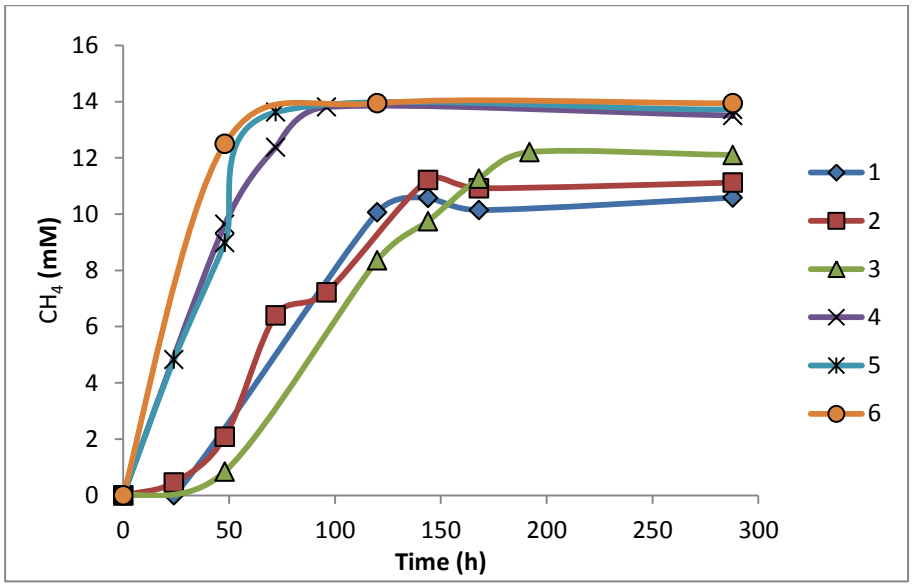


Figure 17 - Effects of inhibition in the production of methane with a fixed concentrations of CO (15%) for *M. marburgensis*

3.3.2 Cellular CO release in methanogenic carboxidotrophic strains growing on H_2/CO_2

Whilst the focused reaction is the consumption of carbon monoxide in order to generate carbon dioxide and the electrons necessary for the methanogenic pathway, in some conditions, the reverse reaction was observed at an early stage (high concentrations of H_2 and CO_2). Indeed, this phenomenon was observed for all strains shown capable of removal of CO from the headspace.

This CO production was always detected alongside vigorous consumption of H_2 and CO_2 (Figure 18), suggesting a dependency on these substrates. Since all of these compounds are being simultaneously utilized for other reactions, a stoichiometry for this reaction cannot be confirmed but it seems to follow:

Equation 7 $$H_2 + CO_2 \rightarrow CO + H_2O$$

For *M. jannaschii*, this production was more substantial. A direct comparison with the other tested strains cannot be performed since this was likely partly due to the overall higher pressure in the system (3 bar versus the 1.7 bar in the other cultures). Since the reaction results in an overall decrease of moles of gas (thus reducing the pressure of the system), it would be encouraged by higher pressures.

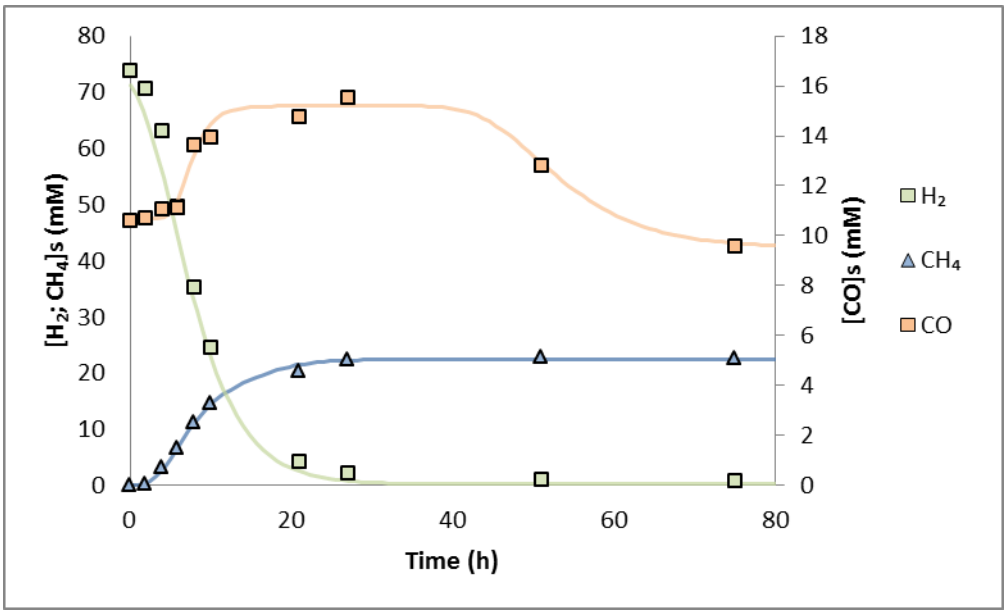


Figure 18 – Carbon monoxide formation from CO_2 and H_2 in *M. jannaschii* at 10% of CO (0.3 bar)

Similar observations have been made previously. Particularly, Thauer and associates have performed extensive studies with *M. marburgensis* to try to determine the origin of CO production (Conrad and Thauer 1983, Stupperich *et al.* 1983, Eikmanns *et al.* 1985, Bott and Thauer 1987).

In their work, it was concluded that CO observed came from the exchange of free with bound CO in the CODH/ACS complex. While often this CO acting as intermediary between CO_2 and acetyl-CoA is kept inside the gas channel, it was demonstrated by use of labelled compounds that such exchange was occurring for that strain and was responsible for the production of CO (Ragsdale 2004). The dependency on H_2 is therefore hypothesized as being mainly in the form of electron equivalent needed for conversion of CO_2 to CO. The methanogenesis pathway generates proton motive force that in turn provides energy that can be used on the formation of CO from CO_2.

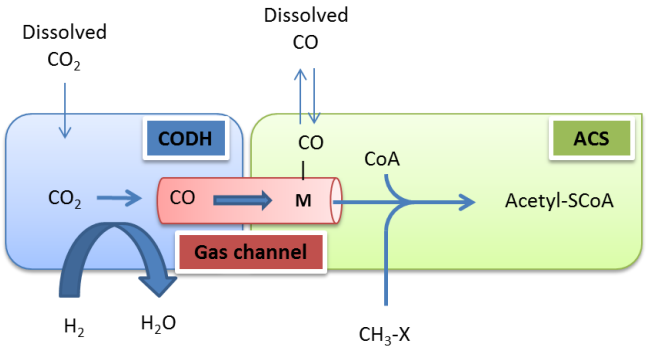


Figure 19 – Representation of equilibrium between bound and dissolved CO in the CODH/ACS complex.

3.4 Adaptation studies to CO

As previously mentioned, out of all tested strains, only *M. thermautotrophicus* and *M. marburgensis* were observed as capable of carboxidotrophic growth in this study. To have a better qualitative overview of the data collected from the GC monitoring of the successive transfers for *M. marbugensis*, the main observations in terms of rate of consumption and amount of CO consumed have been summarized in a heat map.

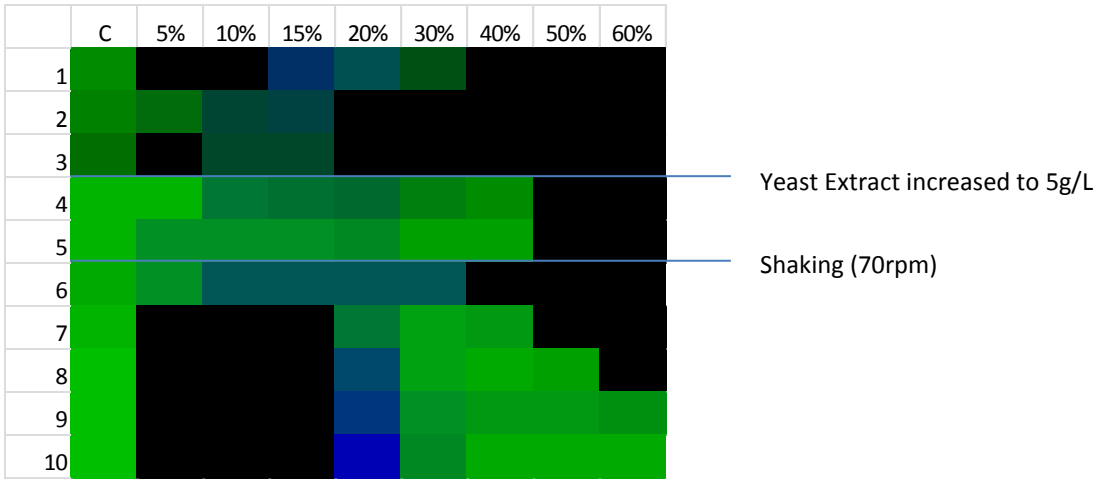


Figure 20 - Heat map of consumption. Black symbolizes untested parameters, green colour intensity corresponds to rate of H_2 consumption (increasing rates from light green to dark green) and blue tone to % of CO consumed (increasing rates from light to dark blue).

By analysis of Figure 20, it is apparent that an optimal range of adaptation seems to be located between 10-20% of CO in headspace (equivalent to partial pressures of 0.17 to 0.34 bar). It was in this range that fastest consumption of CO was detected (in relation to the primary catabolism using H_2 as an energy source). Concordantly, the condition with 15% of CO (0.26 bar of partial pressure) was the first where carboxidotrophic growth was detected as discussed more thoroughly below in section 3.5.

An interesting observation that can be taken from the adaptation study is the decrease in relative CO consumption rates following the 1st and 6th transfer. Contrary to what was being attempted, there seems to be a loss of adaptation/preference to the use of CO as a substrate. Additionally the surge of CO consumption happened with increased shaking but not with the addition of yeast extract. Furthermore, the initial surge in CO consumption following the introduction of shaking, although substantial, seemed to regress almost completely in the subsequent transfer.

Both these instances mark a point immediately following a period of inferior availability of primary substrates. Transfer 1 follows prolonged cell inactivity (shipped inoculum from DSMZ) and transfer 6 corresponds to a surge in dissolution rates of electron donors (H_2 and CO). Thus, both times correspond to substantial relief of less favourable energetic conditions for the cells albeit due to different reasons. Situations of chronic energy stress might provide an incentive for the cells to utilize all possible sources of electrons (including CO). The momentary spike in the use of CO following

transfer 6 would therefore be due to faster replenishing of CO levels in the immediate vicinity of the cells. Once the cells readjust to the added energy availability they would reverse to using H_2 a more thermodynamically advantageous energy donor as a primary source of electrons foregoing CO consumption.

Contrastingly, from the 7th transfer onward, CO consumption rates increase in a sustainable way, even with frequent transfers. CO availability remains constant in these transfers.

The juxtaposition of these two phenomena indicates that some other process is influencing the CO use apart from its availability to the cell and/or the development of resistance mechanisms to inhibition.

3.4.1 Consumption of CO by methanogenic strains

One of the first observations that can be made is that H_2 is consumed at a faster rate than CO and, in the cases where CO levels are depleted, it occurs after H_2 partial pressures have been lowered significantly. This would seem to indicate that H_2 is the preferred electron donor for methanogenesis as would be expected. The reaction that describes the conversion of CO_2 with H_2 for methane production is thermodynamically more favourable than the one using only CO. Therefore, based on this criterion, as long as H_2 is present in non-limiting concentrations, this pathway should take precedence. Nevertheless, it should be noted that the simultaneous formation of carbon monoxide discussed in section 3.3.2, could be, at least partly, masking an early decrease of CO concentrations in the headspace.

Beside longer incubation times, successive transfers of cultures, maintaining the same CO concentrations, were effective reducing the lag time of CO consumption. This indicates a progressive adaptation of the cultures to CO levels.

Exactly what is behind this adaptation mechanism is unclear. Similar observations have been made for other CO utilizing organisms (Rother and Metcalf 2004, Sipma *et al.* 2006). In some cases, CO is metabolized to lower levels, thus diminishing its presence to levels below toxicity with growth occurring afterwards. However, results obtained in this study do not indicate such process since CO levels in the headspace only significantly decrease after H_2 is consumed and cultures are substantially grown.

Apart from the consumption of CO towards the production of methane, as indicated by the schematic in 1.4.4, CO can be converted into acetyl-CoA by the CODH/ACS complex. This reaction requires a methyl group that can be acquired through methyltetrahydro-methanopterin, an intermediary product of methanogenesis (Eikmanns *et al.* 1985).

3.4.1.1 Effect of CO partial pressure on the growth of methanogens

The most direct way of increasing the amount of CO dissolved in the media is to increase the partial pressure of the gas in the headspace. CO solubility drops considerably at high temperatures which can be a hindrance to the use of this substrate for thermophilic and hyperthermophilic species (Figure 21). It's therefore critical to regulate the partial pressures in a way that would circumvent this lower solubility but, at the same time, that would not be so high as to pose an overwhelming toxicity for cell growth. Analysis of Figure 20 indicated that such range is located within the 10-20% CO concentrations. Presumably, 5% would be too low for CO to reach the cells and be replenished in significant way to sustain growth and higher concentrations are toxic needed further attempts for adaptation.

$$k_H = k_H^{\theta} \times e^{\left[\frac{-\Delta_{solution}H}{R}\left(\frac{1}{T}-\frac{1}{T^{\theta}}\right)\right]}$$

Equation 8 - Henry's law equation

Table 7 - Henry's law constants dependency on temperature

Constant	H_2	CO
$k_H^{\theta}\ \left[\frac{M}{atm}\right]$	0,00078	0,00099
$\frac{-\Delta_{solution}H}{R}\ [K]$	2200	1300

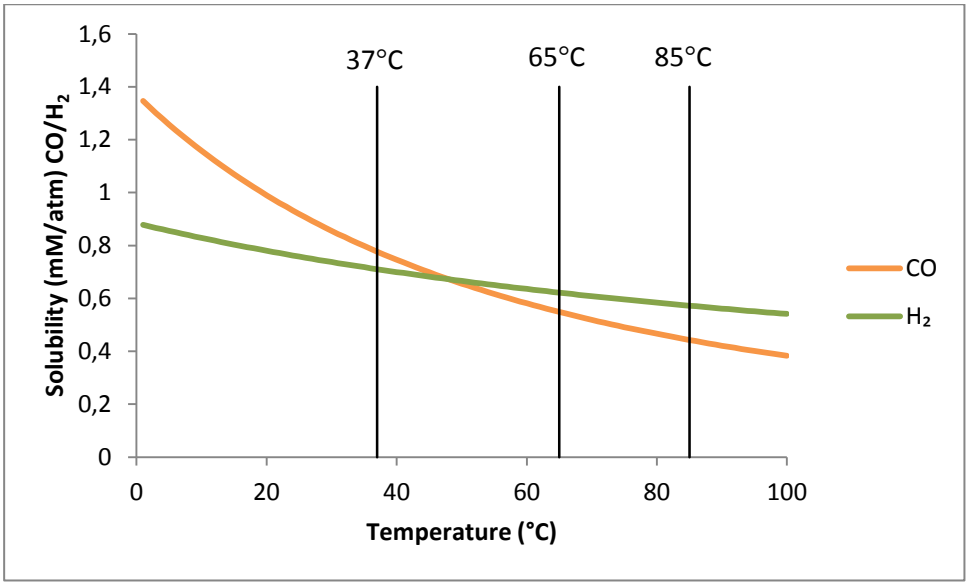


Figure 21 – Dependency of solubility of H_2 and CO on temperature.

3.4.1.2 Effect of shaking speed on CO utilization rates

For *M. marburgensis*, a clear shift in CO consumption was detected when cultures were subjected to a shaking speed of 70 rpm. Further increase to 120 rpm had also significant effect on CO consumption rates.

Shaking speed affects the mass transfer rate between the headspace and the media itself thus reducing the time required for the dynamic equilibrium to be achieved. At a microscopic level, this means that dissolved gas concentrations in the immediate vicinity of the cells are more quickly restored upon their consumption. Since H_2 and CO are both gases, they are both being affected by this change although not necessarily in the same way.

The increase in H_2 availability should result in an overall increase in growth rates. Indeed this can be observed for all concentrations of CO tested. Curiously, the initial transfers of the controls do not seem to share this effect at first with growth speed only increasing substantially after the 8^{th} transfer.

The effect of increased dissolution of CO on growth is not as straightforward due to the triple effect of this gas as detailed in section 3.3. The faster dissolution of gases in the media would have opposite results depending on whether production or consumption of CO is dominant. On the other hand, the dual nature as inhibitory substance and catabolic substrate are in direct opposition to each other and would either hinder or aid the methanogenesis. The inhibitory effect of CO for key enzymes such as hydrogenases should be compounded further with higher concentrations of CO. However the additional availability of a second carbon and energy source could result in extra yields of methane and aid cell carbon material by means of acetyl-CoA synthesis by ACS.

3.4.1.3 Effect of buffer used in the media

While *M. thermautotrophicus* grew slightly slower in the phosphate buffer, depletion of CO after considerable incubation periods was more successful than with bicarbonate buffer within the limited testing performed (Figure 22).

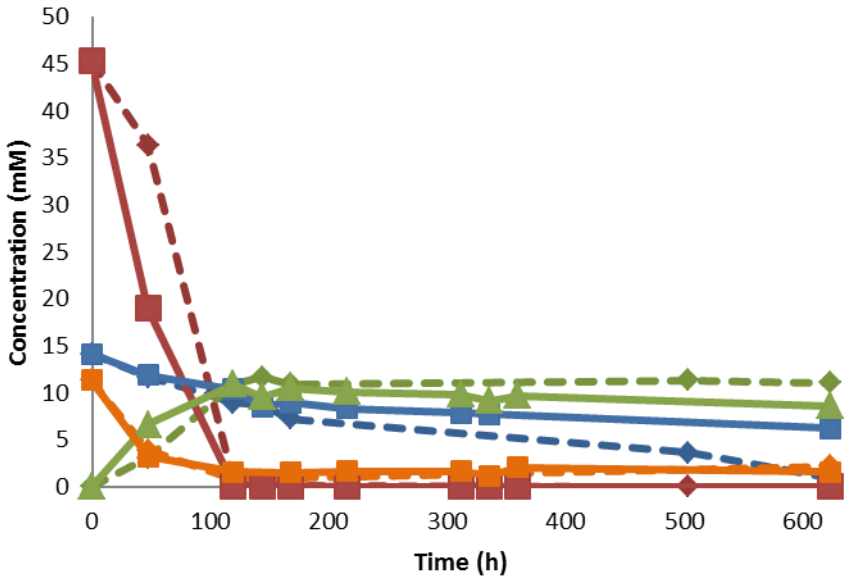


Figure 22 – Difference in growth observed with phosphate buffer vs bicarbonate buffer at CO concentrations of 20%

The impetus behind this difference is likely attributed to lower levels of overall dissolved CO_2 in the media that would drive the equilibrium presented in equation 9 towards the $CO_2 + H_2$ formation and thus contribute to a higher availability of methanogenesis substrates.

3.4.1.4 Effect of supplementing methanogenic cultures with yeast extract

From the first 3 transfers, it could be observed that growth rates were steadily diminishing for all incubation, including the controls without CO. With the assumption that this might be due to lower levels of growth supplements, following the 3rd transfer, yeast extract was increased from 2.5 g/L to 5 g/L. This resulted in an overall increase of the growth rate of the cultures in the 4th transfer series (Figure 20 and Figure 17). Furthermore, growth rates stabilized in the control for subsequent cultures.

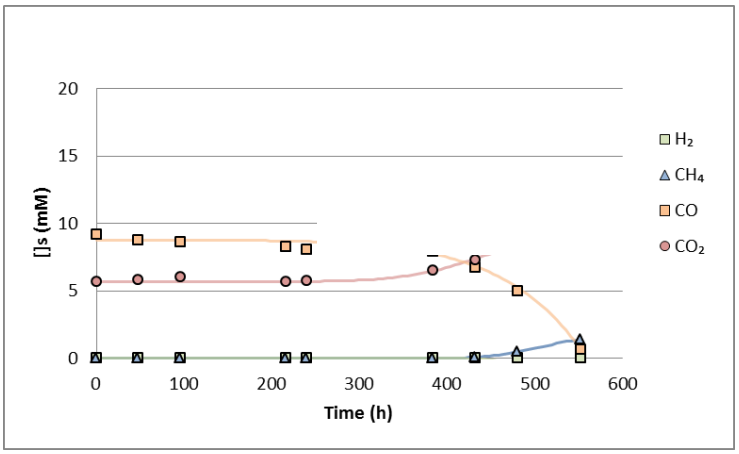
Regarding CO consumption rates, no significant difference could be detected. Yeast extract is composed of a mixture of nutrients. While it increases the availability of nutrients needed for growth, these results indicate that is does not significantly affect the ability of the cells to resist/consume CO.

3.5 Carboxidotrophic growth

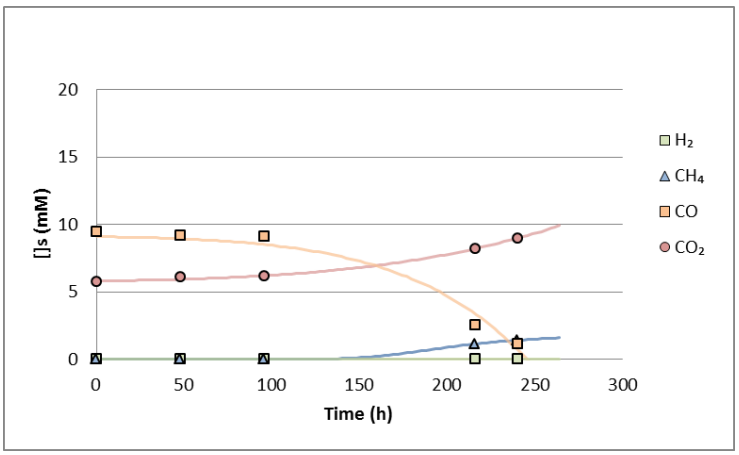
As previously mentioned, autotrophic growth on CO as a sole carbon and energy source (carboxydotrophy) was only successful for two of the tested microorganisms: *M. thermautotrophicus* and *M. marburgensis.*

While *M. thermautotrophicus* ability to grown on this substrate alone was previously reported (Daniels *et al.* 1977), *M. marburgensis* was not part of the group of known carboxidotrophic methanogens with this work being the first description of such capability.

M. marburgensis
Growth with 10% CO
(6th transfer)



M. marburgensis
Growth with 15% CO
(6th transfer)



M. marburgensis
Growth with 20% CO
(6th transfer)

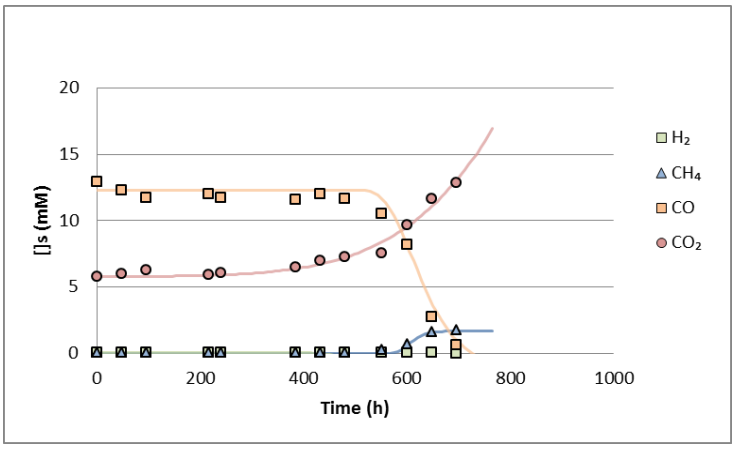


Figure 23 – Concentrations of gases in headspace for cultures growing on CO in the first transfer serie where carboxidotrophic growth was observed.

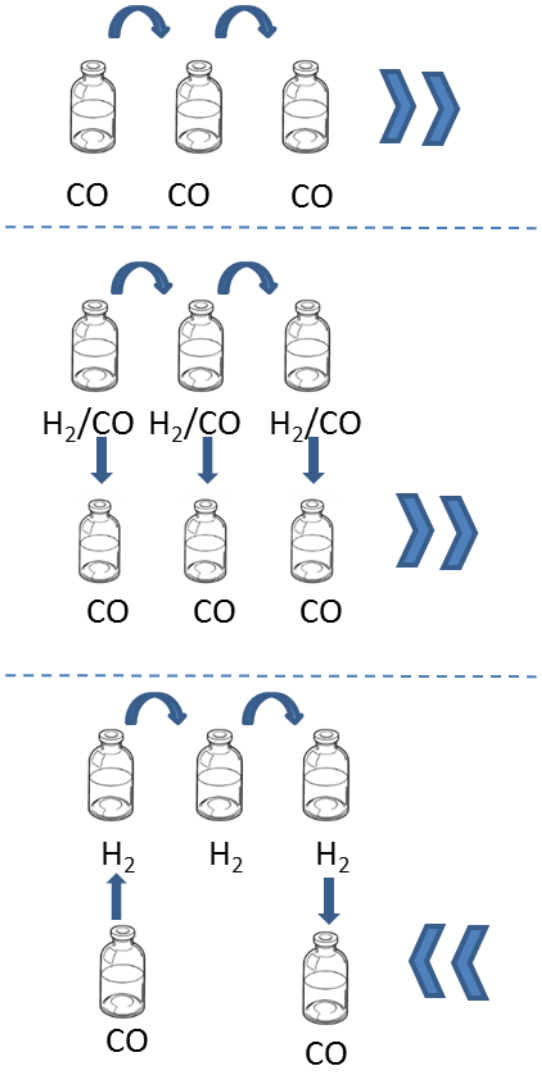


Figure 24 – Adaptation of cultures to CO in the transfer series.

Growth on CO for *M. marburgensis* was first achieved with inoculum from the 5th transfer series after long periods of incubation. Whilst medium turbidity was visualized in advance, methane production was not detectable until an incubation period longer than 150 h for cultures growing at 15% of CO (partial pressures of 0.26 bar). As for concentration of 10 and 20%, it took 450 and 600 h for methane to be detected in the headspace.

From the 6th transfer series onwards, new inoculations from bottles with H_2/CO into bottles with only CO always resulted in carboxidotrophic growth in the range of 10-20% of CO in the headspace. Furthermore, the initial lag period diminished in bottkes inoculated with cultures furter along in the H_2/CO transfer series. Additionally, both the adaptation period and growth rate were faster in new CO bottles inoculated from cultures already growing on CO. Furthermore, transfer of culture growing on CO back to control bottles without CO in the headspace, resulted in a loss of carboxidotrophic ability which indicated a reversal of the adaptation mechanism for this archaea. These observations are summarized in Figure 24.

3.5.1 Presence of hydrogen in headspace

In the cultures growing with CO as the only electron source, basal levels of H_2 were detected in the headspace (Figure 25).

The minute levels of hydrogen present in time 0 could be explained either by the transfer of dissolved hydrogen in the media from the previous inoculum or due to trace levels of hydrogen in the tubing of the gas exchanger.

Nevertheless, low level accumulation of H_2 (< 0.1mM) is clearly visible in the bottles with CO as only electron source. This build-up is sustained until production of CH_4 starts. At this point, the rate of consumption of CO quickly increases and the hydrogen levels drop below the detection limit.

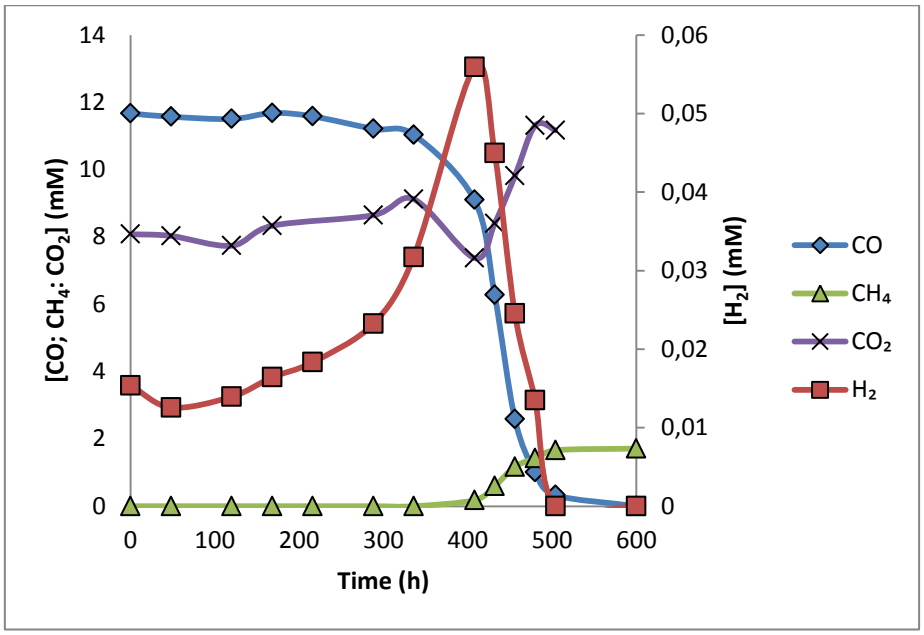


Figure 25 – Production of H_2 in cultures growing carboxidotrophically

Concordantly, CO_2 levels seem to slowly rise at an early stage with a marked increase accompanying the quick consumption of CO and CH_4 production. It should be noted, however, that CO_2 in the headspace is at equilibrium with the bicarbonate in the media, making its assessment for stoichiometric purposes problematic.

Presumably, H_2 production is a consequence of CODH slowly oxidizing CO according to the biological water gas shift reaction (equation 9). Subsequently, that H_2 can be converted in the presence of CO_2 through the well-defined reaction for hydrogenotrophic methanogenesis (Equation 11) explaining the depletion measured.

Another possible route for CO conversion into methanogenesis is detailed by equations equation 9 - Equation 13. In this pathway, the electrons formed by CO oxidation are directed towards the required steps of methanogenesis independently of H_2 formation.

Both of these processes result in the overall stoichiometry represented by Equation 13.

Equation 9 $CO + H_2O \rightarrow CO_2 + H_2$

Equation 10 $CO + H_2O \rightarrow CO_2 + 2\,H^+ + 2\,e^-$

Equation 11 $CO_2 + 4\,H_2 \rightarrow CH_4 + H_2O$

Equation 12 $CO_2 + 8\,H^+ + 8\,e^- \rightarrow CH_4 + 2\,H_2O$

Equation 13 $4\,CO + 2\,H_2O \rightarrow CH_4 + 3CO_2$

This would explain the accumulation of CO_2 despite production of methane. While CO_2 is an intermediary product in the conversion of CO to CH_4, to provide enough electrons for the methanogenesis, a surplus of CO_2 must be produced beyond the carbon needs of the pathways in order to provide reducing power for steps 4 and 5 of methanogenesis. It should be noted that, unlike what happens during growth with H_2/CO_2, the overall stoichiometry indicated by Equation 13 does not result in a variation in total moles of gas. Consequently, closed system serum bottles with cultures growing according to this reaction should maintain a constant pressure in the headspace. That was indeed what was observed experimentally.

There is, however, insufficient information to fully determine if hydrogen formation is a necessary intermediate in carboxydotrophy of M. marburgensis. The trend observed could suggest that H_2 is potentially produced as an electron carrier but is being immediately consumed at a superior rate dropping its levels in the headspace sharply. Hydrogen measured would therefore be excess molecules that are being leaked into the media and subsequently the headspace.

However the hypothesis that an excess of electrons is being generated and partly converted by hydrogenases into molecular hydrogen simply as an unnecessary side product cannot be discarded. Ultimately, the need for production of molecular hydrogen would depend on the mechanism being utilized in the reducing steps of methanogenesis. Mechanisms for H_2 independent methanogenesis in hydrogenotrophy are known to occur using electron carriers such as ferredoxins (Costa *et al.* 2013).

3.5.2 Visual observations

Cultures growing on CO have a notable different outward appearance. Cells acquire lighter shading compared to ones grown on the other tested gas mixtures. Microscopic observation show that cell size decreases with increase the amount of CO in the media/decrease of H_2. Furthermore, there is a very noticeable visual difference between pellets obtained from the different conditions.

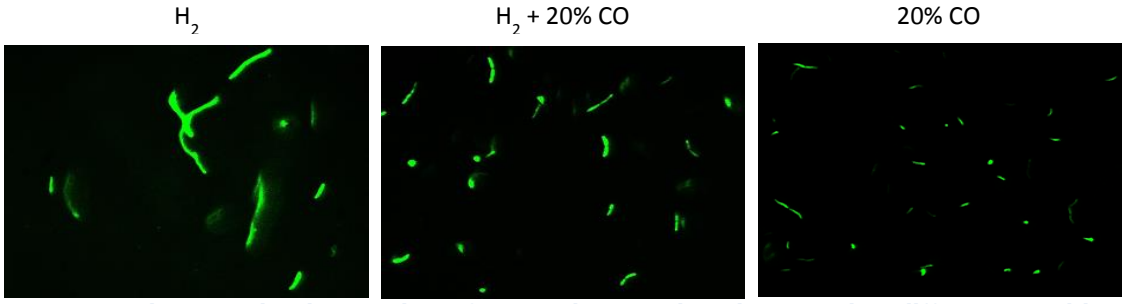


Figure 26 – Microscopic observation of *M. marburgensis* cultures using different combinations of electron donors.

While merely a circumstantial observation, during attempts of cell lysis of cultures grown in atmosphere with and without CO, it was noted that cells grown in CO alone were noticeably harder to lyse (*vide* chapter 3.6.1). This could also be attributed to the changes in the cell wall that make it more resilient.

Adaptations of the cell wall of *M. thermautotrophicus* in response to environmental conditions in syntrophy have been reported previously (Nakamura *et al.* 2006). While the observation was made amidst comparison of pure culture with a co-culture, the author postulated that this archaea increases the thickness of its wall as a response to the low partial pressures of H_2 it is being subjected to.

If indeed that is the case, considering the experimental conditions tested, it seems likely that a similar process would be emerging. Such phenomena could, at least partly, explain the differences detected. Observations conducted in this work would seem to indicate the reported changes extend to *M. marburgensis*.

Whether this is only due to H_2 as hypothesised by Nakamura or if another feature, such as CO presence, also plays a role remains uncertain. Substantial differences between the control and 20% CO would seem to suggest that the presence of CO might also be a factor considering that the decrease in H_2 partial pressures is not substantial. Following the same logic as previously, if the cell wall can change due to low H_2 partial pressure, then it could change as a defence to CO. Being a polar molecule, the same mechanisms that prevent redundant proton exchange through the membrane would help keep CO from entering the cell which in turn would help with CO toxicity. There is, however, not enough data to conclude a direct correlation of this nature.

Differences in archaeal cell walls have been linked to adaptations to chronic energy stress conditions. By having a more tight structure to their walls, archaea can prevent redundant proton exchange, making them more energy efficient (Valentine 2007).

In conditions with low levels of H_2, this is certainly an advantage. It could be postulated that this is a further refinement of this phenomena. When the preferential electron donor, H_2, is removed from the headspace, we induce a state of chronic energy stress in the cells.

3.6 Proteomic studies of carboxidotrophic cultures

3.6.1 Optimization of protein extraction and quantification protocols

Initially, cell lysis was performed by the standard method used in the Microbiology Laboratory of Wageningen University which is based on sonication aided by the presence of surfactants (SDS). While previously tested successfully for bacterial strains and control samples of *M. marburgensis*, this lysis protocol proved ineffective for samples with H_2/CO and CO.

In order to bypass this resistance, increasing the number of sonication cycles was attempted. While the number of cells detected under the microscope diminished with increasing cycles, lysis yield was not substantial until over 30 cycles for cells grown with CO (Figure 27). Furthermore, there was a noticeable difference between lysis for the different conditions. Cells grown on CO were the most resilient (Figure 28). Unfortunately this high number of cycles can also have a detrimental effect towards protein yields.

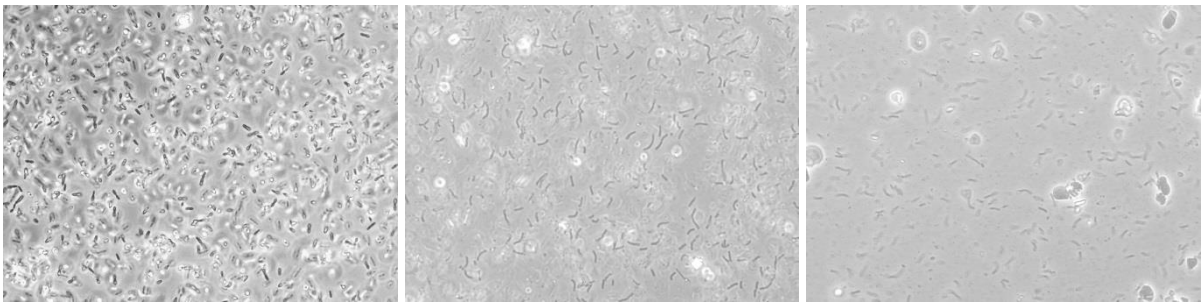


Figure 27– Microscopic observations of cells grown with CO during lysis with sonication. From left to right: before sonication, 18 cycles and 30 cycles.

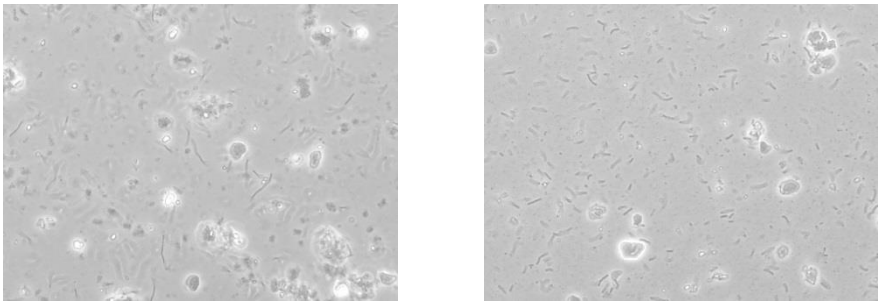


Figure 28 - Microscopic observations of cells subjected to 30 cycles of sonication. From left to right: cells grown on H_2+CO before sonication; cells grown on CO.

Considering the poor results obtained, an alternative method of cell lysis for was needed. There are several methods for cell lysis, all of them with their own set of advantages and drawbacks.

Several other methods were assessed for cell lysis. Ultimately, taking in account previous results in other similar research and the equipment and reagents readily available in the laboratory, a French press method was chosen. This method proved to be highly effective but required a relatively

big volume of sample (> 3-5 ml) therefore producing a very dilute sample for the operational conditions tested.

While the primary purpose for optimization of the lysis protocol was intended for protein extraction towards proteomic analysis, lysis for other purposes might be impossible with this method. More specifically, for enzyme activity assays, anaerobic conditions (or as close to it as possible) need to be maintained. Consequently, the French press method can not be utilized for this purpose.

3.6.2 Proteomic analysis

As was previously mentioned, results from samples sent for proteomic analysis in an external laboratory weren't returned in time for their inclusion in this written dissertation. Consequently, in their place, this chapter will list the proteins identified in previous proteomics analysis of *M. thermautotrophicus* growing in H_2/CO_2 and discuss the expected modifications in the expression profiles of proteins in the other conditions sent for analysis.

A comprehensive list of proteins identified under high and low availabilities of H_2 in a previous study (Farhoud 2011) can be found in Annex II (kindly provided by Hans JCT Wessels from Radboud University).

Figure 29 details the pathways for methanogenesis (using either CO or H_2 as electron donors) in *M. thermautotrophicus* and *M. marburgensis*. For the cells utilizing CO, the oxidation of this gas into CO_2 occurs at CODH/ACS protein complex. As such these should be the main enzymes whose expression is induced in the presence of CO.

Constrastingly, in cultures growing with CO as electron donor, H_2-dependent pathways should be downregulated. This would potentially affect the reducing steps of methanogenesis. In particular, Hmd dependent pathway might not occur under H_2 limiting conditions. It's possible that CO oxidation might directly catalyse all the reductions of the ferredoxins needed for the methanogenesis enzymes, without the aid of H_2. In that case, Eha (energy converting [Ni,Fe]-hydrogenase), Frh (F_{420}-reducing [Ni,Fe]-hydrogenase), Mvh (F_{420}-non reducing [Ni,Fe]-hydrogenase) and Hdr (heterodisulfide reductase) enzymes would play a limited role. However, it should be noted that H_2 production in cells growing on CO was detected. If H_2 is being produced as a necessary intermediary towards one or more of the reducing steps in the methanogenesis pathway, then the corresponding enzymes should still be present in the proteomic analysis.

Transcriptomic analysis of *M. marburgensis* already revealed that Mcr (methyl-coenzyme M reductase isoenzyme I), the protein involved in the last, rate-limiting step of methanogenesis, is induced by the presence of CO (Zhou *et al.* 2013).

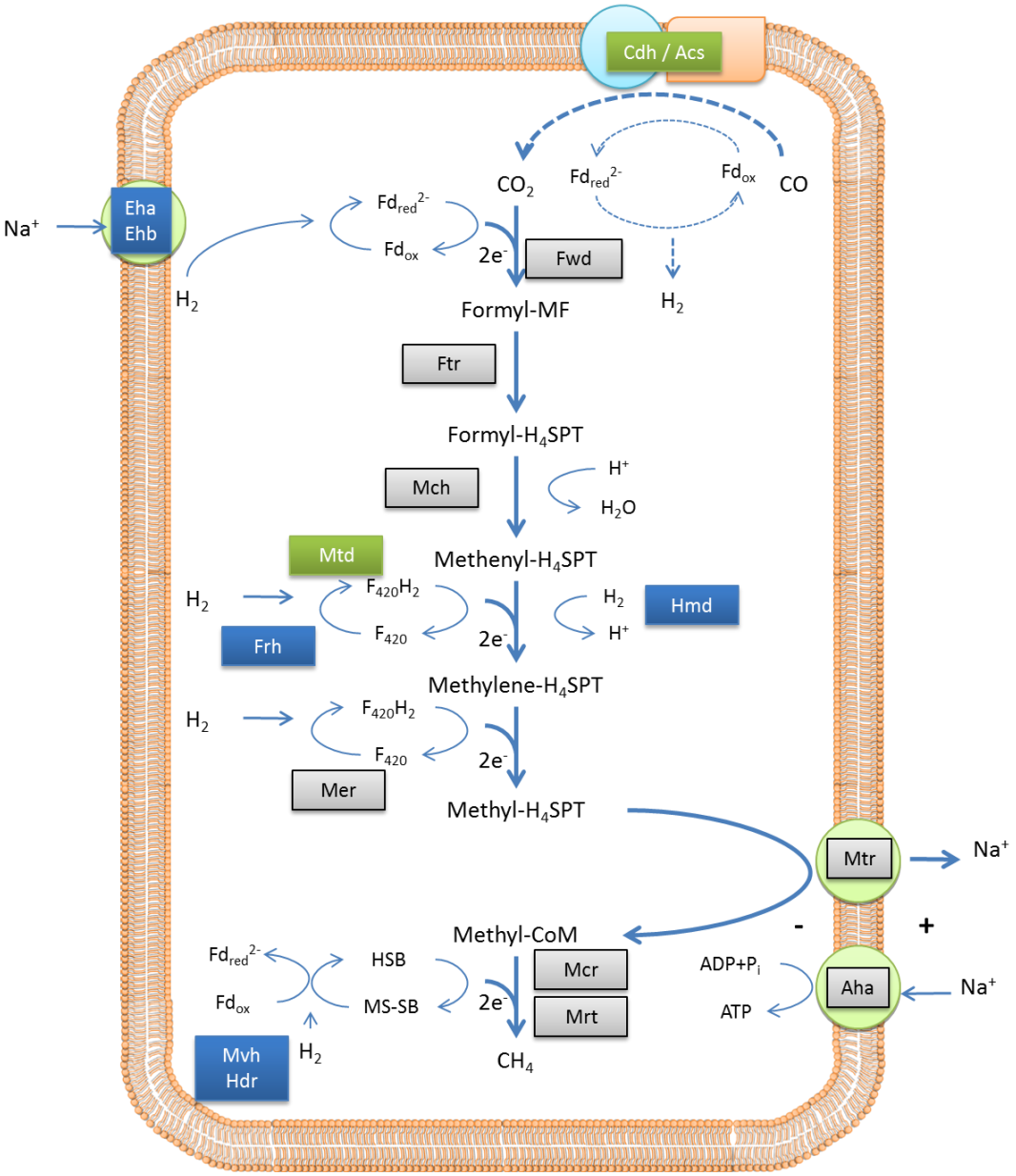


Figure 29 – Expected pathway for *M. thermautotrophicus* and *M. marbugensis*. Proteins whose expression is expected to increase in the presence of CO are marked in green. Those whose expression is expected to decrease are marked in blue.

4 Conclusions and Future Work

It is likely that the number of carboxidotrophic organisms documented in literature is grossly underestimated. Potential to grow on this substrate can easily be overlooked since it is not commonly tested in characterization studies and CO inhibition can induce false negatives. Surpassing this inhibition often requires time consuming and/or labour intensive adaptations through long term incubations or multiple transfers with gradual increase of CO percentages. Consequently, while a physiological approach to this problem is a necessary starting point, a readjustment of the testing parameters with the added knowledge gained from each newly determined carboxydotroph could be extremely beneficial. As such, detailed comparative genomic and proteomic analysis of the strains known to possess the desired ability to grown on CO alone in different experimental conditions are essential to provide further insight into the mechanisms behind carboxydotrophy and expand the knowledge on this field of study.

In this study, several different hydrogenotrophic methanogens were selected through genomic comparison to a known methanogenic carboxydotroph, *M. thermautotrophicus*, and tested for their capability to utilize CO for production of CH_4. Out of the tested species, only *M. thermautotrophicus* and *M. marburgensis* were observed as capable of utilizing CO as a sole energy source. For the latter, this work marks the first report of carboxidotrophic growth.

Growth on CO is a complex process that is not fully understood. Observations in this work for *M. marburgensis* seem to confirm the presence of several parameters that affect such ability. In particular, mass transfer rates (affected by shaking) and specific ranges of CO partial pressures were crucial to the success of carboxydotrophy. Moreover, while *M. thermautotrophicus* ability to adapt to grow on CO alone is extensively reported, within the parameters tested, it was harder to achieve and growth rates were considerably lower than the ones observed for *M. marburgensis*.

It should be noted that, while adaptations for carboxidotrophic growth in other strains were unsuccessful, this does not mean necessarily that they are incapable of such. Merely that, in the conditions tested and within the limited timeframe of this study, such growth could not be observed. It is feasible that extra transfers and longer incubation times might be fruitful were this study was not. Likewise, several operational parameters could be further readjusted such as media composition, shaking speed, partial pressures of headspace components, *etc*. The combination of these factors provides a myriad of potential combinations to test.

For the confirmed carboxidotrophic strains, *M. thermautotrophicus* and *M. marburgensis*, a comparative proteomic was begun. However, the results of that analysis still need to be thoroughly examined and could not be included in this thesis because of time limitations. Carboxydotrophs are slow growers and cell yields were extremely low. This resulted in very long experiments and the need to grow high volumes of cell culture for protein isolation. Another drawback was the need to optimize cell disruption for protein extraction. First trials with sonication did not result for efficiently breaking the archaeal cell membranes. Further tests with the French press were necessary for successful protein extraction from the methanogenic cultures.

With adaptation of *M. marburgensis* to use of CO as sole energy source, it is now possible to further characterize its growth. While methane production was measured closely, to determine growth rates accurately, a more direct measurement of cells still needs to be performed. Also interesting to study is the full range of CO partial pressures for which adaptation can be successful. Concurrently, optimum operational conditions maximizing CO conversion can be perceived with the fully adapted cultures at different CO partial pressures.

Morphological changes observed for *Methanothermobacter* cultures in H_2/CO and CO also offer an interesting avenue for further research. Additional analysis of cell wall can be done either through more refined microscopic methods or through direct study of cell wall components. Transmission electron microscopy (TEM) and membrane specific metabolome analysis, respectively, would be the preferred methods for such study.

Hydrogen formation observed can also be a possible avenue of study. From this work it is still unclear whether molecular hydrogen is a necessary intermediary for carboxidotrophic methanogenesis pathway of *M. marburgensis*. Enzyme activity assays targeting the hydrogenases thorough the build-up and depletion phase would provide us with further insight into this question. Additionally, deletion mutants blocking this H_2 formation from CO, if grown successfully, would give a definitive answer to whether the methanogenesis can occur in a H_2-independent way or not. Moreover, these deletion mutants could potentially be used to selectively remove CO from the syngas mixture.

5 References

Abubackar, H. N., M. C. Veiga and C. Kennes (2011). "Biological conversion of carbon monoxide: rich syngas or waste gases to bioethanol." Biofuels Bioproducts & Biorefining-Biofpr **5**(1): 93-114.

Alves, J. I. (2013). Microbiology of thermophilic anaerobic syngas conversion. PhD, University of Minho.

Balch, W. E. and R. S. Wolfe (1976). "New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of Methanobacterium ruminantium in a pressureized atmosphere." Appl Environ Microbiol **32**(6): 781-791.

Beijerinck, M. and A. van Delden (1903). "On a colorless bacterium, whose carbon-food comes from the atmosphere." Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen(5): 25.

Beijerinck, M. W. and A. van Delden (1914). "Über das Nitratferment und über fiber physiologische Artbildung." Fol. Microbiol(3): 23.

Bott, M., B. Eikmanns and R. K. Thauer (1986). "Coupling of carbon monoxide oxidation to CO2 and H2 with the phosphorylation of ADP in acetate-grown Methanosarcina barkeri." Eur J Biochem **159**(2): 393-398.

Bott, M. and R. K. Thauer (1987). "Proton-motive-force-driven formation of CO from CO2 and H2 in methanogenic bacteria." Eur J Biochem **168**(2): 407-412.

Bult, C. J., O. White, G. J. Olsen, L. Zhou, R. D. Fleischmann, G. G. Sutton, J. A. Blake, L. M. FitzGerald, R. A. Clayton, J. D. Gocayne, A. R. Kerlavage, B. A. Dougherty, J. F. Tomb, M. D. Adams, C. I. Reich, R. Overbeek, E. F. Kirkness, K. G. Weinstock, J. M. Merrick, A. Glodek, J. L. Scott, N. S. Geoghagen and J. C. Venter (1996). "Complete genome sequence of the methanogenic archaeon, Methanococcus jannaschii." Science **273**(5278): 1058-1073.

Carere, C. R., R. Sparling, N. Cicek and D. B. Levin (2008). "Third generation biofuels via direct cellulose fermentation." Int J Mol Sci **9**(7): 1342-1360.

Colby, J., E. Williams and A. P. F. Turner (1985). "Applications of Co-Utilizing Microorganisms." Trends Biotechnol **3**(1): 12-17.

Conrad, R. and R. K. Thauer (1983). "Carbon monoxide production by Methanobacterium thermoautotrophicum." FEMS Microbiol Lett **20**(2): 229-232.

Costa, K. C., T. J. Lie, M. A. Jacobs and J. A. Leigh (2013). "H2-independent growth of the hydrogenotrophic methanogen Methanococcus maripaludis." MBio **4**(2).

Daniel, S. L., T. Hsu, S. I. Dean and H. L. Drake (1990). "Characterization of the H2- and CO-dependent chemolithotrophic potentials of the acetogens Clostridium thermoaceticum and Acetogenium kivui." J Bacteriol **172**(8): 4464-4471.

Daniels, L., G. Fuchs, R. K. Thauer and J. G. Zeikus (1977). "Carbon monoxide oxidation by methanogenic bacteria." J Bacteriol **132**(1): 118-126.

Dashekvicz, M. P. and R. L. Uffen (1979). "Identification of a Carbon Monoxide Metabolizing Bacterium as a Strain of Rhodopseudomonas-Gelatinosa (Molisch) Vanniel." International Journal of Systematic Bacteriology **29**(2): 145-148.

de Poorter, L. M., W. J. Geerts and J. T. Keltjens (2007). "Coupling of Methanothermobacter thermautotrophicus methane formation and growth in fed-batch and continuous cultures under different H2 gassing regimens." Appl Environ Microbiol **73**(3): 740-749.

Diekert, G. B. and R. K. Thauer (1978). "Carbon monoxide oxidation by Clostridium thermoaceticum and Clostridium formicoaceticum." J Bacteriol **136**(2): 597-606.

Dioum, E. M., J. Rutter, J. R. Tuckerman, G. Gonzalez, M. A. Gilles-Gonzalez and S. L. McKnight (2002). "NPAS2: a gas-responsive transcription factor." Science **298**(5602): 2385-2387.

Eikmanns, B., G. Fuchs and R. K. Thauer (1985). "Formation of carbon monoxide from CO2 and H2 by Methanobacterium thermoautotrophicum." Eur J Biochem **146**(1): 149-154.

Ensign, S. A. and P. W. Ludden (1991). "Characterization of the CO oxidation/H2 evolution system of Rhodospirillum rubrum. Role of a 22-kDa iron-sulfur protein in mediating electron transfer between carbon monoxide dehydrogenase and hydrogenase." J Biol Chem **266**(27): 18395-18403.

Farhoud, M. (2011). Disease Biology of Mitochondrial Complex-I: proteomics insights. PhD, Radboud University Nijmegen.

Ferry, J. G. (2010). "CO in methanogenesis." Annals of Microbiology **60**(1): 1-12.

Ferry, J. G. and D. J. Lessner (2008). "Methanogenesis in marine sediments." Ann N Y Acad Sci **1125**: 147-157.

Fischer, F., L. R. and W. K. (1931). "Biologische gasreaktionen. I. Mitteilung: die umsetzung des kohlenoxyds." Biochemische Zeitschrift(236): 21.

Fischer, F., L. R. and W. K. (1932). "Uber die bildung von essigsaure bei der biologischen umsetzung von kohlenoxyd und kohlensaure mit wasserstoff zu methan." Biochemische Zeitschrift(245): 11.

Gonzalez, J. M. and F. T. Robb (2000). "Genetic analysis of Carboxydothermus hydrogenoformans carbon monoxide dehydrogenase genes cooF and cooS." FEMS Microbiol Lett **191**(2): 243-247.

Henstra, A. M., J. Sipma, A. Rinzema and A. J. Stams (2007). "Microbiology of synthesis gas fermentation for biofuel production." Curr Opin Biotechnol **18**(3): 200-206.

Henstra, A. M. and A. J. Stams (2004). "Novel physiological features of Carboxydothermus hydrogenoformans and Thermoterrabacterium ferrireducens." Appl Environ Microbiol **70**(12): 7236-7240.

Hirsch, P. (1968). "Photosynthetic bacterium growing under carbon monoxide." Nature **217**(5128): 555-556.

Hungate, R. E. (1950). "The anaerobic mesophilic cellulolytic bacteria." Bacteriol Rev **14**(1): 1-49.

Hungate, R. E. and J. Macy (1973). "The Roll-Tube Method for Cultivation of Strict Anaerobes." Bulletins from the Ecological Research Committee(17): 123-126.

Hussain, A., S. R. Guiot, P. Mehta, V. Raghavan and B. Tartakovsky (2011). "Electricity generation from carbon monoxide and syngas in a microbial fuel cell." Appl Microbiol Biotechnol **90**(3): 827-836.

Jansen, K., R. K. Thauer, F. Widdel and G. Fuchs (1984). "Carbon Assimilation Pathways in Sulfate Reducing Bacteria - Formate, Carbon-Dioxide, Carbon-Monoxide, and Acetate Assimilation by Desulfovibrio-Baarsii." Arch Microbiol **138**(3): 257-262.

Jensen, A. and K. Finster (2005). "Isolation and characterization of Sulfurospirillum carboxydovorans sp. nov., a new microaerophilic carbon monoxide oxidizing epsilon Proteobacterium." Antonie Van Leeuwenhoek **87**(4): 339-353.

Jung, G. Y., H. O. Jung, J. R. Kim, Y. Ahn and S. Park (1999). "Isolation and characterization of Rhodopseudomonas palustris P4 which utilizes CO with the production of H-2." Biotechnology Letters **21**(6): 525-529.

Kaster, A. K., M. Goenrich, H. Seedorf, H. Liesegang, A. Wollherr, G. Gottschalk and R. K. Thauer (2011). "More than 200 genes required for methane formation from H(2) and CO(2) and energy conservation are present in Methanothermobacter marburgensis and Methanothermobacter thermautotrophicus." Archaea **2011**: 973848.

Kerby, R., W. Niemczura and J. G. Zeikus (1983). "Single-carbon catabolism in acetogens: analysis of carbon flow in Acetobacterium woodii and Butyribacterium methylotrophicum by fermentation and 13C nuclear magnetic resonance measurement." J Bacteriol **155**(3): 1208-1218.

Kim, D. and I. S. Chang (2009). "Electricity generation from synthesis gas by microbial processes: CO fermentation and microbial fuel cell technology." Bioresour Technol **100**(19): 4527-4530.

Klemps, R., H. Cypionka, F. Widdel and N. Pfennig (1985). "Growth with hydrogen, and further physiological characteristics of Desulfotomaculum species." Arch Microbiol **143**(2): 203-208.

Kluyver, A. J. and C. G. Schnellen (1947). "On the fermentation of carbon monoxide by pure cultures of methane bacteria." Arch Biochem **14**(1-2): 57-70.

Lantzsch, K. (1922). "Actinomyces oligocarbophilus, sein Formwechsel und seine Physiologie." Zentralblat fur Bakteriologie(57): 11.

Lessner, D. J., L. Li, Q. Li, T. Rejtar, V. P. Andreev, M. Reichlen, K. Hill, J. J. Moran, B. L. Karger and J. G. Ferry (2006). "An unconventional pathway for reduction of CO2 to methane in CO-grown Methanosarcina acetivorans revealed by proteomics." Proc Natl Acad Sci U S A **103**(47): 17921-17926.

Liou, J. S., D. L. Balkwill, G. R. Drake and R. S. Tanner (2005). "Clostridium carboxidivorans sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen Clostridium scatologenes strain SL1 as Clostridium drakei sp. nov." Int J Syst Evol Microbiol **55**(Pt 5): 2085-2091.

Ljungdahl, L. G. (1986). "The autotrophic pathway of acetate synthesis in acetogenic bacteria." Annu Rev Microbiol **40**: 415-450.

Lorowitz, W. H. and M. P. Bryant (1984). "Peptostreptococcus-Productus Strain That Grows Rapidly with Co as the Energy-Source." Appl Environ Microbiol **47**(5): 961-964.

Lupton, F. S., R. Conrad and J. G. Zeikus (1984). "Co Metabolism of Desulfovibrio-Vulgaris Strain Madison - Physiological-Function in the Absence or Presence of Exogeneous Substrates." FEMS Microbiol Lett **23**(2-3): 263-268.

Maeder, D. L., I. Anderson, T. S. Brettin, D. C. Bruce, P. Gilna, C. S. Han, A. Lapidus, W. W. Metcalf, E. Saunders, R. Tapia and K. R. Sowers (2006). "The Methanosarcina barkeri genome: comparative analysis with Methanosarcina acetivorans and Methanosarcina mazei reveals extensive rearrangement within methanosarcinal genomes." J Bacteriol **188**(22): 7922-7931.

Markowitz, V. M., I.-M. A. Chen, K. Palaniappan, E. S. Ken Chu, Y. Grechkin, A. Ratner, B. Jacob, J. Huang, P. Williams, M. Huntemann, I. Anderson, K. Mavromatis, N. N. Ivanova and N. C. Kyrpides (2014). IMG: the integrated microbial genomes database and comparative analysis system

Maron, P. A., L. Ranjard, C. Mougel and P. Lemanceau (2007). "Metaproteomics: a new approach for studying functional microbial ecology." Microbial Ecology **53**(3): 486-493.

Matschiavelli, N., E. Oelgeschlager, B. Cocchiararo, J. Finke and M. Rother (2012). "Function and regulation of isoforms of carbon monoxide dehydrogenase/acetyl coenzyme A synthase in Methanosarcina acetivorans." J Bacteriol **194**(19): 5377-5387.

Mehta, P., A. Hussain, B. Tartakovsky, V. Neburchilov, V. Raghavan, H. Wang and S. R. Guiot (2010). "Electricity generation from carbon monoxide in a single chamber microbial fuel cell." Enzyme and Microbial Technology **46**(6): 450-455.

Meyer, O. and K. Fiebig (1985). Enzymes Oxidizing Carbon Monoxide. Gas Enzymology. H. Degn, R. P. Cox and H. Toftlund, Springer Netherlands**:** 147-168.

Meyer, O. and H. G. Schlegel (1983). "Biology of aerobic carbon monoxide-oxidizing bacteria." Annu Rev Microbiol **37**: 277-310.

Mohammadi, M., G. D. Najafpour, H. Younesi, P. Lahijani, M. H. Uzir and A. R. Mohamed (2011). "Bioconversion of synthesis gas to second generation biofuels: A review." Renewable & Sustainable Energy Reviews **15**(9): 4255-4273.

Mörsdorf, G., K. Frunzke, D. Gadkari and O. Meyer (1992). "Microbial growth on carbon monoxide." Biodegradation **3**(1): 61-82.

Nakamura, K., T. Terada, Y. Sekiguchi, N. Shinzato, X. Y. Meng, M. Enoki and Y. Kamagata (2006). "Application of pseudomurein endoisopeptidase to fluorescence in situ hybridization of methanogens within the family Methanobacteriaceae." Appl Environ Microbiol **72**(11): 6907-6913.

O'Brien, J. M., R. H. Wolkin, T. T. Moench, J. B. Morgan and J. G. Zeikus (1984). "Association of hydrogen metabolism with unitrophic or mixotrophic growth of Methanosarcina barkeri on carbon monoxide." J Bacteriol **158**(1): 373-375.

O'Farrell, P. H. (1975). "High resolution two-dimensional electrophoresis of proteins." J Biol Chem **250**(10): 4007-4021.

Oelgeschlager, E. and M. Rother (2008). "Carbon monoxide-dependent energy metabolism in anaerobic bacteria and archaea." Arch Microbiol **190**(3): 257-269.

Parshina, S. N., S. Kijlstra, A. M. Henstra, J. Sipma, C. M. Plugge and A. J. Stams (2005). "Carbon monoxide conversion by thermophilic sulfate-reducing bacteria in pure culture and in co-culture with Carboxydothermus hydrogenoformans." Appl Microbiol Biotechnol **68**(3): 390-396.

Parshina, S. N., J. Sipma, Y. Nakashimada, A. M. Henstra, H. Smidt, A. M. Lysenko, P. N. Lens, G. Lettinga and A. J. Stams (2005). "Desulfotomaculum carboxydivorans sp. nov., a novel sulfate-reducing bacterium capable of growth at 100% CO." Int J Syst Evol Microbiol **55**(Pt 5): 2159-2165.

Ragsdale, S. W. (2004). "Life with carbon monoxide." Crit Rev Biochem Mol Biol **39**(3): 165-195.

Ragsdale, S. W., J. E. Clark, L. G. Ljungdahl, L. L. Lundie and H. L. Drake (1983). "Properties of purified carbon monoxide dehydrogenase from Clostridium thermoaceticum, a nickel, iron-sulfur protein." J Biol Chem **258**(4): 2364-2369.

Rother, M. and W. W. Metcalf (2004). "Anaerobic growth of Methanosarcina acetivorans C2A on carbon monoxide: an unusual way of life for a methanogenic archaeon." Proc Natl Acad Sci U S A **101**(48): 16929-16934.

Rother, M., E. Oelgeschlager and W. M. Metcalf (2007). "Genetic and proteomic analyses of CO utilization by Methanosarcina acetivorans." Arch Microbiol **188**(5): 463-472.

Santiago, B., U. Schubel, C. Egelseer and O. Meyer (1999). "Sequence analysis, characterization and CO-specific transcription of the cox gene cluster on the megaplasmid pHCG3 of Oligotropha carboxidovorans." Gene **236**(1): 115-124.

Schauder, R., A. Preuss, M. Jetten and G. Fuchs (1989). "Oxidative and Reductive Acetyl Coa Carbon Monoxide Dehydrogenase Pathway in Desulfobacterium-Autotrophicum .2. Demonstration of the Enzymes of the Pathway and Comparison of Co Dehydrogenase." Arch Microbiol **151**(1): 84-89.

Sipma, J., A. M. Henstra, S. M. Parshina, P. N. Lens, G. Lettinga and A. J. Stams (2006). "Microbial CO conversions with applications in synthesis gas purification and bio-desulfurization." Crit Rev Biotechnol **26**(1): 41-65.

Sowers, K. R., S. F. Baron and J. G. Ferry (1984). "Methanosarcina acetivorans sp. nov., an Acetotrophic Methane-Producing Bacterium Isolated from Marine Sediments." Appl Environ Microbiol **47**(5): 971-978.

Stephenson, M. and L. H. Stickland (1933). "Hydrogenase: The bacterial formation of methane by the reduction of one-carbon compounds by molecular hydrogen." Biochem J **27**(5): 1517-1527.

Stupperich, E., K. E. Hammel, G. Fuchs and R. K. Thauer (1983). "Carbon monoxide fixation into the carboxyl group of acetyl coenzyme A during autotrophic growth of Methanobacterium." FEBS Lett **152**(1): 21-23.

Svetlichny, V. A., T. G. Sokolova, M. Gerhardt, N. A. Kostrikina and G. A. Zavarzin (1991). "Anaerobic extremely thermophilic carboxydotrophic bacteria in hydrotherms of Kuril Islands." Microbial Ecology **21**(1): 1-10.

Svetlitchnyi, V., C. Peschel, G. Acker and O. Meyer (2001). "Two membrane-associated NiFeS-carbon monoxide dehydrogenases from the anaerobic carbon-monoxide-utilizing eubacterium Carboxydothermus hydrogenoformans." J Bacteriol **183**(17): 5134-5144.

Techtmann, S. M., A. S. Colman and F. T. Robb (2009). "'That which does not kill us only makes us stronger': the role of carbon monoxide in thermophilic microbial consortia." Environ Microbiol **11**(5): 1027-1037.

Techtmann, S. M., A. V. Lebedinsky, A. S. Colman, T. G. Sokolova, T. Woyke, L. Goodwin and F. T. Robb (2012). "Evidence for horizontal gene transfer of anaerobic carbon monoxide dehydrogenases." Front Microbiol **3**: 132.

Tersteegen, A. and R. Hedderich (1999). "Methanobacterium thermoautotrophicum encodes two multisubunit membrane-bound [NiFe] hydrogenases. Transcription of the operons and sequence analysis of the deduced proteins." Eur J Biochem **264**(3): 930-943.

Thauer, R. K., K. Jungermann and K. Decker (1977). "Energy conservation in chemotrophic anaerobic bacteria." Bacteriol Rev **41**(1): 100-180.

Tiquia-Arashiro, S. (2014). Biotechnological Applications of Thermophilic Carboxydotrophs. Thermophilic Carboxydotrophs and their Applications in Biotechnology, Springer International Publishing**:** 29-101.

Uffen, R. L. (1976). "Anaerobic Growth of a Rhodopseudomonas Species in Dark with Carbon-Monoxide as Sole Carbon and Energy Substrate." Proc Natl Acad Sci U S A **73**(9): 3298-3302.

Uffen, R. L. (1981). "Metabolism of Carbon-Monoxide." Enzyme and Microbial Technology **3**(3): 197-206.

Valentine, D. L. (2007). "Adaptations to energy stress dictate the ecology and evolution of the Archaea." Nat Rev Microbiol **5**(4): 316-323.

Verma, A., D. Hirsch, C. Glatt, G. Ronnett and S. Snyder (1993). "Carbon monoxide: a putative neural messenger." Science **259**(5093): 381-384.

Wang, V. C., S. W. Ragsdale and F. A. Armstrong (2013). "Investigations of two bidirectional carbon monoxide dehydrogenases from Carboxydothermus hydrogenoformans by protein film electrochemistry." Chembiochem **14**(14): 1845-1851.

Wood, H. G. (1991). "Life with CO or CO2 and H2 as a source of carbon and energy." FASEB J **5**(2): 156-163.

Yagi, T. (1958). "Enzymic Oxidation of Carbon Monoxide." Biochim Biophys Acta **30**(1): 194-195.

Yagi, T. (1959). "Enzymic Oxidation of Carbon Monoxide .2." Journal of Biochemistry **46**(7): 949-955.

Yagi, T. and N. Tamiya (1962). "Enzymic Oxidation of Carbon Monoxide .3. Reversibility." Biochim Biophys Acta **65**(3): 508-&.

Yang, H. C. and H. L. Drake (1990). "Differential-Effects of Sodium on Hydrogen-Dependent and Glucose-Dependent Growth of the Acetogenic Bacterium Acetogenium-Kivui." Appl Environ Microbiol **56**(1): 81-86.

Zhao, Y., R. Cimpoia, Z. Liu and S. R. Guiot (2011). "Kinetics of CO conversion into H2 by Carboxydothermus hydrogenoformans." Appl Microbiol Biotechnol **91**(6): 1677-1684.

Zhou, Y., A. E. Dorchak and S. W. Ragsdale (2013). "In vivo activation of methyl-coenzyme M reductase by carbon monoxide." Front Microbiol **4**: 69.

Zhu, W., C. I. Reich, G. J. Olsen, C. S. Giometti and J. R. Yates, 3rd (2004). "Shotgun proteomics of Methanococcus jannaschii and insights into methanogenesis." J Proteome Res **3**(3): 538-548.

Annex I

Table 8 - Sequence alignment results for putative CODH gene coding results in reference to the CODH of *M. methanothermobacter thermoautotrophicus*.

Homolog	Product Name	Percent Identity	Alignment On Query Gene	Alignment On Subject Gene	Length	E-value	Bit Score	Genome	Scaffold ID	Scaffold GC
640792748	acetyl-CoA decarbonylase/synthase, subunit alpha	93.70%			777aa	0.0e+00	1503	Methanothermobacter marburgensis Marburg DSM 2133	NC_014408	0.49
650872298	acetyl-CoA decarbonylase/synthase complex subunit alpha	73.50%			783aa	0.0e+00	1219	Methanobacterium sp. SWAN-1	NC_015574	0.36
650750581	acetyl-CoA decarbonylase/synthase complex subunit alpha	73.10%			781aa	0.0e+00	1209	Methanobacterium sp. AL-21	NC_015216	0.36
2563556927	CO dehydrogenase/acetyl-CoA synthase complex subunit alpha	71.30%			778aa	0.0e+00	1178	Methanococcus maripaludis S2	BX950229	0.33
640166306	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	71.20%			778aa	0.0e+00	1178	Methanococcus maripaludis C5	NC_009135	0.33
649738126	acetyl-CoA decarbonylase/synthase alpha subunit	70.80%			774aa	0.0e+00	1143	Methanothermus fervidus V24S, DSM 2088	NC_014658	0.32

641284174	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	70.40%			778aa	0.0e+00	1165	Methanococcus maripaludis C6	NC_009975	0.33
640785975	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	70.30%			779aa	0.0e+00	1151	Methanococcus vannielii SB	NC_009634	0.31
2511671884	acetyl-CoA decarbonylase/synthase complex subunit alpha	70.30%			779aa	0.0e+00	1152	Methanococcus maripaludis X1	NC_015847	0.33
640792748	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	70.20%			778aa	0.0e+00	1164	Methanococcus maripaludis C7	NC_009637	0.33
650857088	acetyl-CoA decarbonylase/synthase complex subunit alpha	62.60%			782aa	2.8e-293	1013	Methanotorris igneus Kol5, DSM 5666	NC_015562	0.32
638201440	acetyl-CoA decarbonylase/synthase, subunit alpha (cdhA)	62.30%			774aa	3.6e-288	996	Methanocaldococcus jannaschii DSM 2661	NC_000909	0.31
646622637	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	61.80%			773aa	4.8e-285	986	Methanocaldococcus sp. FS406-22	NC_013887	0.32
644969638	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	61.70%			774aa	6.6e-288	995	Methanocaldococcus fervens AG86	NC_013156	0.32
646367701	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	61.00%			774aa	3.5e-281	973	Methanocaldococcus vulcanius M7, DSM 12094	NC_013407	0.31
650917575	acetyl-CoA decarbonylase/synthase complex subunit alpha	58.90%			774aa	5.9e-275	952	Methanothermococcus okinawensis IH1	NC_015636	0.29

637896327	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	57.00%			787aa	1.9e-255	887	Methanospirillum hungatei JF-1	NC_007796	0.45
640788579	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	57.00%			786aa	1.4e-268	931	Methanococcus aeolicus Nankai-3	NC_009635	0.30
646777846	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	56.60%			758aa	1.9e-257	894	Methanocaldococcus infernus ME	NC_014122	0.34
2509038682	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	56.40%			783aa	1.7e-256	891	Methanoregula formicica SMSP, DSM 22288	Metfor_Contig51.1	0.55
648151855	acetyl-CoA decarbonylase/synthase complex, alpha subunit	55.90%			139aa	2.7e-38	166	Methanothermobacter marburgensis Marburg DSM 2133	NC_014408	0.49
640868606	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	55.90%			786aa	8.9e-253	879	Methanoregula boonei 6A8	NC_009712	0.55
2505970200	CO dehydrogenase/acetyl-CoA synthase alpha subunit	55.40%			770aa	4.7e-254	883	Methanocella arvoryzae MRE50 (reannotation)	LRC50LV__640427159standard	0.55
2504145711	Acetyl-CoA decarbonylase/synthase complex subunit alpha	53.80%			797aa	1.8e-239	834	Archaeoglobus veneficus SNP6, DSM 11195	Aven11195_unknown1	0.47
2522261782	acetyl-CoA decarbonylase/synthase alpha subunit (EC 1.2.99.2)	53.10%			800aa	3.4e-236	823	Archaeoglobus sulfaticallidus PM70-1, DSM 19444 (Asulf_version 1)	Asulf_Archaeoglobus.1	0.43
641592958	CO dehydrogenase/acetyl-CoA synthase complex, epsilon	52.40%			766aa	3.8e-235	820	Candidatus Desulforudis audaxviator MP104C	NC_010424	0.61

	subunit									
2588655581	acetyl-CoA decarbonylase/synthase alpha subunit (EC 1.2.99.2)	52.10%			798aa	1.3e-232	812	Archaeoglobus fulgidus DSM 8774	CP006577	0.48
2509662624	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	52.10%			804aa	1.9e-234	818	Methanomethylovorans hollandica DSM 15978	Metho_Contig 141.1	0.43
640787866	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	52.10%			768aa	7.1e-242	842	Methanococcus aeolicus Nankai-3	NC_009635	0.30
638158998	acetyl-CoA decarbonylase/synthase, subunit alpha (cdhA-2)	52.00%			798aa	9.0e-229	799	Archaeoglobus fulgidus VC-16, DSM 4304	AE000782	0.49
638168739	CO dehydrogenase/acetyl-CoA synthase alpha subunit	52.00%			760aa	6.7e-232	809	Methanopyrus kandleri AV19	AE009439	0.61
648151854	acetyl-CoA decarbonylase/synthase complex, alpha subunit	51.30%			311aa	9.7e-74	284	Methanothermobacter marburgensis Marburg DSM 2133	NC_014408	0.49
646611862	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	51.30%			804aa	2.1e-224	784	Ferroglobus placidus AEDII12DO, DSM 10642	NC_013849	0.44
638157644	acetyl-CoA decarbonylase/synthase, subunit alpha (cdhA-1)	51.20%			802aa	6.6e-226	789	Archaeoglobus fulgidus VC-16, DSM 4304	AE000782	0.49
648055108	CO dehydrogenase/acetyl-CoA synthase complex subunit epsilon	51.20%			801aa	8.4e-235	819	Methanohalobium evestigatum Z-7303, DSM 3721	NC_014253	0.37
2588654165	acetyl-CoA decarbonylase/synthase alpha subunit (EC 1.2.99.2)	51.00%			802aa	9.3e-226	789	Archaeoglobus fulgidus DSM 8774	CP006577	0.48

2519474196	CO dehydrogenase/acetyl-CoA synthase complex subunit epsilon	51.00%			802aa	6.6e-234	816	Methanolobus psychrophilus R15	CP003083	0.45
646707218	acetyl-CoA decarbonylase/synthase alpha subunit	50.60%			801aa	1.8e-232	811	Methanohalophilus mahii SLP, DSM 5219	NC_014002	0.43
2502871140	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	49.50%			803aa	5.8e-230	803	Methanosalsum zhilinae WeN5, DSM 4017	Sent_unknown	0.39
637958643	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	49.20%			802aa	3.1e-223	780	Methanococcoides burtonii DSM 6242	NC_007955	0.41
638178729	carbon-monoxide dehydrogenase, subunit alpha	48.20%			805aa	2.1e-216	758	Methanosarcina acetivorans C2A	AE010299	0.43
639699298	CO dehydrogenase/acetyl-CoA synthase complex, epsilon subunit	48.00%			805aa	2.9e-221	774	Methanosaeta thermophila PT	NC_008553	0.54
638166633	CO dehydrogenase/acetyl-COA synthase alpha subunit	47.90%			806aa	6.7e-216	756	Methanosarcina mazei Go1, DSM 3647	AE008384	0.41
650797560	CO dehydrogenase/acetyl-CoA synthase complex subunit alpha	47.80%			793aa	2.2e-214	751	Methanosaeta concilii GP-6	NC_015416	0.51
638179299	carbon-monoxide dehydrogenase, subunit alpha	47.80%			805aa	1.8e-216	758	Methanosarcina acetivorans C2A	AE010299	0.43
637699328	acetyl-CoA decarbonylase/synthase complex subunit alpha	47.50%			806aa	7.6e-214	749	Methanosarcina barkeri Fusaro, DSM 804	NC_007355	0.39
2540562562	acetyl-CoA decarbonylase/synthase complex subunit alpha	47.30%			807aa	2.9e-213	747	Methanosarcina mazei Tuc01	CP004144	0.42

638165176	CO dehydrogenase/acetyl-COA synthase alpha subunit	47.30%			807aa	1.8e-213	748	Methanosarcina mazei Go1, DSM 3647	AE008384	0.41
638175771	carbon-monoxide dehydrogenase, subunit alpha	47.20%			806aa	4.0e-215	753	Methanosarcina acetivorans C2A	AE010299	0.43
637699095	acetyl-CoA decarbonylase/synthase complex subunit alpha	47.00%			806aa	1.1e-213	749	Methanosarcina barkeri Fusaro, DSM 804	NC_007355	0.39
2512009248	Acetyl-CoA decarbonylase/synthase alpha subunit	46.80%			809aa	4.6e-217	760	Methanosaeta harundinacea 6Ac	CP003117	0.61
648151853	acetyl-CoA decarbonylase/synthase complex, alpha subunit	46.60%			395aa	1.8e-96	359	Methanothermobacter marburgensis Marburg DSM 2133	NC_014408	0.49
2512010787	Acetyl-CoA decarbonylase/synthase complex subunit alpha	46.50%			811aa	2.6e-214	751	Methanosaeta harundinacea 6Ac	CP003117	0.61

Annex II

Table 9 – Protein detected by LC-MS/MS for *Methanothermobacter thermautotrophicus* for high and low availability of H_2 as electron donor (data from (Farhoud 2011)). Proteins known to be involved in H_2 activation are marked in aqua, proteins involved in the methanogenesis pathway from CO_2 are marked in green and genes involved in CO oxidation into CO_2 are marked in red

General information			Proteomics			
locus tag	Gi	Product name	LC-MS/MS H_2 membrane	LC-MS/MS H_2 cytoplasm	LC-MS/MS Low H_2 membrane	LC-MS/MS Low H_2 cytoplasm
MTH1	15678032	conserved protein; predicted RNA-binding protein		1		1
MTH2	15678033	50S ribosomal protein L3 (E.coli L3P)	1	1	1	1
MTH3	15678034	50S ribosomal protein L4 (E.coli L4) (L1E)	1	1	1	1
MTH4	15678035	50S ribosomal protein L23a (E.coli L23P)				1
MTH5	15678036	50S ribosomal protein L8 (E.coli L2P)	1	1	1	
MTH6	15678037	30S ribosomal protein S15 (E.coli S19P)		1	1	
MTH7	15678038	50S ribosomal protein L17 (E.coli L22P)		1	1	1
MTH8	15678039	30S ribosomal protein S3 (E.coli S3P)	1	1	1	1
MTH12	15678043	30S ribosomal protein S11 (E.coli S17P)	1	1	1	1
MTH13	15678044	50S ribosomal protein L23 (E.coli L14P)		1		1
MTH14	15678045	50S ribosomal protein L26 (E.coli L24P)	1	1		1
MTH15	15678046	30S ribosomal protein S4e	1	1	1	1
MTH16	15678047	50S ribosomal protein L11 (E.coli L5P)	1	1	1	1
MTH18	15678049	30S ribosomal protein S15a (E.coli S8P)		1		1
MTH19	15678050	50S ribosomal protein L9 (E.coli L6P)	1	1	1	1
MTH20	15678051	50S ribosomal protein L32E				1
MTH21	15678052	50S ribosomal protein L19E			1	
MTH22	15678053	50S ribosomal protein L5 (L18P)	1	1	1	1
MTH23	15678054	30S ribosomal protein S2 (E.coli S5P)	1	1	1	1
MTH24	15678055	50S ribosomal protein L7 (E.coli L30P)		1		1
MTH25	15678056	50S ribosomal protein L27a (E.coli L15P)	1	1	1	
MTH26	15678057	preprotein translocase subunit secY; protein transport protein SEC61 subunit alpha homolog		1		1
MTH27	15678058	adenylate kinase	1	1	1	
MTH28	15678059	conserved archaea-specific membrane-spanning protein MTH28 (COG1422, DUF107)		1		
MTH30	15678061	cytidylate kinase		1		1
MTH31	15678062	50S ribosomal protein L14E			1	
MTH34	15678064	30S ribosomal protein S18P (E.coli S13P)	1	1	1	
MTH35	15678065	30S ribosomal protein S9 (E.coli S4P)		1	1	
MTH36	15678066	30S ribosomal protein S14 (E.coli S11P)		1		1
MTH37	15678067	DNA-dependent RNA polymerase, subunit D (RpoD)		1		1
MTH38	15678068	50S ribosomal protein L18E (E.coli L17)		1		1
MTH39	15678069	30S ribosomal protein S16 (E.coli S9); fused L13/S9 ribosomal protein	1	1	1	1
MTH43	15678072	enolase (eno)	1	1	1	1
MTH44	15678073	30S ribosomal protein Sa (E.coli S2P)	1	1	1	1
MTH45	15678074	conserved protein (UPF0103); DNA-repair protein MutT related		1		1
MTH46	15678075	mevalonate kinase		1		
MTH47	15678076	mevalonate-5-phosphate kinase, phosphomevalonate kinase (PMVK)		1		1
MTH48	15678077	isopentenyl-diphosphate delta-isomerase (EC 5.3.3.2) (IPP isomerase)		1		1
MTH49	15678078	predicted mevalonatediphosphate decarboxylase, diphosphomevalonate decarboxylase	1	1	1	1
MTH50	15678079	bifunctional short chain isoprenyl diphosphate synthase (IdsA)	1	1	1	
MTH51	15678080	glutamyl-tRNA synthetase	1	1	1	
MTH52	15678081	LL-diaminopimelate aminotransferase	1	1	1	1
MTH54	15678083	conserved methanogen-specific membrane protein MTH54 containing metal chelatase domain (COG5643)	1	1		
MTH55	15678084	conserved methanogen-specific protein MTH55 containing cell surface anchor domain (COG5643)	1	1		

MTH56	15678085	conserved methanogen-specific protein MTH56 (COG5643)		1		
MTH59	15678088	conserved membrane-bound protein MTH59 associated with fimbrin MTH60		1		1
MTH60	15678089	fimbrin (fimbriae protein) (verified)		1		1
MTH63	15678092	conserved protein (gamma-carboxymuconolactone decarboxylase homolog; alkylhydroperoxidase AhpD core)		1		
MTH72	15678100	O-linked GlcNAc transferase containing TPR-domain (11x)		1		1
MTH75	15678103	membrane-associated cell wall-binding surface protease (double gly) related protein (pkd repeat; pseudomurein binding repeat, PF09373)		1		
MTH87	15678115	cell wall-binding surface (double gly) protease related protein (pkd repeat; pseudomurein binding repeat, PF09373)		1		
MTH101	15678129	conserved methanogen-specific protein MTH101 (COG4883)	1	1		1
MTH102	15678130	conserved methanogen-specific small zinc-finger protein (COG4855)	1			
MTH105	15678133	glutamate synthase (NADPH), alpha subunit		1	1	1
MTH106	15678134	tungsten formylmethanofuran dehydrogenase, subunit C homolog; glutamate synthase subunit (COG0070)		1		
MTH115	15678143	conserved protein MTH115 (SOUL heme-binding protein; pfam04832)	1			1
MTH117	15678145	membrane-associated cell surface protein containing pseudomurein binding repeat (PF09373)		1		1
MTH119	15678147	ATP phosphoribosyltransferase related protein	1	1	1	1
MTH120	15678148	conserved protein (NADP oxidoreductase/nitroreductase; FMN binding) (COG0778)		1		
MTH121	15678149	conserved (Methanosphaera) small zinc-finger protein	1	1		1
MTH126	15678154	inosine-5'-monophosphate dehydrogenase related protein VII (contains 2xCBS-domain; HcrA-ArsR regulat. domain)		1		1
MTH129	15678157	orotidine 5' monophosphate decarboxylase (PyrF)		1		
MTH133	15678161	cobalt transport ATP-binding protein O (CbiO)	1			1
MTH134	15678162	riboflavin synthase (EC 2.5.1.9)		1		
MTH135	15678163	NADP-dependent FMN oxidoreductase, multimeric flavodoxin (WrbA family)		1	1	
MTH138	15678166	glycosyl transferase (GT4 family); GlcNAc-phosphatidylinositol related biosynthetic protein		1		
MTH140	15678168	conserved protein (4Fe-4S domain) (COG1139, DUF162), predicted fumarate reductase subunit		1		1
MTH141	15678169	conserved protein (COG1891, UPF0264, DUF556); methanopterin/ methanofuran biosynthesis protein		1		1
MTH142	15678170	inosine-5'-monophosphate dehydrogenase (guaB)	1	1	1	1
MTH143	15678171	molybdopterin-guanine dinucleotide biosynthesis MobA related protein		1		
MTH145	15678173	conserved membrane-bound protein MTH145		1	1	1
MTH146	15678174	precorrin-8W decarboxylase (CbiT), precorrin methyltransferase	1			
MTH147	15678175	aromatic acid decarboxylase (EC 4.1.1.-); 3-octaprenyl-4-hydroxybenzoate carboxy-lyase	1	1		1
MTH149	15678177	molybdenum cofactor biosynthesis protein MoaE		1		
MTH150	15678178	nicotinamide mononucleotide adenyltransferase (NadM)		1		
MTH151	15678179	methyl coenzyme M reductase system, component A2 homolog; ATPase subunit of an ABC-type transport system with duplicated ATPase domain	1	1		
MTH153	15678181	universal stress protein (UspA); ATP-dependent chaperone		1		1
MTH155	15678183	rubredoxin		1		1
MTH157	15678185	flavoprotein A homolog (III); F420H2 oxidase (III)		1		
MTH158	15678186	ferritin-like protein (RsgA)	1	1		1
MTH159	15678187	alkyl hydroperoxide reductase, probable peroxiredoxin (PRX)	1	1		1
MTH160	15678188	superoxide dismutase [Fe] (EC 1.15.1.1).	1	1		
MTH161	15678189	coenzyme F390 synthetase III (FtsAIII)		1		
MTH162	15678190	regulatory subunit (ACT domain family) linked with coenzyme F390 synthetase III		1		1
MTH163	15678191	helix-turn-helix transcriptional regulator (type 11 domain; Crp/Fnr family)		1		1
MTH167	15678195	S-adenosyl-L-methionine uroporphyrinogen methyltransferase (CysGB)	1	1		1
MTH168	15678196	phosphoribosylformylglycinamidine synthase I, PurQ subunit		1		1
MTH169	15678197	phosphoribosylformylglycinamide synthase I, PurS subunit		1		1
MTH170	15678198	phosphoribosylaminoimidazolesuccinocarboxamide synthase (SAICAR synthetase) (PurC)	1		1	
MTH171	15678199	glucosamine--fructose-6-phosphate aminotransferase [isomerizing] (EC 2.6.1.16); L-glutamine-D-fructose-6-phosphate amidotransferase/ glucosamine-6-phosphate synthase	1	1		1
MTH173	15678201	glycosyl transferase (GT4 family); LPS biosynthesis RfbU related protein		1		
MTH174	15678202	sensory transduction histidine kinase (PAS/PAC domain) MTH_proteome		1		
MTH176	15678204	7-cyano-7-deazaguanine tRNA-ribosyltransferase (EC 2.4.2.-); Archaeal		1		1

		tRNA-guanine transglycosylase				
MTH177	15678205	nascent polypeptide-associated complex (NAC) protein	1	1	1	1
MTH179	15678207	conserved methanogen-specific protein MTH179 containing zinc finger (CCCH-type)		1		
MTH180	15678208	conserved methanogen-specific membrane-bound protein MTH180		1		1
MTH183	15678211	acetylglutamate kinase (EC 2.7.2.8) (NAG kinase) (AGK); N- acetyl-L-glutamate 5-phosphotransferase	1	1		
MTH193	15678221	coenzyme F420-reducing hydrogenase, beta subunit homolog; putative glutamate synthase, subunit 2		1		
MTH194	15678222	glutamate synthase (F420 dependent), subunit 1		1		
MTH198	15678226	conserved zinc finger CDGSH-type domain protein MTH198 (COG3369, 2x)		1		
MTH199	15678227	conserved (Methanobrevibacter) membrane-spanning protein MTH199	1	1		
MTH204	15678232	conserved (N-terminal) membrane protein (COG1714) fused(C-terminal) with 5-formyl-H4folate cycloligase		1		
MTH205	15678233	hydrogenase expression/formation protein HypE (HypE)	1	1	1	
MTH207	15678235	30S ribosomal protein S8E	1	1	1	1
MTH209	15678237	conserved protein (HAD-superfamily hydrolase/phosphatase, COG1011)		1		1
MTH210	15678238	predicted AAA+ class ATPase with chaperone activity	1	1	1	1
MTH211	15678239	uncharacterized membrane-bound protein MTH211		1		
MTH212	15678240	exodeoxyribonuclease III (xthA); DNA uridine endonuclease		1		1
MTH213	15678241	ferrous iron transport protein B (FeoB)		1		1
MTH214	15678242	iron dependent transcriptional regulator (Fe2+-binding) (FeoA)		1		
MTH218	15678246	thermosome alpha subunit, chaperonine (HSP60) I	1	1	1	1
MTH219	15678247	uncharacterized protein MTH219 containing TonB-dependent receptor protein motif (PF00593)	1	1	1	1
MTH220	15678248	flavoprotein A homolog (II); F420H2 oxidase II	1	1		1
MTH222	15678250	conserved methanogen-specific hypothetical protein MTH222 (COG4740); predicted metalloprotease fused to aspartyl protease		1		1
MTH223	15678251	cyclic 2,3-diphosphoglycerate-synthetase	1	1		1
MTH226	15678254	aspartyl-tRNA synthetase	1	1	1	
MTH227	15678255	precorrin isomerase; precorrin-8X methylmutase (CbiC; CobH)		1		1
MTH228	15678256	glutamate-1-semialdehyde aminotransferase (HemL)		1		
MTH230	15678258	conserved Methanobacteriaceae-specific membrane-spanning protein MTH230 containing TPR repeat	1	1	1	1
MTH231	15678259	conserved methanogen-specific protein (COG4002) predicted phosphotransacetylase		1		
MTH232	15678260	undecaprenyl pyrophosphate synthetase (UPP synthetase) (Di-trans-poly-cis-decaprenylcistransferase)		1		1
MTH235	15678263	2,5-diamino-6-hydroxy-4-(5-phosphoribosylamino)pyrimidine 1-reductase (RibD) (EC 1.1.1.193)		1		
MTH236	15678264	conserved methanogen-specific protein MTH236 (COG1790, DUF483)		1		1
MTH237	15678265	hydrogenocobyrinic acid a,c-diamide cobaltochelatase (CobN)	1	1		
MTH241	15678269	replication factor C, small subunit	1	1	1	1
MTH242	15678270	shikimate 5-dehydrogenase (chorismate synthesis) (AroE)		1		
MTH243	15678271	conserved DNA-binding protein containing PIN domain (COG1458, UPF0278)	1	1	1	1
MTH244	15678272	histidyl-tRNA synthetase (HisRS)	1	1		1
MTH246	15678274	twitching mobility (PilT) related protein; bacterial type II secretion system ATPase	1	1	1	1
MTH247	15678275	conserved protein (COG1082); predicted TIM barrel sugar phosphate isomerase/epimerase or endonuclease (endonuclease IV)		1		
MTH248	15678276	F420-dependent NADP reductase	1	1	1	1
MTH249	15678277	hexulose-6-phosphate isomerase		1		1
MTH250	15678278	tRNA-splicing endonuclease (EC 3.1.27.9); tRNA-intron endonuclease			1	
MTH251	15678279	tryptophanyl-tRNA synthetase (TrpRS)	1	1	1	1
MTH253	15678281	threonine synthase (thrC)	1	1	1	1
MTH254	15678282	histone HMtB		1		1
MTH255	15678283	50S ribosomal protein L7Ae; snoRNA-dependent rRNA methylase		1		1
MTH256	15678284	30S ribosomal protein S28E		1		1
MTH257	15678285	50S ribosomal protein L24E		1		
MTH258	15678286	nucleoside diphosphate kinase	1	1	1	
MTH259	15678287	translation initiation factor IF2 homolog		1		1
MTH260	15678288	30S ribosomal protein S6e	1	1	1	1
MTH261	15678289	translation initiation factor eIF-2, gamma subunit	1	1	1	1
MTH263	15678291	inorganic pyrophosphatase	1	1	1	1
MTH264	15678292	DNA-dependent RNA polymerase, subunit E' (RpoE')		1		1
MTH265	15678293	DNA-dependent RNA polymerase, subunit E'' (RpoE'')		1		1

MTH267	15678295	30S ribosomal protein S24E		1		1
MTH269	15678297	argininosuccinate lyase (ArgH)		1		
MTH272	15678300	hexarepeat-containing acetyl transferase		1		
MTH273	15678301	phosphatidylethanolamine-binding protein, PBP family (COG1881)		1		1
MTH277	15678305	digeranylgeranylglycerophospholipid reductase/ FAD-dependent geranylgeranyl reductase (FixC)	1	1		
MTH280	15678308	coenzyme F420-dependent sulfite reductase (Fsr) (verified)		1	1	
MTH292	15678320	sensory transduction histidine kinase		1		1
MTH301	15678329	cell wall-binding surface protease related protein (pseudomurein binding repeat, PF09373)		1		1
MTH302	15678330	uncharacterized protein MTH302, putative adhesin		1		
MTH309	15678337	uncharacterized protein MTH309 (DUF587)		1		
MTH311	15678339	signal transduction histidine kinase (COG0642)		1		
MTH326	15678354	CRISPR-associated protein Csx11		1		
MTH333	15678361	GDP-mannose 4,6-dehydratase		1		1
MTH340	15678368	polysaccharide-pyruvyl transferase (S-layer biosynthesis)		1		1
MTH341	15678369	coenzyme F420-reducing hydrogenase, beta subunit homolog		1		
MTH346	15678374	formate acetyltransferase 2; pyruvate-formate lyase (COG1882)		1		
MTH351	15678379	membrane-bound metal chelatase belonging to the CobN/BchH family	1	1		
MTH357	15678385	cell wall-binding pseudomureine (pseudomurein binding repeat, PF09373) endopeptidase containing transglutaminase and GH18-type chitinase domains		1	1	1
MTH359	15678387	cell wall-binding pseudomureine endopeptidase (pseudomurein binding repeat, PF09373, 2x)				1
MTH360	15678388	sensory transduction histidine kinase		1		1
MTH361	15678389	teichoic acid biosynthesis protein RodC related protein; CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase		1		
MTH362	15678390	glycosyl transferase (GT4 family); capsular polysaccharide biosynthesis protein		1		
MTH365	15678393	teichoic acid biosynthesis protein RodC related protein; CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase		1		
MTH368	15678396	glycerol-3-phosphate dehydrogenase, NAD(P)		1		
MTH369	15678397	UDP-N-acetylglucosamine pyrophosphorylase related protein	1	1		1
MTH370	15678398	glycosyl transferase (GT4 family); LPS biosynthesis RfbU related protein/ N-acetylglucosaminyl-phosphatidylinositol biosynthetic protein		1		
MTH373	15678401	dTDP-glucose 4,6-dehydratase related protein	1	1	1	
MTH374	15678402	membrane-bound dolichyl-phosphate beta-D-mannosyltransferase	1	1		1
MTH375	15678403	UDP-glucose 4-epimerase homolog	1	1	1	1
MTH376	15678404	conserved protein (predicted glycosyltransferase, COG0438)	1	1		1
MTH389	15678417	conserved membrane protein (EhaF)		1		1
MTH397	15678425	formate hydrogenlyase, subunit 7 (EhaN); small subunit NiFe H2ase		1		1
MTH398	15678426	formate hydrogenlyase, subunit 5 (EhaO); large (alpha) subunit NiFe H2ase		1		1
MTH399	15678427	polyferredoxin (EhaP)		1		1
MTH400	15678428	polyferredoxin (EhaQ)		1		
MTH401	15678429	polyferredoxin (EhaQ)		1		1
MTH403	15678431	formylmethanofuran:tetrahydromethanopterin formyltransferase II (EhaS, ftr II)		1		1
MTH405	15678433	polyferredoxin		1		
MTH406	15678434	conserved membrane-bound protein containing signal transduction CBS-domain (2x)	1	1	1	1
MTH412	15678440	cell wall-binding pseudomureine endopeptidase (pseudomurein binding repeat, PF09373)		1		
MTH413	15678441	conserved protein, pyridoxamine 5'-phosphate oxidase-related (FMN-binding; COG3576)	1	1	1	
MTH414	15678442	asparagine synthetase (glutamine-hydrolyzing) AsnB		1		
MTH415	15678443	Gln/asn-dependent aspartyl-tRNA(Asn) amidotransferase subunit C (GatC)		1		
MTH416	15678444	predicted allosteric regulator of homoserine dehydrogenase	1	1	1	1
MTH417	15678445	homoserine dehydrogenase (thrA2, metL2) homolog		1		1
MTH419	15678447	CTP synthase (pyrG)		1		1
MTH421	15678449	membrane-bound prepilin-cleaving (type IV) signal peptidase (EppA/ TFPP-like signal peptidase)		1		1
MTH423	15678451	conserved archaeal membrane-spanning hypothetical protein MTH423 (COG4025)		1		1
MTH426	15678454	uncharacterized protein MTH426		1		1
MTH427	15678455	conserved membrane-spanning hypothetical protein MTH427		1		1
MTH428	15678456	uncharacterized membrane-bound protein MTH428		1		1
MTH429	15678457	conserved membrane-spanning protein MTH429		1		

MTH435	15678463	conserved archaeal protein (COG1701); pantothenate synthase (PS; PanC) (verified)		1		
MTH437	15678465	tetrahydral aminopeptidase (TET); family M42 deblocking aminopeptidase/ glutamyl aminopeptidase	1	1	1	1
MTH439	15678467	recombinase/ RecA superfamily ATPase		1		1
MTH440	15678468	sensory transduction regulatory protein contaning PAS/PAC domain	1	1	1	1
MTH442	15678470	excinuclease ABC subunit B (UvrB)		1		
MTH443	15678471	excinuclease ABC subunit A (UvrA)	1	1	1	1
MTH444	15678472	sensory transduction histidine kinase				1
MTH445	15678473	sensory transduction regulatory protein	1	1		
MTH446	15678474	sensory transduction regulatory protein containing PAS/PAC domain				1
MTH447	15678475	sensory transduction regulatory protein		1		
MTH477	15678505	tungstate transport ABC system ATP-binding (TupC)		1		1
MTH478	15678506	tungstate transport ABC system permease protein (TupB)		1		
MTH479	15678507	tungstate transport ABC system TupA protein	1	1	1	1
MTH480	15678508	conserved protein MTH480 containing ATP cone domain (PF03477)				1
MTH487	15678515	superfamily I DNA and RNA helicase; Very Short Patch Repair (Vsr), endonuclease subunit	1	1	1	1
MTH488	15678516	superfamily I DNA and RNA helicase; Very Short Patch Repair (Vsr), endonuclease subunit				1
MTH504	15678532	H(2)-dependent N5,N10-methylenetetrahydromethanopterin dehydrogenase homolog (III)		1		
MTH511	15678539	ATP-dependent DNA helicase II (UvrD, PcrA)		1		
MTH517	15678545	predicted regulator of CRISPR system (COG2462 fused to ArsR-HTH5 (COG1517)	1	1	1	1
MTH519	15678547	conserved Methanobacteriaceae-specific protein MTH519	1	1		1
MTH526	15678554	conserved protein MTH526 (COG2043, DUF169)	1	1	1	1
MTH527	15678555	uncharacterized protein MTH527		1		1
MTH528	15678556	uncharacterized MobA-related protein (COG2068); UDP-N-acetylglucosamine pyrophosphorylase/ N-acetylglucosamine 1-phosphuridyltransferase		1		1
MTH529	15678557	conserved methanogen-specific protein MTH529				1
MTH530	15678558	UDP-N-acetylmuramate-alanine ligase	1	1	1	1
MTH531	15678559	UDP-N-acetylmuramoylalanine-D-glutamate ligase	1		1	
MTH532	15678560	UDP-N-acetylmuramoylalanine-D-glutamate ligase homolog		1		
MTH540	15678568	DNA double-strand break repair rad50 ATPase (Rad50)		1		1
MTH541	15678569	Mla(2)- Mre11 fusion protein		1		
MTH542	15678570	DNA segregation ATPase Mla(1)/ FtsK (gene split)		1		
MTH544	15678572	conserved protein MTH544 (DUF74 UPF0145)		1	1	
MTH552	15678580	geranylgeranylglyceryl phosphate synthase		1		1
MTH554	15678582	conserved protein MTH554 (COG2042, DUF367, UPF0293)	1	1		
MTH556	15678584	protoporphyrin IX magnesium-chelatase subunit ChlI	1	1		
MTH562	15678590	3-hydroxy-3-methylglutaryl CoA reductase (HmgA)	1	1		1
MTH563	15678591	succinyl-CoA synthetase, alpha subunit (sucC)	1	1	1	1
MTH566	15678594	3-dehydroquinate dehydratase, chorismate synthesis (AroD)		1		1
MTH567	15678595	signal peptidase I		1		1
MTH569	15678597	heat shock protein X; protease htpX homolog (EC 3.4.24.-)		1		1
MTH570	15678598	conserved adhesin-like protein		1		1
MTH574	15678602	conserved flavoredoxin family protein (FMN binding, Ni binding) (COG1853)	1	1		
MTH579	15678607	fructose-1,6-bisphosphate aldolase/ 2-amino-3,7-dideoxy-D-threo-hept-6-ulosonate (ADTH) synthase (aroA')	1	1	1	1
MTH580	15678608	3-dehydroquinate synthase (AroB')	1	1	1	
MTH582	15678610	conserved protein related to HPr kinase (C-terminal part)		1		
MTH583	15678611	2',5'-RNA ligase	1	1		1
MTH584	15678612	CCA-adding enzyme/ tRNA CCA-pyrophosphorylase (tRNA adenylyl-/cytidylyl- transferase) (EC 2.7.7.25)		1		
MTH587	15678615	methionyl-tRNA synthetase (MetG)	1	1	1	
MTH592	15678620	RecA/ Rad55 superfamily ATPase involved in homologous recombination (COG0467)		1		1
MTH593	15678621	RecA/ Rad55 superfamily ATPase involved in homologous recombination (COG0467)		1		
MTH594	15678622	RecA/ Rad55 superfamily ATPase/GTPase (COG0467; COG2229)	1	1	1	
MTH595	15678623	conserved protein (COG2018); regulatory roadblock-LC7 domain		1		
MTH599	15678627	uncharacterized protein MTH599				1
MTH604	15678632	zinc (Mn) ABC transporter, periplasmic zinc-binding protein		1		
MTH605	15678633	zinc (Mn) ABC transporter, ATP-binding protein ABC transporter		1		

MTH611	15678639	prolyl-tRNA synthetase (+cysteinyl-tRNA)	1	1	1	1
MTH612	15678640	uncharacterized membrane-bound protein MTH612		1		
MTH613	15678641	conserved protein (COG1920, DUF121); GTP:lactate guanylyl transferase involved in F420 biosynthesis (CofC)		1		1
MTH614	15678642	phosphomethylpyrimidine kinase (HMPP-kinase) (ThiD/ transcriptional regulator)		1		
MTH615	15678643	adenylosuccinate synthetase (EC 6.3.4.4) (IMP-aspartate ligase) (PurA)		1		1
MTH616	15678644	predicted AAA+ class ATPase with chaperone activity			1	
MTH619	15678647	sensory transduction histidine kinase containing PAS/PAC domain		1		1
MTH622	15678650	thiosulfate sulfurtransferase; 3-mercaptopyruvate sulfurtransferase (rhodanese-related sulfurtransferase)		1		1
MTH627	15678655	partially conserved membrane-bound protein MTH627		1		
MTH631	15678659	UDP-glucose 4-epimerase		1		1
MTH632	15678660	conserved protein MTH632; putative (sugar) kinase (COG0464)		1		
MTH634	15678662	UDP-glucose pyrophosphorylase, UTP-glucose-1-phosphate uridylyltransferase (GalU)		1		1
MTH635	15678663	membrane-associated porin-like protein		1		1
MTH643	15678670	nitrogenase NifH subunit; nitrogenase iron protein (EC 1.18.6.1) (nitrogenase component II) (NifH)		1		1
MTH644	15678671	inosine-5'-monophosphate dehydrogenase related protein with regulatory CBS domain		1	1	1
MTH646	15678673	amidophosphoribosyltransferase (glutamine phosphoribosylpyrophosphate amidotransferase) (PurF)	1	1		
MTH647	15678674	topoisomerase-primase (TOPRIM)-domain-containing protein, potential nuclease (gene split with MTH838, MTH839)		1		1
MTH648	15678675	50S ribosomal protein L37E		1		
MTH649	15678676	small nuclear ribonucleoprotein (snRNP/Sm protein)		1		1
MTH650	15678677	RNA-binding translation factor (PUA domain)		1		1
MTH651	15678678	2-amino-5-formylamino-6-ribosylamino-4(3H)-pyrimidinone 5'-monophosphate formamide hydrolase		1		1
MTH653	15678680	putative membrane-bound dolichyl-phosphate-mannose-protein mannosyltransferase		1		1
MTH654	15678681	conserved methanogen-specific membrane-spanning protein;adhesin-like protein		1	1	1
MTH657	15678684	long-chain-fatty-acid-CoA ligase	1	1	1	1
MTH658	15678685	acyl-CoA thioesterase		1		1
MTH659	15678686	helix-turn-helix transcriptional regulator, XRE family (COG1396)	1	1	1	
MTH664	15678691	nitrogen regulatory protein P-II 2		1		1
MTH666	15678693	pyridoxal biosynthesis lyase pdxS (EC 4.-.-.-); pyridoxal-5-phosphate biosynthesis protein (pdx2)	1	1	1	
MTH670	15678697	conserved membrane-spanning protein MTH670		1		
MTH671	15678698	predicted B12 transporter (btuT)	1	1	1	1
MTH673	15678700	B12 transporter (BtuS; CbiN-related)	1	1		
MTH674	15678701	conserved protein MTH674 containing a metal-binding domain as in formylmethanofuran dehydrogenase subunit E (COG5643)	1	1		
MTH675	15678702	conserved protein (COG0500); SAM-dependent methyltransferase, putative nicotianamine synthase (plant iron chelator)		1		
MTH678	15678705	prefoldin beta subunit (GimC; Cpn beta subunit)		1		1
MTH680	15678707	Brix domain-containing ribosomal biogenesis protein (IMP4/RPC10) (known structure)		1		
MTH681	15678708	50S ribosomal protein L37Ae		1		
MTH682	15678709	exosome complex exonuclease 2 Rrp41 (EC 3.1.13.-)	1	1	1	
MTH683	15678710	exosome complex exonuclease 1 Rrp42 (EC 3.1.13.-)	1	1	1	
MTH684	15678711	exosome complex RNA-binding protein 1 Rrp4	1	1		1
MTH685	15678712	conserved protein (COG1500)	1	1	1	
MTH686	15678713	proteasome, alpha subunit	1	1	1	1
MTH688	15678715	ribonuclease P protein component 3 (EC 3.1.26.5) (RNase P component 3) exosome subunit Rpp30		1		
MTH691	15678718	conserved protein (COG599); gamma-carboxymuconolactone decarboxylase homolog/ alkylhydroperoxidase AhpD core		1		1
MTH692	15678719	p-stomatin (stomatine band 7 homolog) (COG0330)		1		1
MTH693	15678720	conserved membrane-bound stomatin operon partner protein (STOPP) (COG1585, DUF107), regulatory protease		1		
MTH694	15678721	uncharacterized protein MTH694 (contains HTH-3 domain) (COG1476)	1	1		
MTH695	15678722	conserved protein (COG0577; PF02687); ABC transporter, permease protein		1		
MTH696	15678723	ABC-type transport system, ATPase component		1		
MTH697	15678724	conserved Archaeoflavoprotein AfpA (COG1036), dihydromethanopterin reductase	1	1		1

MTH698	15678725	conserved Methanobacteriaceae-specific hypothetical protein MTH698		1		1
MTH699	15678726	conserved protein (COG1839, DUF355}		1		
MTH700	15678727	transcriptional regulator, PbsX/ XRE family (COG1396)		1		1
MTH701	15678728	acetyl-CoA synthetase 2 (N-terminal part) (COG0365)	1	1	1	
MTH704	15678731	2-oxoisovalerate oxidoreductase, beta subunit	1	1		
MTH705	15678732	2-oxoisovalerate oxidoreductase, alpha subunit		1		
MTH706	15678733	Asn/gln-dependent glutamyl-tRNA(Gln) amidotransferase subunit D (EC 6.3.5.-) (Glu-ADT subunit D)		1		1
MTH707	15678734	Asn/gln-dependent glutamyl-tRNA(Gln) amidotransferase subunit E (EC 6.3.5.-) (Glu-ADT subunit E)	1	1		1
MTH708	15678735	thioredoxin reductase		1		
MTH709	15678736	GMP synthase [glutamine-hydrolyzing] subunit A (EC 6.3.5.2) (Glutamine amidotransferase)	1	1		1
MTH710	15678737	GMP synthase [glutamine-hydrolyzing] subunit B (EC 6.3.5.2)		1		1
MTH714	15678741	membrane-bound metal chelatase (CobN/BchH family) containing cell adhesion related domain (CardB)	1	1		
MTH715	15678742	membrane-bound methanogen-specific cell surface protein	1	1		
MTH716	15678743	cell wall-binding glycoprotein (s-layer protein) (2x pkd repeat;CASH, NosD and CARBD domains; pseudomurein binding repeat, PF09373)	1	1		
MTH717	15678744	membrane-bound methanogen-specific cell surface protein (COG1572) containing cell adhesion related domain (CardB)		1		
MTH719	15678746	membrane-bound cell wall-binding glycoprotein (s-layer protein) containing CASH and NosD domains; pseudomurein binding repe (PF09373)		1		1
MTH721	15678748	conserved pyridoxal phosphate-dependent protein (COG0075, COG1167); putative amino-sugar biosynthesis prot.		1		
MTH723	15678750	R-citramalate (CimA)/ R-homocitrate synthase		1		
MTH726	15678753	conserved membrane-bound protein MTH726 containing DUF304 domain (COG3428, PF3703)		1		
MTH728	15678755	ATP-dependent 26S protease regulatory subunit 4, proteasome-activating nucleotidase		1		
MTH729	15678756	eukaryotic multiprotein bridging factor MBF1 homolog		1		
MTH730	15678757	conserved archaeal protein MTH730 (COG1844, DUF356)		1		
MTH731	15678758	conserved methanogen-specific membrane-spanning protein MTH731		1		1
MTH733	15678760	conserved methanogen-specific membrane-spanning protein MTH733		1		1
MTH735	15678761	phospho-N-acetylmuramoyl-pentapeptide-transferase; UDP-MurNAc-pentapeptide phosphotransferase		1		
MTH739	15678765	nickel responsive regulator 2 NikR (copG-HTH4)	1	1	1	1
MTH740	15678766	conserved protein containing signal transduction CBS domain (2x)				1
MTH741	15678767	conserved Methanobacteriaceae-specific protein MTH741				1
MTH742	15678768	phenylalanyl-tRNA synthetase alpha chain (EC 6.1.1.20); phenylalanine--tRNA ligase alpha chain		1		1
MTH743	15678769	ATP:dephospho-CoA triphosphoribosyl transferase (CitG; DPCK)	1	1	1	
MTH744	15678770	porphobilinogen synthase; delta-aminolevulinic acid dehydratase (EC 4.2.1.24) (HemB)	1	1	1	
MTH747	15678773	conserved protein (COG2013, DUF124)/ HTH DNA-binding protein		1		1
MTH748	15678774	chorismate synthase, 5-enolpyruvylshikimate-3-phosphate phospholyase (AroC)		1		
MTH752	15678777	conserved methanogen-specific (not Methanosphaera) protein MTH752 (COG4079)	1	1		
MTH753	15678778	conserved protein (COG1331, DUF255), DsbD gamma family protein involved in electron transfer to thiol-disulfide isomerase (N-terminal part)		1		1
MTH755	15678780	copper (Cu2+) transporting CPX-type ATPase (CopB)		1		
MTH756	15678781	rubrerythrin		1		
MTH757	15678782	rubredoxin oxidoreductase; desulfoferrodoxin homolog (Dfx)		1		
MTH758	15678783	S-D-lactoylglutathione methylglyoxal lyase		1		
MTH759	15678784	partially (C-terminal) conserved protein involved in exopolysaccharide biosynthesis		1		
MTH763	15678788	archaea-specific RecJ protein, ss-DNA specific exonuclease	1	1	1	1
MTH765	15678790	small GTP-binding protein Rab-related protein; translation initiation factor 2	1	1	1	1
MTH766	15678791	5-enolpyruvylshikimate 3-phosphate synthase (3-phosphoshikimate 1-carboxyvinyltransferase (EC 2.5.1.19)(aroA)		1		
MTH767	15678792	valyl-tRNA synthetase	1	1	1	1
MTH770	15678795	phenylalanyl-tRNA synthetase beta chain (EC 6.1.1.20); phenylalanine-tRNA ligase beta chain	1	1	1	
MTH771	15678796	conserved protein (COG0432, UPF0047)		1		
MTH773	15678798	N5,N10-methenyl-tetrahydromethanopterin cyclohydrolase	1	1	1	1
MTH774	15678799	thymidylate synthase (EC 2.1.1.45) (TS) (TSase).		1		

MTH775	15678800	methylcobalamin:homocysteine methyltransferase (EC 2.1.1.-); cobalamin-independent methionine synthase		1	1	1
MTH777	15678802	conserved methanogen-specific protein (COG4081)		1		1
MTH781	15678805	conserved archaeal protein MTH781 (COG1817, DUF354)		1		
MTH782	15678806	hydrogenase expression/formation protein HypB		1		1
MTH784	15678808	ribose-phosphate pyrophosphokinase (EC 2.7.6.1) (RPPK) (Phosphoribosyl pyrophosphate synthetase) (P-Rib-PP synthetase) (PRPP synthetase)		1		1
MTH785	15678809	ATP-dependent protease LA/ Lon protease		1	1	1
MTH786	15678810	sensory transduction histidine kinase related protein conaining PAS/PAC domain		1		1
MTH792	15678814	3-hydroxy-3-methylglutaryl-CoA-synthase	1	1	1	1
MTH793	15678815	lipid-transfer protein (sterol or nonspecific)	1	1	1	1
MTH794	15678816	thermosome subunit 2, chaperonine (HSP60) I)	1	1	1	1
MTH797	15678819	conserved hypothetical protein (COG1196), putative chromosome segregation ATPase		1	1	1
MTH799	15678821	aspartate-semialdehyde dehydrogenase (asd)		1		1
MTH800	15678822	dihydrodipicolinate reductase (DapB)		1		
MTH801	15678823	dihydrodipicolinate synthase (DapA)	1	1	1	1
MTH802	15678824	aspartokinase II alpha subunit (thrA1, metL) containing regulatory ACT domain	1	1		1
MTH805	15678827	shikimate kinase (GHMP kinase) (EC 2.7.1.71) (AroK)		1	1	
MTH806	15678828	type IV signal peptide peptidase (SPP)	1	1		1
MTH807	15678829	thioredoxin (glutaredoxin-like protein).		1		1
MTH808	15678830	cobalt-precorrin-6A synthase [deacetylating], anaerobic ring methylation		1		
MTH809	15678831	molybdenum cofactor biosynthesis protein MoaC				1
MTH810	15678832	SF2- (ski2)-type DNA helicase (Hel308; EC 3.6.1.-)		1		1
MTH814	15678836	conserved methanogen-specific protein MTH814 (COG4014)		1		1
MTH815	15678837	conserved Methanobacteriaceae-specific protein (COG1873) implicated in RNA metabolism		1		1
MTH817	15678839	histone acetyltransferase Elp3 homolog, component RNA polymerase II complex		1		
MTH818	15678840	2-deoxyribose-5-phosphate aldolase (deoC)		1		
MTH821	15678843	histone HMtA1		1		1
MTH822	15678844	rubrerythrin		1		1
MTH827	15678847	protein L-isoaspartyl carboxyl methyltransferase (SAM-dependent methyltransferase)	1	1		1
MTH829	15678849	3-isopropylmalate dehydratase small subunit 1 (EC 4.2.1.33) (Isopropylmalate isomerase 1)	1	1	1	1
MTH831	15678851	molybdenum cofactor biosynthesis-related protein, MoaA; radical SAM protein	1	1	1	1
MTH834	15678854	conserved protein (COG1548); pantheonate/ sugar kinase-like protein		1		1
MTH836	15678856	UDP-N-acetyl-D-mannosaminuronic/ talosaminuronic acid dehydrogenase		1		1
MTH840	15678860	pseudouridylate synthase I, tRNA pseudouridine synthase A (EC 4.2.1.70)		1		
MTH843	15678863	phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase (HisA)		1		1
MTH845	15678865	cell surface pseudomureine (transglutaminase) endopeptidase		1		
MTH846	15678866	N-acetyl-gamma-glutamyl-phosphate reductase (ArgC)	1	1	1	
MTH847	15678867	flavodoxin	1	1		1
MTH848	15678868	protein-export membrane protein, SecF	1	1	1	1
MTH849	15678869	protein-export membrane protein, SecD		1		1
MTH850	15678870	aspartate carbamoyltransferase regulatory subunit (PyrI)	1	1	1	1
MTH851	15678871	cell surface protein containing pseudomurein binding repeat (PF09373)		1		1
MTH852	15678872	membrane-bound metal-dependent hydrolase of the aminoacylase-2/ carboxypeptidase Z family		1		
MTH854	15678874	ferredoxin		1		1
MTH855	15678875	inosine-5'-monophosphate dehydrogenase related protein VIII containing (2x) signal transduction CBS-domain	1	1	1	1
MTH857	15678877	conserved AMMECR1 domain protein (COG2078, DUF51)		1		
MTH858	15678878	archaeal GTP-binding protein (TGS domain), GTP1/OBG family		1		
MTH859	15678879	heat shock protein, class I (HSP20)	1	1	1	1
MTH860	15678880	glucosamine-fructose-6-phosphate aminotransferase (SIS domain)		1		
MTH862	15678882	conserved methanogen-specific protein MTH862 (COG4090)		1		
MTH867	15678887	lysine-oxoglutarate dehydrogenase/ saccharopine dehydrogenase bifunctional enzyme (LOR/SDH)		1		
MTH869	15678889	translation initiation factor 5A (eIF-5A), hypusine-containing protein	1	1	1	1
MTH871	15678891	bifunctional inositol-1 monophosphatase/fructose-1,6-bisphosphatase		1		
MTH872	15678892	inorganic polyphosphate/ATP-NAD kinase (EC 2.7.1.23); poly(P)/ATP NAD		1		

		kinase)				
MTH873	15678893	UDP-N-acetylmuramyl tripeptide synthetase related protein		1		
MTH875	15678895	NAD-dependent 3"-UDP-N-acetyl-D-glucosamine dehydrogenase		1		1
MTH876	15678896	orotate phosphoribosyltransferase homolog (PyrE-like protein)	1	1	1	1
MTH878	15678898	peptide chain release factor eRF, subunit 1; translation termination factor aRF1	1	1	1	1
MTH879	15678899	uridine monophosphate kinase (pyrH)		1		1
MTH880	15678900	conserved methanogen-specific small zinc-finger protein(COG4068)		1		
MTH883	15678903	conserved methanogen-specific membrane-spanning protein MTH883 (COG2985)		1		
MTH885	15678905	transcription initiation factor TFIIB		1	1	1
MTH888	15678908	conserved protein; 2-demethylmenaquinone methyltransferase (menG) (COG0684, PF03737)	1			1
MTH889	15678909	conserved archaeal protein (COG1888, DUF211)		1		1
MTH891	15678911	bacterial DNA primase (DnaG, Toprim domain)	1	1	1	1
MTH892	15678912	ATP-dependent protease LA related protein		1		1
MTH897	15678917	pyrroline-5-carboxylate reductase (ProC)		1		
MTH898	15678918	universal stress protein (UspA); chaperone (ATP-dependent)		1	1	1
MTH901	15678921	sensory transduction regulatory protein		1		
MTH905	15678925	conserved protein (COG1268); biotine transport protein (BioY)		1		1
MTH909	15678929	tRNA acetylating (4-acetylcytidine) enzyme		1		
MTH911	15678931	cell surface adhesin-like protein	1	1		
MTH914	15678934	conserved methanogen-specific membrane-bound protein MTH914		1		
MTH916	15678936	DNA-binding protein tfx (HTH3; Mo-uptake regulator)	1	1	1	1
MTH917	15678937	molybdenum formylmethanofuran dehydrogenase, subunit E	1	1	1	1
MTH918	15678938	molybdenum formylmethanofuran dehydrogenase, subunit C	1	1	1	1
MTH919	15678939	molybdenum formylmethanofuran dehydrogenase, subunit B	1	1	1	1
MTH922	15678942	molybdenum transport system permease protein (COG2014), modB (N-terminus) related	1	1	1	
MTH923	15678943	molybdenum transport system permease protein, modB (C-terminus) related		1	1	
MTH924	15678944	molybdate-binding periplasmic protein (modA)	1	1		1
MTH925	15678945	conserved protein (COG2014, DUF364)	1	1		1
MTH926	15678946	tungsten formylmethanofuran dehydrogenase subunit F homolog; polyferredoxin		1	1	1
MTH927	15678947	ferredoxin		1		
MTH928	15678948	membrane-bound metal chelatase belonging to the CobN/BchH family	1	1		
MTH943	15678963	phophoenolpyruvate carboxylase (PpcA) (verified)	1	1	1	1
MTH944	15678964	conserved membrane-spanning protein MTH944	1	1		
MTH949	15678967	conserved archaeal protein MTH949 (COG2083, DUF61, UPF0216)		1		
MTH950	15678968	uncharacterized protein MTH950		1		1
MTH952	15678970	conserved methanogen-specific ATPase-operon protein (COG1417, DUF22)	1	1	1	
MTH953	15678971	ATP synthase, subunit D (delta)	1	1	1	1
MTH954	15678972	ATP synthase, subunit B (beta)	1	1	1	1
MTH955	15678973	ATP synthase, subunit A (alfa)	1	1	1	1
MTH956	15678974	ATP synthase, subunit F		1		1
MTH957	15678975	ATP synthase, subunit C (gamma)		1	1	1
MTH958	15678976	ATP synthase, subunit E (epsilon)	1	1	1	1
MTH960	15678978	ATP synthase, subunit I	1	1	1	1
MTH969	15678987	conserved methanogen-specific protein MTH969		1		
MTH970	15678988	D-3-phosphoglycerate dehydrogenase (SerA) (EC 1.1.1.95) containg regulatory ACT domain	1	1	1	1
MTH972	15678990	conserved protein (COG4021, DUF549), predicted tRNA(His) guanylyltransferase				1
MTH973	15678991	NAD(P)-dependent aspartate dehydrogenase (NadB)		1		
MTH974	15678992	conserved methanogen-specific protein (COG1873) implicated in RNA metabolism	1	1	1	1
MTH975	15678993	phenylalanine--tRNA ligase (EC 6.1.1.20) beta chain; Tyrosyl-tRNA synthetase (EC 6.1.1.1)		1		1
MTH977	15678995	membrane-bound cell surface glycoprotein (s-layer protein)		1	1	1
MTH978	15678996	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (gapN), lactaldehyde dehydrogenase (CofA)	1	1		
MTH980	15678998	type II secretion system protein F, GspF (COG1459)		1		
MTH984	15679002	1,3-propanediol dehydrogenase/ iron-containing alcohol dehydrogenase (COG1454)		1		
MTH993	15679011	universal stress protein (UspA); chaperone (ATP-dependent)		1		1
MTH996	15679014	carbamoyl-phosphate synthase, large subunit (carB)	1	1	1	1

MTH997	15679015	carbamoyl-phosphate synthase, large subunit (carB)	1	1	1	1
MTH998	15679016	carbamoyl-phosphate synthase, small subunit (carB)				1
MTH1001	15679019	ATPase, E1-E2 type: calcium-translocating P-type ATPase, PMCA-type	1	1	1	1
MTH1003	15679021	molybdenum cofactor biosynthesis protein MoeA				1
MTH1005	15679023	serine/threonine protein kinase (Rio1 family)		1		1
MTH1006	15679024	RNA-binding protein; hydratase (S1 and KH domains) (COG1094)		1		1
MTH1007	15679025	type II DNA topoisomerase VI subunit B (EC 5.99.1.3)	1	1	1	1
MTH1008	15679026	type II DNA topoisomerase VI subunit A (EC 5.99.1.3)	1	1	1	1
MTH1009	15679027	glyceraldehyde 3-phosphate dehydrogenase (gapA)	1	1	1	1
MTH1014	15679032	predicted methanogen-specific metal-binding transcription factor (COG4008)	1	1	1	1
MTH1015	15679033	methyl coenzyme M reductase system, component A2; ATPase subunit of an ABC-type Ni2+ transport system	1	1	1	1
MTH1020	15679038	IMP cyclohydrolase (PurO)		1		1
MTH1022	15679040	biopolymer transport protein, MotA/TolQ/ExbB family	1	1		1
MTH1024	15679042	rod shape-determining protein (MreB protein)	1	1	1	1
MTH1025	15679043	uncharacterized conserved protein MTH1025		1	1	1
MTH1028	15679046	conserved archaeal membrane-spanning protein MTH1028 (COG1627, DUF515)		1		1
MTH1030	15679048	sortase (surface protein transpeptidase), SrtA involved in cell wall protein attachment (PF04203)		1		
MTH1033	15679051	2-oxoglutarate synthase subunit korA (EC 1.2.7.3) (2-ketoglutarate oxidoreductase alpha chain) (KOR) (2-oxoglutarate-ferredoxin oxidoreductase subunit alpha)	1	1		
MTH1034	15679052	2-oxoglutarate synthase subunit korB (EC 1.2.7.3) (2-ketoglutarate oxidoreductase beta chain) (KOR) (2-oxoglutarate-ferredoxin oxidoreductase subunit beta)		1		1
MTH1035	15679053	2-oxoglutarate synthase subunit korC (EC 1.2.7.3) (2-ketoglutarate oxidoreductase gamma chain) (KOR) (2-oxoglutarate-ferredoxioxidoreductase subunit gamma)		1		1
MTH1036	15679054	succinyl-CoA synthetase, beta subunit (sucD)	1	1	1	1
MTH1037	15679055	conserved methanogen-specific ABC transporter, permease		1		
MTH1038	15679056	conserved membrane-spanning protein MTH1038 (COG4086, DUF1002)				1
MTH1040	15679058	transglutaminase/protease-like protein		1		1
MTH1041	15679059	triosephosphate isomerase (tpi)	1	1	1	
MTH1042	15679060	3-phosphoglycerate kinase (pgk)		1		1
MTH1049	15679062	DNA-dependent RNA polymerase, subunit B" (RpoB")	1	1	1	1
MTH1050	15679063	DNA-dependent RNA polymerase, subunit B' (RpoB')	1	1	1	1
MTH1051	15679064	DNA-dependent RNA polymerase, subunit A' (RpoA')	1	1	1	1
MTH1052	15679065	DNA-dependent RNA polymerase, subunit A" (RpoA")	1	1	1	1
MTH1053	15679066	50S ribosomal protein L30e		1		1
MTH1054	15679067	transcription termination factor NusA	1	1	1	1
MTH1055	15679068	30S ribosomal protein S23 (E.coli S12P)		1	1	1
MTH1056	15679069	30S ribosomal protein S5 (E.coli S7P)	1	1	1	1
MTH1057	15679070	translation elongation factor, EF-2	1	1	1	1
MTH1058	15679071	translation elongation factor, EF-1 alpha	1	1	1	1
MTH1059	15679072	30S ribosomal protein S20 (E.coli S10P)		1		1
MTH1062	15679073	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase, subunit A homolog		1		
MTH1064	15679075	conserved methanogen-specific protein MTH1064 (COG1432, DUF88)		1		1
MTH1065	15679076	universal stress protein (UspA); chaperone (ATP-dependent)		1	1	
MTH1066	15679077	predicted transcriptional activator (COG4987)				1
MTH1068	15679079	conserved (UPF0334) kinase-like protein/ ATPase-kinase involved in pili biogenesis (pilT) (COG1618, COG0630)		1		
MTH1070	15679081	zinc metalloprotease (U62 family), modulator of DNA gyrase		1		
MTH1072	15679083	hydrogenase expression/formation protein HypD (HypD)		1		1
MTH1080	15679091	CRISPR-associated RAMP protein Csm3 (COG1337)		1	1	
MTH1081	15679092	CRISPR-associated protein Csm2 (COG1421, DUF310)		1		1
MTH1082	15679093	CRISPR-associated protein, Csm1, metal-dependent hydrolase HD superfamily/ possibly novel polymerase (COG1353, PF01966)				1
MTH1086	15679097	CRISPR-associated protein Cas3, DNA-helicase (COG1203) (acquired virus resistance system)		1		
MTH1094	15679105	circadian clock protein KaiC, RecA/ Rad55 superfamily ATPase involved in homologous recombination		1		
MTH1097	15679108	conserved protein (COG3467), pyridoxamine 5'-phosphate oxidase-related (FMN-binding) (crystal structure known)		1		
MTH1104	15679115	partially conserved membrane-bound protein MTH1104		1		
MTH1105	15679116	1L-myo-inositol-1-phosphate synthase	1	1	1	1

MTH1107	15679118	pyruvate carboxylase subunit B (EC 6.4.1.1)(PycB)		1	1	
MTH1108	15679119	archaeosine biosynthesis protein queC (EC 3.5.-.-).		1		1
MTH1110	15679121	conserved methanogen-specific protein (COG4075); nitrogen regulatory protein PII homolog		1		1
MTH1113	15679124	conserved archaeal protein (COG1303, UPF0106); SpoU-like RNA methylase, archaeal tRNA-methylase for position 56 (Trm56)		1		1
MTH1117	15679128	phosphoenolpyruvate synthase, C-terminal part (ppsA)	1	1	1	
MTH1118	15679129	phosphoenolpyruvate synthase (ppsA)	1	1	1	1
MTH1119	15679130	50S ribosomal protein L10e		1	1	1
MTH1121	15679132	conserved Methanobacteriaceae-specific protein MTH1121		1		
MTH1122	15679133	type-2 seryl-tRNA synthetase (EC 6.1.1.11) (Seryl-tRNA(Ser/Sec) synthetase) (SerRS)	1	1	1	
MTH1123	15679134	conserved protein, putative ATPase, glucocorticoid receptor-like DNA-binding domain		1		1
MTH1125	15679136	fkbp-type peptidyl-prolyl cis-trans isomerase	1		1	
MTH1129	15679140	methyl coenzyme M reductase II, alpha subunit	1	1	1	1
MTH1130	15679141	methyl coenzyme M reductase II, gamma subunit	1	1	1	1
MTH1131	15679142	methyl coenzyme M reductase II, D protein		1	1	1
MTH1132	15679143	methyl coenzyme M reductase II, beta subunit	1	1	1	1
MTH1133	15679144	polyferredoxin (MvhB)	1	1	1	1
MTH1134	15679145	methyl viologen-reducing hydrogenase, alpha subunit	1	1	1	1
MTH1135	15679146	methyl viologen-reducing hydrogenase, gamma subunit	1	1		
MTH1136	15679147	methyl viologen-reducing hydrogenase, delta subunit	1	1	1	1
MTH1137	15679148	conserved methanogen-specific protein (FlpA) (COG4018)	1	1		
MTH1138	15679149	methyl viologen-reducing hydrogenase, delta subunit homolog FlpD		1		
MTH1139	15679150	formate dehydrogenase, beta subunit (F420-reducing) related protein FlpB	1	1		
MTH1140	15679151	formate dehydrogenase, alpha subunit related protein/ nitrate reductase catalytic subunit, FlpC	1	1		
MTH1142	15679153	H(2)-dependent N5,N10-methylenetetrahydromethanopterin dehydrogenase	1	1	1	1
MTH1144	15679155	conserved methanogen-specific hypothetical protein MTH1144 (COG4019)		1		
MTH1146	15679157	conserved hypothetical protein MTH1146 (COG0323); NIF3-related protein (NGG1p interacting factor 3)		1		1
MTH1147	15679158	conserved methanogen-specific hypothetical protein (COG4015)/ UBA/THIF-type NAD/FAD binding fold		1		
MTH1149	15679160	ABC transporter involved in Fe-S cluster assembly, ATPase component (SufC)	1	1	1	1
MTH1150	15679161	ABC transporter involved in Fe-S cluster assembly, permease component (SufBD)	1	1	1	1
MTH1153	15679164	conserved methanogen-specific protein MTH1153 (COG4065)		1		1
MTH1154	15679165	universal stress protein (UspA); chaperone (ATP-dependent)	1	1	1	1
MTH1156	15679167	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase, subunit H	1	1	1	1
MTH1157	15679168	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase, subunit G	1	1	1	
MTH1158	15679169	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase, subunit F	1	1		1
MTH1159	15679170	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase, subunit A	1	1	1	1
MTH1161	15679172	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase, subunit C	1	1	1	1
MTH1162	15679173	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase, subunit D	1	1		1
MTH1163	15679174	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase, subunit E		1	1	1
MTH1164	15679175	methyl coenzyme M reductase I, alpha subunit	1	1	1	1
MTH1165	15679176	methyl coenzyme M reductase I, gamma subunit	1	1	1	1
MTH1166	15679177	methyl coenzyme M reductase I, C protein		1		
MTH1167	15679178	methyl coenzyme M reductase I, D protein	1	1	1	1
MTH1168	15679179	methyl coenzyme M reductase I, beta subunit	1	1	1	1
MTH1175	15679186	dinitrogenase iron-molybdenum cofactor biosynthesis protein (NifB/ NifX/ NifY family)		1		1
MTH1180	15679191	conserved methanogen-specific protein related to methyl coenzyme M reductase II operon protein C (mtrC) (COG4052)		1	1	
MTH1182	15679193	2-phosphosulfolactate phosphatase (EC 3.1.3.-) (ComB)		1		1
MTH1193	15679204	transcriptional regulator (AsnC-HTH), leucine regulator (Lrp)	1	1	1	1
MTH1196	15679207	type 1 GTP cyclohydrolase (FolE2, MptA) (COG1469, UPF0343, DUF198); archaeosine (7-deazaguanosine) biosynthesis protein	1	1		
MTH1199	15679210	conserved archaeal protein (COG2410, DUF429), ribosomal protein S7		1		

MTH1201	15679212	5-formaminoimidazole-4-carboxamide-1-beta-D-ribofuranosyl 5'-monophosphate synthetase (FAICAR) (PurP)	1	1	1	1
MTH1202	15679213	proteasome, beta subunit	1	1	1	1
MTH1203	15679214	mbl-RNAse (metallo beta lactamase), mRNA 3'-end processing factor	1	1	1	1
MTH1204	15679215	phosphoribosylformylglycinamidine cyclo-ligase (AIR synthase) (PurM)	1	1		1
MTH1205	15679216	L-sulfolactate dehydrogenase/ 2-ketoglutarate reductase involved in HS-CoM and methanopterin biosynthesis		1	1	
MTH1210	15679221	5-methylcytosine-specific restriction enzyme (mrr)		1		
MTH1212	15679223	dihydroorotate dehydrogenase electron transfer subunit (PyrK)		1		
MTH1216	15679227	phosphopantothenoylcysteine synthase/decarboxylase (PPCS/PPCDC; CoaBC)		1		
MTH1217	15679228	archaeal transcription terminator NusA (COG0195)	1	1	1	1
MTH1219	15679230	conserved Methanobacteriaceae-specific membrane-spanning protein MTH1219 putatively involved in cell wall metabolism		1		
MTH1221	15679232	archaeal intramolecular RNA (single-stranded DNA) ligase		1		
MTH1222	15679233	inosine-5'-monophosphate dehydrogenase related protein I containing signal transduction CBS-domain (4x)	1	1	1	
MTH1223	15679234	inosine-5'-monophosphate dehydrogenase related protein II containing signal transduction CBS-domain (4x)	1	1	1	1
MTH1224	15679235	inosine-5'-monophosphate dehydrogenase related protein III containing signal transduction CBS-domain (4x)	1	1	1	1
MTH1225	15679236	inosine-5'-monophosphate dehydrogenase related protein IV containing signal transduction CBS-domain (4x)	1	1	1	1
MTH1226	15679237	inosine-5'-monophosphate dehydrogenase related protein V containing signal transduction CBS-domain (2x) fused to a Zn-ribbon (rubredoxin) domain	1	1	1	
MTH1229	15679240	conserved methano-archaeal protein MTH1229 (COG2029, DUF366)		1		
MTH1234	15679245	uncharacterized protein MTH1234		1		
MTH1237	15679248	NADH dehydrogenase (ubiquinone), subunit 1 related protein (EhbO) ('quinone binding')		1		1
MTH1238	15679249	formate hydrogenlyase, subunit 5 (EhbN); large (alfa) subunit NiFe H2ase		1		1
MTH1239	15679250	formate hydrogenlyase, subunit 7 (EhbM); small subunit NiFe H2ase		1		
MTH1240	15679251	ferredoxin-like protein (EhbL)		1		1
MTH1241	15679252	polyferredoxin (EhbK)		1		
MTH1254	15679265	argininosuccinate synthase (EC 6.3.4.5), citrulline-aspartate ligase (ArgG)	1	1	1	
MTH1256	15679267	conserved protein; N-terminal YjeF related protein fused to C-terminal sugar kinase (COG0063, COG0062)		1		
MTH1259	15679270	formylmethanofuran:tetrahydromethanopterin formyltransferase	1	1	1	1
MTH1260	15679271	sensory transduction histidine kinase		1		
MTH1263	15679274	conserved protein (SAM-dependent methyltransferase); Trm2-RNA methylase (TRAM domain)		1		
MTH1280	15679284	Gln/asn-dependent aspartyl-tRNA(Asn) amidotransferase subunit B (EC 6.3.5.-) (Asp/Glu-ADT subunit B).	1	1		1
MTH1282	15679286	inosine-5'-monophosphate dehydrogenase related protein VI; signal transduction CBS-domain (2x) associated with a ParBc (ParB-nuclease) HTH domain		1		1
MTH1287	15679291	hydrogenase maturation factor, HypF		1		
MTH1289	15679293	heat shock protein GrpE		1		
MTH1290	15679294	DnaK protein (Hsp70)	1	1	1	1
MTH1291	15679295	DnaJ protein		1		
MTH1294	15679296	conserved methanogen-specific membrane-spanning protein MTH1294		1		1
MTH1295	15679297	conserved methanogen-specific membrane-spanning protein MTH1295 (COG1196)		1		1
MTH1297	15679299	coenzyme F420-reducing hydrogenase, beta subunit	1	1		
MTH1298	15679300	coenzyme F420-reducing hydrogenase, gamma subunit	1	1		
MTH1300	15679302	coenzyme F420-reducing hydrogenase, alpha subunit	1	1		1
MTH1301	15679303	conserved Methanobacteriaceae-specific membrane-spanning protein; peptidase propeptide (PepSY) surface prot.	1	1		1
MTH1302	15679304	archaeal ATP-Grasp superfamily protein (COG1938)/ HS-CoB biosynthesis protein (AksE)	1	1		1
MTH1306	15679306	probable 3-isopropylmalate dehydratase (LeuD)/ HS-CoB biosynthesis protein (AksE)	1	1	1	1
MTH1308	15679308	translation initiation factor eIF-2, alpha subunit	1	1	1	1
MTH1309	15679309	30S ribosomal protein S27E		1		1
MTH1310	15679310	50S ribosomal protein L36a (L44E)		1		
MTH1312	15679312	proliferating-cell nuclear antigen (PCNA); DNA polymerase sliding clamp	1	1	1	1
MTH1314	15679314	transcription elongation factor TFIIS; DNA-directed RNA polymerase subunit M (EC 2.7.7.6) (RpoM)		1		
MTH1316	15679316	conserved protein (COG1628, DUF99, UPF0215); predicted endonuclease		1		1

		V homolog				
MTH1317	15679317	DNA-dependent RNA polymerase, subunit L (RpoL), exosome subunit RPC19		1		1
MTH1318	15679318	RNA-binding protein, exonuclease, Csl4	1	1	1	1
MTH1319	15679319	diphthamide synthase, subunit DPH2, translation factor EF-2 modification		1		
MTH1320	15679320	adenine/ (verified) hypoxanthine phosphoribosyltransferase (APRT) (EC 2.4.2.8)	1	1		
MTH1321	15679321	signal recognition particle protein SRP54		1		1
MTH1322	15679322	55-pseudouridylate synthetase (pus10)		1		
MTH1324	15679324	RNA polymerase Rpb4 (RpoH)		1		1
MTH1325	15679325	conserved protein, predicted RNA-binding protein (COG1491)	1	1	1	1
MTH1326	15679326	dimethyladenosine transferase (EC 2.1.1.-), S-adenosylmethionine-6-N',N'-adenosyl(rRNA)dimethyltransferase) (16S rRNA dimethylase)		1		
MTH1329	15679329	methyltransferase related protein (SAM-dependent methyltransferase)/ methylase of polypeptide chain release factors (HemK)/r/tRNA cytosine-C5 methylase		1		1
MTH1334	15679334	diaminopimelate epimerase (DapF)	1		1	
MTH1335	15679335	diaminopimelate decarboxylase (EC 4.1.1.20) (DAP decarboxylase)	1		1	1
MTH1336	15679336	mutator MutT protein homolog; A,G-mismatch DNA glycosylase		1		
MTH1337	15679337	N-acetylornithine aminotransferase (ArgD)	1	1	1	1
MTH1343	15679343	imidazoleglycerol-phosphate synthase (cyclase) (HisF)		1		
MTH1345	15679344	conserved protein (molybdopterin binding domain), formylmethanofuran dehydrogenase subunit E homolog		1	1	
MTH1346	15679345	conserved methanogen-specific membrane-bound protein MTH1346 (COG5643)	1	1		
MTH1347	15679346	ATP-dependent helicase (RAD3, XPD)		1		
MTH1348	15679347	precorrin-2 methyltransferase (CbiL)		1		1
MTH1349	15679348	transcription regulator (ArsR/ MarR-HTH5 family)		1		
MTH1350	15679349	flavoprotein AI; F420H2 oxidase (fprA)	1	1	1	1
MTH1351	15679350	rubrerythrin (fprB)	1	1	1	1
MTH1352	15679351	rubredoxin (fprC)		1	1	1
MTH1354	15679353	NADH: rubredoxin oxidoreductase (NPOR)		1	1	1
MTH1361	15679360	ferrous iron transport protein B (FeoB)	1	1	1	1
MTH1362	15679361	ferrous iron transport protein A (FeoA)		1		1
MTH1369	15679368	molybdenum cofactor biosynthesis MoeA		1		
MTH1370	15679369	ABC transporter (ATP-binding protein)		1		1
MTH1371	15679370	ABC-type Na+ efflux pump, permease component		1		
MTH1372	15679371	ABC-type Na+ efflux pump, permease component		1		
MTH1374	15679373	phosphoribosylformylglycinamidine synthase II (PurL)	1	1	1	1
MTH1375	15679374	isoleucyl-tRNA synthetase	1	1	1	1
MTH1376	15679375	S-adenosylmethionine synthetase (EC 2.5.1.6) (MetK)	1	1	1	1
MTH1377	15679376	conserved protein MTH1377 (COG1430, DUF192, UPF0127)		1		
MTH1378	15679377	PBS lyase HEAT domain protein repeat-containing protein/ phycocyanin alpha phycocyanobilin lyase CpcE		1		
MTH1380	15679379	serine hydroxymethyltransferase (GlyA)	1		1	
MTH1381	15679380	CoB--CoM heterodisulfide reductase iron-sulfur subunit A (EC 1.8.98.1)	1	1	1	1
MTH1383	15679382	DNA repair protein RadA (RadA, RecA, Rad51)	1	1	1	1
MTH1384	15679383	replication factor A related protein (C-terminal part), ssDNA-binding replication initiation	1	1	1	1
MTH1385	15679384	replication factor A related protein, ssDNA-binding replication initiation	1	1	1	1
MTH1386	15679385	3-isopropylmalate dehydratase large subunit 1 (EC 4.2.1.33) (Isopropylmalate isomerase 1)	1	1	1	1
MTH1387	15679386	3-isopropylmalate dehydratase large subunit 2 (EC 4.2.1.33) (Isopropylmalate isomerase 2)	1	1	1	1
MTH1388	15679387	3-isopropylmalate dehydrogenase, NAD-dependent (LeuB)	1	1	1	1
MTH1389	15679388	nifS protein;cysteine desulfurase (EC 4.4.1.-)	1	1	1	1
MTH1390	15679389	riboflavin synthase beta subunit; 6,7-dimethyl-8-ribityllumazine synthase (EC 2.5.1.9) (RibH)		1		1
MTH1391	15679390	conserved protein (COG1571, DUF1743), predicted DNA binding protein (Zn-ribbon)/ archaeal-specific RecJ-like exonuclease		1		1
MTH1392	15679391	glycosyl transferase (GT2 family); dolichyl-phosphate mannosyltransferase related protein		1		1
MTH1393	15679392	phosphoribosylaminoimidazole carboxylase (AIR carboxylase) fused to phosphoribosylcarboxyaminoimidazole (NCAIR) mutase (PurE)	1			
MTH1394	15679393	putative 3-octaprenyl-4-hydroxybenzoate carboxylyase, 4-hydroxybenzoate decarboxylase (UbiD)		1		1
MTH1395	15679394	pyruvate formate-lyase activating enzyme related protein/ radical SAM protein		1		
MTH1397	15679396	sirohydrochlorin cobaltochelatase (EC 4.99.1.3) (CbiXS)		1		

MTH1398	15679397	conserved protein MTH1398 (COG1545, DUF35); putative nucleide binding protein (Zn-ribbon)	1	1	1	1
MTH1402	15679401	potassium ion transport protein (TrkA-C domain) fused to PhoU regulatory domain; ATP binding	1	1	1	1
MTH1404	15679403	conserved Methanobacteriaceae-specific membrane-spanning protein MTH1404		1		
MTH1406	15679405	fuculose-1-phosphate aldolase, class II aldolase/adducin family		1		
MTH1410	15679409	conserved archaea-specific hypothetical protein MTH1410 (COG5440)	1	1	1	1
MTH1412	15679411	cell division control protein 6 homolog 1 (CDC6 homolog 1) (ORC1)		1		
MTH1413	15679412	aspartate carbamoyltransferase (EC 2.1.3.2) (PyrB)		1		
MTH1414	15679413	tRNA(1-methyladenosine) methyltransferase (m1A57A58) (complex GCD14 subunit protein)		1		
MTH1415	15679414	ATP-dependent ss-DNA endonuclease (XPF; RAD1), eIF-4A family		1	1	
MTH1417	15679416	peroxiredoxin, predicted regulator of disulfide bond formation conserved protein (DorE/DsrF)	1	1	1	1
MTH1418	15679417	conserved protein MTH1418 (COG2043, DUF169)	1	1	1	1
MTH1423	15679422	30S ribosomal protein S13 (E.coli S15); S15P/S13E		1		
MTH1425	15679424	Kae1 protein, DNA-binding protein with apurinic (AP)-endonuclease activity (AP-lyase) fused with protein kinase Bud32		1		
MTH1430	15679429	branched-chain amino-acid aminotransferase (IlvE)	1	1	1	
MTH1434	15679431	type II restriction endonuclease		1		1
MTH1442	15679439	ketol-acid reductoisomerase (EC 1.1.1.86), acetohydroxy-acid isomeroreductase (IlvC)	1	1	1	1
MTH1443	15679440	acetolactate synthase, small subunit (IlvH) (regulatory ACT domain)	1	1	1	1
MTH1444	15679441	acetolactate synthase, large subunit (IlvB)		1	1	1
MTH1445	15679442	glycinamide ribonucleotide synthetase; phosphoribosylamine-glycine ligase (EC 6.3.4.13) (GARS) (PurD)	1			
MTH1447	15679444	arginyl-tRNA synthetase (ArgRS)		1		1
MTH1448	15679445	signal peptidase I		1		
MTH1449	15679446	dihydroxy-acid dehydratase (EC 4.2.1.9) (DAD) (IlvD)		1		1
MTH1452	15679449	conserved barrel domain protein (Cupin-2) (COG1917)		1		1
MTH1453	15679450	hybrid cluster (6Fe-6S, prismane)-containing protein; hydroxylamine reductase (COG1151)	1	1		1
MTH1455	15679452	threonyl-tRNA synthetase				1
MTH1460	15679457	cobyrinic acid a,c-diamide synthase (glutamine-hydrolyzing) (CbiA/CbiP/cobB)	1	1		
MTH1464	15679461	coenzyme F420-dependent N5,N10-methylene tetrahydromethanopterin dehydrogenase	1	1	1	1
MTH1467	15679464	imidazoleglycerol-phosphate dehydratase (HisB, TrpB2)		1		
MTH1474	15679471	bifunctional enzyme fae/hps [Includes: Formaldehyde-activating enzyme (E4C.3.-.-) (Fae); 3-hexulose-6-phosphate synthase (EC 4.1.2.-) (HPS) (D-arabino-3-hexulose-6-osphate formaldehyde lyase)]	1	1	1	1
MTH1476	15679473	tryptophan synthase, beta 2 subunit (TrpB2)	1	1	1	1
MTH1479	15679476	conserved metal-dependent phosphoesterase (PHP family, C terminal part) (COG0613, PF02231)		1		
MTH1481	15679478	2-isopropylmalate synthase (EC 2.3.3.13)	1	1		1
MTH1483	15679480	DNA/RNA-binding protein Alba (dsDNA binding protein, exponential growth)	1	1	1	
MTH1485	15679482	membrane-bound serine/threonine protein kinase related protein containing Class III signal sequence		1		
MTH1486	15679483	conserved ABC-transporter (permease)		1		1
MTH1487	15679484	ABC-type multidrug transport system, ATPase component		1		1
MTH1489	15679486	AP endonuclease family 2 endonuclease IV		1		
MTH1493	15679490	cation transporting P-type ATPase related protein		1		
MTH1496	15679493	Gln/asn-dependent aspartyl-tRNA(Asn) amidotransferase subunit A (EC 6.3.5.-) (Glu-ADT subunit A)	1	1		1
MTH1499	15679496	GTP cyclohydrolase II;3,4-dihydroxy-2-butanone 4-phosphate synthase (DHBP synthase) (RibB)		1		
MTH1501	15679498	O-phosphoseryl-tRNA synthetase (SepRS)		1		1
MTH1502	15679499	thiol:fumarate reductase subunit A protein/ succinate dehydrogenase flavoprotein subunit	1			1
MTH1505	15679502	N-ethylammeline chlorohydrolase homolog (COG0402;)5-methylthioadenosine/S-adenosylhomocysteine deaminase		1		
MTH1506	15679503	ATP phosphoribosyltransferase (EC 2.4.2.17) (HisG)		1		1
MTH1508	15679505	leucyl-tRNA synthetase (LeuS)	1	1	1	1
MTH1510	15679507	NH(3)-dependent NAD+ synthetase (NadE)		1		
MTH1511	15679508	arsenical pump-driving ATPase		1		1
MTH1512	15679509	H(2)-dependent N5,N10-methylenetetrahydromethanopterin dehydrogenase homolog (II)	1	1	1	1

MTH1514	15679511	precorrin-6Y methylase (CbiE)		1		
MTH1520	15679517	calcium-gated potassium channel mthK (ATP binding)		1		
MTH1523	15679520	glucose-1-phosphate adenylyltransferase related protein/ sugar phosphate nucleotydyl transferase	1	1	1	
MTH1524	15679521	imidazoleglycerol-phosphate synthase; amidotransferase hisH (EC 2.4.2.-) (HisH)		1		
MTH1525	15679522	hydrogenase expression/formation protein HypE related protein (HypE)		1		
MTH1528	15679524	coenzyme F390 synthetase I	1	1		1
MTH1534	15679530	aryldialkylphosphatase related protein/ Pro-Hyp metallo dipeptidase (MEROPS family M38) (COG1228)		1		
MTH1535	15679531	heavy-metal (Zn, Cu+) transporting CPX-type ATPase (zntA, CopA)		1		1
MTH1536	15679532	DNA polymerase II large subunit (EC 2.7.7.7) (Pol II)		1		
MTH1537	15679533	adenylosuccinate lyase (PurB)	1	1	1	1
MTH1538	15679534	Na+ dependent nucleoside/dicarboxylate transporter (COG0609)	1	1		1
MTH1539	15679535	anaerobic ribonucleoside-triphosphate reductase		1		
MTH1541	15679537	partially conserved protein MTH1541			1	
MTH1542	15679538	lysyl-tRNA synthetase (EC 6.1.1.6) (Lysine--tRNA ligase) (LysRS)	1	1	1	1
MTH1543	15679539	thiamine biosynthesis protein (thiC1)	1	1	1	1
MTH1544	15679540	ribokinase/ broad-substrate nucleoside kinase	1	1	1	
MTH1546	15679542	6-phospho-3-hexulose isomerase (PHI) (HTH-RpiR family)	1	1	1	
MTH1547	15679543	nitrate reductase (NarQ)/ formate dehydrogenase (FdhD) accessory protein		1		1
MTH1549	15679545	NADH dehydrogenase I chain F (nuoF)		1		
MTH1550	15679546	molybdenum cofactor biosynthesis MoaA		1		
MTH1551	15679547	molybdopterin-guanine dinucleotide biosynthesis protein B (MobB) related		1		
MTH1552	15679548	formate dehydrogenase, alpha subunit homolog	1	1	1	
MTH1554	15679550	tungsten formylmethanofuran dehydrogenase, subunit F	1	1	1	1
MTH1555	15679551	tungsten formylmethanofuran dehydrogenase, subunit G		1	1	
MTH1556	15679552	tungsten formylmethanofuran dehydrogenase, subunit D		1		1
MTH1557	15679553	tungsten formylmethanofuran dehydrogenase, subunit A	1	1	1	1
MTH1558	15679554	tungsten formylmethanofuran dehydrogenase, subunit C	1	1	1	1
MTH1559	15679555	tungsten formylmethanofuran dehydrogenase, subunit B	1	1	1	1
MTH1568	15679564	conserved methanogen-specific protein MTH1568 (COG1413)		1		1
MTH1569	15679565	repressor nitrogen metabolism (gln, nif) (NrpR)		1		
MTH1570	15679566	glutamine synthetase (EC 6.3.1.2) (Glutamate--ammonia ligase) (GS)	1	1	1	1
MTH1571	15679567	molybdopterin biosynthesis protein MoeB homolog		1		1
MTH1575	15679570	inosine-5'-monophosphate dehydrogenase related protein X containing (2x) signal transduction CBS-domain		1		1
MTH1577	15679572	glutaredoxin, NrdH-redoxin (NrdH)		1		
MTH1580	15679575	DNA ligase Cdc9 (EC 6.5.1.1) (ADP-dependent)		1		
MTH1584	15679579	phosphomannomutase/ phosphoglucomutase homolog		1		
MTH1588	15679583	ferripyochelin/ siderophore-binding protein/ transacetylase (COG0663)		1		
MTH1589	15679584	bifunctional acetylating glucosamine-1-phosphate uridylyltransferase		1		
MTH1591	15679586	2,3-bisphosphoglycerate-independent phosphoglycerate mutase 1		1		
MTH1593	15679588	30S ribosomal protein S3Ae	1	1	1	1
MTH1595	15679590	NADP-dependent FMN oxidoreductase/ multimeric flavodoxin (WrbA)		1	1	1
MTH1597	15679592	conserved protein (COG1690, UPF0027); RtcB family protein implicated in RNA metabolism	1	1	1	1
MTH1601	15679596	phosphoserine aminotransferase (SerC)/ archaeal aspartate aminotransferase (AspC)	1	1	1	1
MTH1608	15679603	signal recognition particle protein SRP54 (SRP alpha, docking protein; ftsY)		1		
MTH1609	15679604	prefoldin subunit alpha (GimC subunit alpha)		1		1
MTH1611	15679606	translation initiation factor 6 (EIF6)		1		1
MTH1612	15679607	50S ribosomal protein L31; translation initiation factor 6 (eIF-6)		1		1
MTH1614	15679609	predicted subunit tRNA(5-aminomethyl-2-thiouridylate)methyltransferase		1		1
MTH1616	15679611	30S ribosomal protein S19 (S19E)	1	1	1	1
MTH1620	15679615	thiamine (thiazole) biosynthetic enzyme (Thi4)/ ribulose-1,5-biphosphate synthetase	1	1	1	
MTH1621	15679616	GTP-binding protein, GTP1/OBG family; GTPase putatively involved in cell cycle control	1	1		1
MTH1622	15679617	conserved protein contain regulatory signal transduction CBS-domain (2x)		1		
MTH1623	15679618	membrane-bound oligosaccharyl transferase STT3 subunit related protein; archaeal oligosaccharide transferase (AglB)		1	1	1
MTH1624	15679619	DNA topoisomerase I		1		
MTH1626	15679621	phosphoserine phosphatase (SerB) fused with chromosome segregation ATPase smc	1	1	1	

MTH1627	15679622	TATA-binding transcription initiation factor	1	1	1	
MTH1629	15679624	adenylate cyclase, class 2 (thermophilic) CyaB (COG1437, PF01928)		1		
MTH1630	15679625	2-isopropylmalate synthase (leuA) (EC 2.3.3.13); HS-CoB and MFR biosynthesis (AksA)		1		1
MTH1631	15679626	3-isopropylmalate dehydratase (LeuC) large subunit (EC 4.2.1.33) (Isopropylmalate isomerase); HS-CoB and MFR biosynthesis (AksD)		1		
MTH1632	15679627	conserved protein (COG0316, DUF98); chorismate-pyruvate lyase/ beta-ribofuranosylaminobenzene 5'-phosphate synthase family		1		1
MTH1634	15679629	transcriptional control factor (enhancer-binding protein); DNA2/NAM7 helicase family		1		
MTH1636	15679631	S-adenosylhomocysteine hydrolase		1		1
MTH1639	15679634	cell division control protein Cdc48 (AAA-ATPase)	1	1	1	1
MTH1645	15679640	ABC1-type protein kinase (AarF-related)		1		
MTH1646	15679641	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase (Undecaprenyl-PP-MurNAc-pentapeptide-UDPGlcNAc GlcNAc transferase) (N-terminal part)		1		1
MTH1647	15679642	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase (Undecaprenyl-PP-MurNAc-pentapeptide-UDPGlcNAc GlcNAc transferase) (C-terminal part)		1		
MTH1649	15679644	hydrogenase expression/formation protein HypC (HypC)		1		
MTH1657	15679652	indole-3-glycerol phosphate synthase (TrpC)		1		1
MTH1659	15679654	tryptophan synthase, beta subunit 1 (TrpB1)		1		
MTH1660	15679655	tryptophan synthase, subunit alpha (TrpA)		1		
MTH1664	15679658	conserved protein (COG2151, DUF59); predicted Suf system metal-sulfur cluster biosynthetic enzyme		1		1
MTH1666	15679660	glutamate synthase (NADPH), alpha subunit related protein		1		
MTH1669	15679663	transcription initiation factor IIE, alpha subunit		1		
MTH1671	15679665	conserved methanogen-specific Fe-S protein (COG2000) related to carbon monoxide dehydrogenase subunit gamma (cdhE)		1		1
MTH1672	15679666	Ni2+-binding GTPase involved in regulation of expression and maturation of hydrogenase HypB (COG0378)	1	1	1	1
MTH1673	15679667	SN-glycerol-3-phosphate transport ATP-binding protein (ugpC)	1	1		1
MTH1674	15679668	(2R)-phospho-3-sulfolactate synthase (ComA)		1		1
MTH1675	15679669	conserved FMN-binding protein (COG1751, DUF1867)	1	1	1	
MTH1676	15679670	cell division protein FtsZ	1	1	1	1
MTH1678	15679672	transcription termination factor NusG	1	1	1	
MTH1679	15679673	50S ribosomal protein L12 (E.coli L11P)	1	1	1	1
MTH1680	15679674	50S ribosomal protein L10a (E.coli L1P)	1	1	1	1
MTH1681	15679675	50S ribosomal protein Lp0 (E.coli L10) (acidic ribosomal protein P0) homolog (L10E)	1	1	1	1
MTH1682	15679676	50S ribosomal protein Lp1;L12P ('A' type)	1	1	1	1
MTH1683	15679677	alanyl-tRNA synthetase	1	1	1	1
MTH1686	15679680	fructose-1,6-bisphosphatase	1	1	1	1
MTH1689	15679683	conserved protein (COG1392, DUF47); phosphate transport regulator (distinct to PhoU)		1		
MTH1694	15679688	aspartate aminotransferase related protein		1		
MTH1695	15679689	RNase L inhibitor/ ATPase RIL	1	1		1
MTH1696	15679690	histone HMtA2		1		1
MTH1698	15679692	delta 1-pyrroline-5-carboxylate synthetase; aspartate/glutamate/uridylate kinase (COG2054)		1		
MTH1698a	15679901	Zn-ribbon RNA-binding protein involved in translation		1		1
MTH1699	15679693	translation elongation factor EF-1b				1
MTH1702	15679696	secretory protein kinase/ type II secretion system protein E	1	1	1	1
MTH1705	15679699	cobalt transport membrane protein (Cbi)/ predicted nickel transport protein NikQ		1		
MTH1707	15679701	cobalamin biosynthesis protein M (CbiM)/ predicted nickel transport protein NikM		1		
MTH1708	15679702	acetyl-CoA decarbonylase/synthase complex, alpha subunit (AcsB)		1	1	1
MTH1709	15679703	acetyl-CoA decarbonylase/synthase complex, epsilon subunit (AcsE)	1	1		
MTH1710	15679704	acetyl-CoA decarbonylase/synthase complex, beta subunit (AcsA)	1	1	1	1
MTH1712	15679706	acetyl-CoA decarbonylase/synthase complex, delta subunit (AcsD); corrinoid/iron-sulfur protein, small subunit		1	1	1
MTH1713	15679707	acetyl-CoA decarbonylase/synthase complex, gamma subunit (AcsC); corrinoid/iron-sulfur protein, large subunit	1	1	1	1
MTH1714	15679708	formate hydrogenlyase, iron-sulfur subunit 2		1		1
MTH1722	15679714	transcriptional regulator 3',5'-cyclic-nucleotide phosphodiesterase (Icc)		1		

		related protein				
MTH1727	15679719	periplasmic phosphate-binding protein PstS	1	1	1	1
MTH1728	15679720	periplasmic phosphate-binding protein PstS homolog	1	1	1	1
MTH1729	15679721	phosphate transporter permease PstC		1		
MTH1730	15679722	phosphate transporter permease PstC homolog		1		1
MTH1731	15679723	phosphate transport system ATP-binding	1	1	1	1
MTH1732	15679724	phosphate transport system regulator	1	1		1
MTH1733	15679725	conserved archaeal protein MTH1733 (COG1667)		1		1
MTH1734	15679726	phosphate transport system regulator phoU homolog	1	1	1	1
MTH1735	15679727	fumarate hydratase, class I (fumA)		1		
MTH1737	15679729	formate hydrogenlyase, iron-sulfur subunit I/ pyruvate oxidoreductase complex subunit		1		1
MTH1738	15679730	pyruvate oxidoreductase, beta subunit	1		1	
MTH1739	15679731	pyruvate oxidoreductase, alpha subunit	1	1	1	1
MTH1740	15679732	pyruvate oxidoreductase, gamma + delta subunit	1	1	1	
MTH1741	15679733	methanopterin biosynthesis protein; dihydropteroate synthase	1	1		1
MTH1742	15679734	conserved protein/ P-loop ATPase involved in RNA modification (thiouridine synthesis)	1	1		
MTH1745	15679737	protein disulfide-isomerase, thioredoxin-related (DsbD/CcdA)/ chaperonine (COG0785)		1		1
MTH1752	15679744	coenzyme F420-dependent N5,N10-methylene tetrahydromethanopterin reductase	1	1	1	1
MTH1757	5183324 15679745	alpha,alpha-trehalose-phosphate synthase		1		
MTH1764	15679752	sensory transduction regulatory protein (PAS/PAC/GAF domain, cGMP phosphodiesterase)		1		1
MTH1767	15679755	tyrosyl-tRNA synthetase (TyrRS)		1		1
MTH1768	15679756	NMD protein affecting ribosome stability and mRNA decay (COG1499)		1		1
MTH1769	15679757	translation initiation factor eIF-2, beta subunit		1		1
MTH1770	15679758	DNA replication initiator (Cdc21/Cdc54/minichromosome maintenance), MCM helicase		1	1	1
MTH1772	15679760	conserved Methanobacteriaceae-specific membrane-spanning protein MTH1772		1	1	1
MTH1773	15679761	cell division protein J; ribosomal RNA methyltransferase (EC 2.1.1.-) (rRNA (uridine-2'-O-)-methyltransferase)	1	1	1	
MTH1774	15679762	conserved protein (COG0622, UPF0025)/ predicted phosphoesterase, YfcE		1		
MTH1777	15679765	conserved methanogen-specific protein MTH1777		1		
MTH1782	15679770	uncharacterized membrane-bound protein MTH1782		1		
MTH1783	15679771	uncharacterized proten MTH1783		1		
MTH1789	15679777	dTDP-glucose 4,6-dehydratase RfbB	1		1	
MTH1790	15679778	dTDP-4-dehydrorhamnose 3,5-epimerase RfbC		1		1
MTH1791	15679779	glucose-1-phosphate thymidylyltransferase RfbA		1		1
MTH1792	15679780	dTDP-4-dehydrorhamnose reductase RfbD		1		
MTH1798	15679786	flavodoxin, fldA (COG0716) C-terminal part		1		
MTH1799	15679787	flavodoxin, fldA (COG0716) N-terminal part	1	1		1
MTH1806	15679794	phycocyanin alpha phycocyanobilin lyase CpcE related protein; PBS lyase HEAT domain protein repeat-containing protein (COG1413)	1	1	1	
MTH1807	15679795	phytoene dehydrogenase/ desaturase	1	1		
MTH1824	15679812	Dps-family bacterioferritin protein (COG1633)/ rubrerythrin		1		1
MTH1826	15679814	conserved protein with 4Fe-4S ferredoxin domain (COG1633)		1		
MTH1827	15679815	quinolinate synthetase (NadA)		1		1
MTH1830	15679818	mechanosensitive channel protein MscS		1		
MTH1833	15679821	conserved hypothetical protein MTH1833 (COG1196)	1	1	1	1
MTH1834	15679822	ZPR1-like zinc finger protein (COG1779)	1	1	1	1
MTH1836	15679824	conserved protein (COG2018) with regulatory roadblock-LC7 domain		1		1
MTH1837	15679825	P-loop containing nucleoside triphosphate hydrolase (COG3367, DUF1611), predicted HypB/UreG family	1			
MTH1838	15679826	conserved Methanobacteriaceae-specific protein MTH1838 (COG2450)		1		1
MTH1840	15679828	cell division ATPase MinD	1	1	1	1
MTH1842	15679830	TatD-related protein export protein (COG1099)		1		
MTH1846	15679834	glycyl-tRNA synthetase	1	1		1
MTH1847	15679835	bifunctional deoxycytidine triphosphate deaminase/dephosphatase		1		1
MTH1850	15679838	fumarate reductase (frdB); thiol:fumarate reductase subunit B protein		1	1	1
MTH1852	15679840	indolepyruvate oxidoreductase, alpha subunit (Indolepyruvate ferredoxin oxidoreductase alpha subunit)		1		
MTH1854	15679842	regulatory subunit (ACT domain family) linked with coenzyme F390 synthetase II	1	1	1	

MTH1855	15679843	coenzyme F390 synthetase II	1	1		1
MTH1858	15679846	phage infection protein homolog/ ABC-2 type transporter, permease (ATP-dependent Na+ efflux)		1		1
MTH1859	15679847	conserved methanogen-specific protein (COG1873) implicated in RNA metabolism		1		1
MTH1860	15679848	orotate phosphoribosyltransferase (EC 2.4.2.10) (OPRT) (OPRTase); orotidine 5'-monophosphate synthase	1	1	1	1
MTH1861	15679849	molybdenum cofactor biosynthesis MoaB	1	1		1
MTH1865	15679853	predicted methanogen-specific peptidyl-prolyl cis-trans isomerase (UPF0288)	1	1		
MTH1866	15679854	conserved methanogen-specific protein MTH1866 (COG4029)			1	
MTH1867	15679855	conserved methanogen-specific protein MTH1867 (COG4048)		1		1
MTH1868	15679856	conserved methanogen-specific protein MTH1868 (COG4050)	1	1	1	
MTH1869	15679857	activator of (R)-2-hydroxyglutaryl-CoA dehydratase; CoA-substrate-specific enzyme activase	1	1	1	1
MTH1870	15679858	conserved methanogen-specific protein MTH1870 (COG4051)	1			
MTH1871	15679859	nitrogenase iron-molybdenum cofactor biosynthesis protein NifB (NifB) (radical SAM protein)		1		
MTH1872	15679860	translation initiation factor eIF-2B, alpha subunit		1		
MTH1878	15679866	CoB--CoM heterodisulfide reductase iron-sulfur subunit C (EC 1.8.98.1)	1	1	1	1
MTH1879	15679867	CoB--CoM heterodisulfide reductase subunit B (EC 1.8.98.1)	1	1	1	1
MTH1880	15679868	conserved methanogen-specific protein (COG4009, DUF749)		1		1
MTH1881	15679869	conserved methanogen-specific protein MTH1881 (COG4010)		1		1
MTH1882	15679870	conserved protein (COG2191); probable phosphoesterase (ICC related)	1	1		1
MTH1884	15679872	conserved protein; signal transduction CBS-domain (2x); cyclic nucleotide-binding domain protein		1		1
MTH1894	15679876	putative L-threonine-O-3-phosphate decarboxylase, cobalamin biosynthesis protein D (CobD)		1		
MTH1900	15679882	uncharacterized protein MTH1900	1	1	1	
MTH1901	15679883	membrane-bound partially conserved protein (COG0810); phosphatidylserine/phosphatidylglycerophosphate/cardiolipin related synthase		1		1
MTH1902	15679884	conserved protein; Zn-dependent hydrolase (beta lactamase fold) (COG2220, UPF0173))	1	1		1
MTH1903	15679885	GTP-binding protein E.coli Ras-family (Era)	1	1		1
MTH1904	15679886	conserved archaea-specific protein MTH1904 (COG3365)		1		
MTH1907	15679889	conserved protein (COG1293, DUF814), predicted RNA-binding protein, eukaryotic snRNP-like protein		1		
MTH1910	15679892	fumarate hydratase, class I related protein, beta subunit	1	1	1	1
MTH1911	15679893	conserved protein MTH1911 (COG0800); TIM barrel protein/ metal-dependent phosphoesterase (PHP family)		1		
MTH1912	15679894	conserved Methanobacteriaceae-specific protein MTH1912		1	1	1
MTH1913	15679895	conserved protein MTH1913 (COG1432, DUF88)	1	1	1	1
MTH1914	15679896	pyridoxal phosphate-dependent protein MTH1914 (COG1921, UPF0425)		1		1
MTH1916	15679898	biotin acetyl-CoA carboxylase ligase; biotin operon repressor bifunctional protein		1		
MTH1917	15679899	pyruvate carboxylase subunit A (EC 6.4.1.1) (Pyruvic carboxylase A) (PycA)	1	1	1	1
MTH1918	15679900	protein methyltransferas/ RNA methylase		1		