Methanobacterium formicicum as a target rumen methanogen for the development of new methane mitigation interventions: A review

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ABSTRACT

Methanobacterium formicicum (Methanobacteriaceae family) is an endosymbiotic methanogenic Archaea found in the digestive tracts of ruminants and elsewhere. It has been significantly implicated in global CH4 emission during enteric fermentation processes. In this review, we discuss current genomic and metabolic aspects of this microorganism for the purpose of the discovery of novel veterinary therapeutics. This microorganism encompasses a typical H2 scavenging system, which facilitates a metabolic symbiosis across the H2 producing cellulolytic bacteria and fumarate reducing bacteria. To date, five genome-scale metabolic models (iAF692, iMG746, iMB745, iVS941 and iMM518) have been developed. These metabolic reconstructions revealed the cellular and metabolic behaviors of methanogenic archaea. The characteristics of its symbiotic behavior and metabolic crosstalk with competitive rumen anaerobes support understanding of the physiological function and metabolic fate of shared metabolites in the rumen ecosystem. Thus, systems biological characterization of this microorganism may provide a new insight to realize its metabolic significance for the development of a healthy microbiota in ruminants. An in-depth knowledge of this microorganism may allow us to ensure a long term sustainability of ruminant-based agriculture.

1. Introduction

CH4 is the second largest anthropogenic greenhouse gas and its global warming potential is 25 times more than that of CO2 (Forster et al., 2007; IPCC 2007). The US-Energy Protection Agency (EPA) stated that China, India, the United States, Brazil, Russia, Mexico, Ukraine and Australia are the major CH4 emitters in the world. CH4 emission is projected to increase by 15% to 7904 MMT (Million Metric Ton)-CO2Eq. by 2020 (US-EPA, 2014). About 25% of enteric CH4 emission accounted globally from the ruminants represents a loss of 5–7% of dietary energy (Hristov et al., 2013; Thorpe, 2009).

A total CH4 emission is estimated to be 163.3 MMT-CO2 Eq. from enteric fermentation and 61.2 MM-TCO2 Eq. from manure management (US-EPA, 2014). Beef (116.7 MMT-CO2 Eq.) and dairy cattle (41.6 MMT-CO2 Eq.) are being as main sources for enteric CH4 emission (Table 1). The management of manure from anaerobic digester (61.2 MMT-CO2 Eq.), dairy cattle (32.2 MMT-CO2 Eq.) and swine (22.4 MMT-CO2 Eq.) is also contributed to global CH4 emission. Cattle (77.3 kg × 10⁶) and buffalo (12.1 kg × 10⁶) will be the major sources for the projected global CH4 emission from enteric fermentation in 2025. CH4 emission budget (105 kg × 10⁶) will be 107 times more from manure management (12,849 kg × 10⁶)(Table 2). Hence, reducing CH4 emissions from ruminants is not only benefits for the environment, but also to ensure the long-term sustainability of ruminant-based agriculture (Zhang et al., 2015).

1.1. Rumen microbiota

The typical rumen microbiota consists of 10–50 billion bacteria, 1 million protozoa and variable numbers of yeasts and fungi in each milliliter of rumen content (Ekarius, 2010). Anaerobic microbes are degrading polysaccharides (cellulose, hemicellulose, starch and pectin), proteins, and lipids from food/feed and of producing organic acids (formate, pyruvate, acetate, propionate, butyrate, and succinate) from which CH4 gas is produced by rumen methanogenic archaea (Chellapandi, Prabaharan, & Uma, 2010). The rumen microbes rapidly ferment amino acids and soluble proteins and form various acidic fermentation products (NH3, H2 and CO2). The turnover rate of fermentation products cannot be measured due to exchange reactions across the microorganisms (Andrade-Montemayor, Gasca, & Kawas, 2009). Pyruvate produced from anaerobes is carboxylated to form oxaloacetate and further converted to malate, fumarate and succinate. The rumen microbes that are not producing succinate can use CO2 as a sole carbon source (Ungerfeld, 2015). Gut microbiota are able to synthesis

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CO2 through methanogenesis. This process depends on the availability of carbon substrates such as formate, pyruvate, methylamine, acetate, and by-products of the rumen bacteria, for CH4 production in rumen gut environment. CO2 is released by the animal into the atmosphere. MFI is a clinically important microorganism because it can cause gastrointestinal and metabolic disorders in animals and humans (Kelly et al., 2014; Mitsumori & Sun, 2008). This microorganism is able to ferment acetate, carbohydrate, amino acid, ethanol, methanol, propionate, butyrate and lactate. MFI contains all essential genes need for methanogenic process with exception of [Fe]-hydrogenase dehydrogenase (hmd). Both CH4 production and formate consumption are linear functions of its growth rate. The molar growth yield of MFI for CH4 of formate cultures is 4.8 g dry weight per mol, and that of H2–CO2 culture was 3.5 g dry weight per mol (Schauer & Ferry, 1980). Pseudomurein and polysaccharide biosynthesis genes are similar to those found in M. ruminantium (Leahy et al., 2010). MFI strain BRM9 does not have homologs of N-acyleuraminic acid cytidyl transferase coding genes (neuA/neuB) as in the strain DSM 3637 (Kandiba & Eichler, 2013). MFI consists of 3 ectoine biosynthetic genes usually existed in halo-tolerant microorganisms, but ectoine is not yet reported to be produced by methanogenic archaea (Lo, Bonner, Xie, D’Souza, & Jensen, 2009; Moe et al., 2009). The genome of this microorganism contains a large number of genes for two-component signal transduction system. This system helps to monitor the changes in the redox potential, oxygen and overall cellular energy level of MFI (Taylor & Zhuin, 1999). It has a characteristic metabolism of nitrogen, particularly in ammonium transporters and glutamine synthase/glutamate synthase pathway.

### 1.2. Rumen methanogenic archaea

Methanogenic archaea are capable of producing CH4 from the low carbon substrates such as formate, pyruvate, methylamine, acetate, and CO2 through methanogenesis. This process depends on the availability of ATP derived from enteric fermentation of rumen anaerobic bacteria (Balch, Fox, & Magrum, 1979; Hook, Wright, & McBride, 2010). The overall effects of methanogenic archaea play a crucial role in the physiology and health of the ruminants (Delzenne & Cani, 2011). Methanomicrobium mobile, Methanobacterium lacus, Methanobacterium formicicum (MFI), Methanomicrobium bryantii, Methanobrevibacter ruminantium, Methanobrevibacter smithii, Methanocarcinace barkeri and Methanosarcina mazei are culturable rumen methanogenic archaea have been studied in detail (Henderson et al., 2015). Methanobacterium and Methanobrevibacter are predominant genera usually inhabited in the rumen ecosystem.

### 2. Methanobacterium formicicum

MFI is a representative species of methanogenic archaea found in the gut of ruminants and humans (Pimentel et al., 2012; Strohi, Goel, & Pandey, 2012). This microorganism can utilize CO2 with H2, fermented by-products of the rumen bacteria, for CH4 production in rumen gut environment. CO2 is released by the animal into the atmosphere. MFI is a clinically important microorganism because it can cause gastrointestinal and metabolic disorders in animals and humans (Kelly et al., 2014; Mitsumori & Sun, 2008). This microorganism is able to ferment acetate, carbohydrate, amino acid, ethanol, methanol, propionate, butyrate and lactate. MFI contains all essential genes need for methanogenic process with exception of [Fe]-hydrogenase dehydrogenase (hmd). Both CH4 production and formate consumption are linear functions of its growth rate. The molar growth yield of MFI for CH4 of formate cultures is 4.8 g dry weight per mol, and that of H2–CO2 culture was 3.5 g dry weight per mol (Schauer & Ferry, 1980). Pseudomurein and polysaccharide biosynthesis genes are similar to those found in M. ruminantium (Leahy et al., 2010). MFI strain BRM9 does not have homologs of N-acyleuraminic acid cytidyl transferase coding genes (neuA/neuB) as in the strain DSM 3637 (Kandiba & Eichler, 2013). MFI consists of 3 ectoine biosynthetic genes usually existed in halo-tolerant microorganisms, but ectoine is not yet reported to be produced by methanogenic archaea (Lo, Bonner, Xie, D’Souza, & Jensen, 2009; Moe et al., 2009). The genome of this microorganism contains a large number of genes for two-component signal transduction system. This system helps to monitor the changes in the redox potential, oxygen and overall cellular energy level of MFI (Taylor & Zhuin, 1999). It has a characteristic metabolism of nitrogen, particularly in ammonium transporters and glutamine synthase/glutamate synthase pathway.

#### 2.1. Genomic features

Currently, 7 complete genome sequences are available for different species of Methanobacterium and Methanobrevibacter (Gutiérrez, 2012; Leaby et al., 2010, Leaby, Kelly, Li, 2013) (Fig. 1; Maus et al., 2013a). Researchers have identified 3 different strains (DSM3637, DSM1535 and BRM9) of MFI from the bovine rumen (Kelly et al., 2014; Maus et al., 2013, 2014). Comparison of 16S rRNA gene sequences indicate 99.8% sequence similarity between strain BRM9 and strain DSM1535 (Bryant & Boone, 1987). MFI strain KOR-1 strain isolated from an anaerobic digester using pig slurry has shown 98% rRNA gene and 97% mcrA gene sequence similarities to other strains (Battumur, Yoon, & Kim, 2016). The genome of strain DSM3637 (2.47 Mbp) comprises of a total of 2556 protein-coding genes in which 643 proteins assigned to be hypothetical proteins (Gutiérrez, 2012). The strain DSM1535 is 2.4 Mbp in genome size with 41.23% GC content and encoded for several adaptation genes responsible for abiotic stress (Maus et al., 2014). The BRM9 strain consists of a single 2.44 Mbp circular chromosome with 2352 protein coding genes (83%). A putative function is assigned to 1715 of the protein-coding genes, with the remainder annotated as hypothetical proteins (Kelly et al., 2014). Conserved hypothetical proteins are ranged from 413 to 736 in the genera of Methanobacterium and Methanobrevibacter.

#### Table 1

| Livestock         | Enteric fermentation | Manure management |
|-------------------|----------------------|-------------------|
| Cattle            | 77.3                 | 7002              |
| Buffalo           | 12.1                 | 933               |
| Sheep             | 6.18                 | 183               |
| Goat              | 5.19                 | 222               |
| Swine             | 1.29                 | 3700              |
| Poultry           | –                    | 537               |
| Camel             | 1.17                 | 62                |
| Horse             | 1.03                 | 9                 |
| Mule              | 0.09                 | 8                 |
| Alpaca            | 0.13                 | 12,849            |

*Accounts for CH4 reductions due to capture and destruction of CH4 at facilities using anaerobic digesters.

EF: Enteric fermentation; MM: Manure management.
2.2. Transcription regulatory systems

The MFI genome possesses a maximum number of transcription units compared to the genera of Methanobacterium and Methanobrevibacter (Kelly et al., 2014; Kern, Linge, & Rother, 2015; Worm, Stams, Cheng, & Plugge, 2011). Transcriptional regulation of coenzyme F420-dependent formate dehydrogenase (fdhCAB) (Patel & Ferry, 1988; Schauer & Ferry, 1982; White & Ferry, 1992), glycer-aldehyde-3-phosphate dehydrogenase (Fabry, Lang, Niermann, Vingron, & Hensel, 1989), archaeal histones (hfOAB) (Darcey, Sandman, & Reeve, 1995; Zhu, Sandman, Lee, Reeve, & Summers, 1998) has been extensively characterized in this microorganism. NrpR is a transcriptional regulator that represses transcription of nitrogen fixation genes, glutamine synthase and ammonium transporters. This regulator binds to inverted repeat operators in the promoter regions located upstream from the starts of glnA,nifH, pdxT, amt1 and amt2 (Andrade-Montemayor et al., 2009; Lie et al., 2010; Magingo & Stumm, 1991). MFI contains nif operon, nitrogenase and nitrogenase cofactor biosynthesis genes as similar to Methanococcus maripaludis (Lie et al., 2010; Magingo & Stumm, 1991). An intensive analysis of the current genomic data of MFI provided a new avenue for the development of veterinary vaccines and small-molecule inhibitors for CH4 mitigation (Leaky, Kelly, Ronimus et al., 2013; Wedlock et al., 2010). Hence, the MFI genome is considered as a suitable candidate for studying systems biological characterization of rumen methanogens. Such systems-level information is currently useful to discover new veterinary vaccines and chemogenomic targets for new CH4 mitigation interventions (Bharathi & Chellapandi, 2017; Sedano-Núñez, Boeren, Stams, & Plugge, 2018).

2.3. Metabolic regulatory systems

Metabolic pathway data including reactions, enzymes, and metabolites provide insight into the growth and metabolic physiology of MFI (Fig. 1b). This genome consists of abundant genes for the biosynthesis of carbohydrate and nucleotides (Fig. 1c). Genes involved in anabolism of this microorganism are considerably lower than that of other species in Methanobacterium genus, indicative of a characteristic system exists for carbohydrate biosynthesis. It has been well-established that systems for catabolism of amino acids and nucleic acids, which are relatively low to related genera (Fig. 1d). Methanobacterium lacus and Methanobacterium sp. SWAN-1 have 5 more additional genes for energetic CH4 biosynthesis, compared to the MFI genome. MFI is a target rumen methanogen for the development of new CH4 mitigation interventions owing to the existence of conserved nature of genes required for methanogenesis, central metabolism and Pseudomurein cell wall formation (Kelly et al., 2014).

Fig. 1. Genome-scale metabolic information of MFI and related rumen methanogens, collected from the MetaCyc database (https://metacyc.org/). We compared genome (a), metabolome (b), anabolism (c) and catabolism (d) to infer genomic similarities and dissimilarities across them. (MEL: Methanobacterium lacus, MEW: Methanobacterium sp. SWAN-1, METH: Methanobacterium sp.MB1, MSI: Methanobrevibacter smithii, MRU: Methanobrevibacter ruminantium, MEB: Methanobrevibacter sp. AbM4).
2.4. Gut microbial symbiosis

Gut microbiota are shaped by both genetic background and lifestyle, which in turns impair intestinal barrier function (Burcelin, 2010; Stenman, Burcelin, & Lahtinen, 2015) and modulates epithelial cell proliferation (Sommer & Bäckhed, 2013) and metabolic inflammation (Stenman et al., 2015). It is well known that healthy gut microbiota are essential one to protect against the pathogenic microorganisms in the intestine (Tremaroli & Bäckhed, 2012) and modulate gut-brain axis (Hsiao et al., 2013). Several studies have focused on the metabolic crosstalk between gut microbiota and host to reveal the metabolic disorders of human (Burcelin, 2010; Cani & Delzenne, 2009; Koeth, Wang, & Levison, 2013; Stenman et al., 2015), but none has been reported for animals. A gut microbial composition may restrict the production of certain bacterial metabolites (Heinken, Sahoo, Fleming, & Thiele, 2013). Microbial mutualism can occur through metabolic interactions between host and gut microbe and between microbe and microbe (Bath, Morrison, Ross, Hayes, & Cocks, 2012; Morgavi et al., 2015). Endo-symbiotic methanogenic archaea are usually habituated in the gastrointestinal tracts of ruminants, which are contributing in the syntrophic degradation and improved metabolic function. MFI is an endosymbiotic methanogenic archaea of free-living anaerobic flagellate *Psalteriomonas vulgaris* (Broers et al., 1993) associating syntrophically with *Syntrophomonas zehnderi* (Sousa, Smidt, Alves, & Stams, 2007).

2.5. H₂ scavenging systems

Interspecies H₂ transfer is a metabolic process occurring between hydrogenotrophic methanogenic archaea and cellulosytic/acetogenic bacteria. Hydrogenotrophic methanogenic archaea maintain the partial pressure of H₂ by utilizing H₂ produced by cellulosytic bacteria. *Ruminococcus albus* and *R. flavefaciens* are H₂-producing cellulosytic anaerobe for interspecies H₂ transfer of MFI (Chaucheyras-Durand, Masségia, Fonty, & Forano, 2010; Joblin, Naylor, & Williams, 1999; Pavlostathis et al., 1996; Williams, Withers, & Joblin, 1994; Wolin, Miller, & Stewart, 1997). *Fibrobacter succinogenes*. *Wolinella succinogenes* and *Mitsukella jalaludini* are fumarate reducing rumen anaerobic bacteria. These microorganisms are able to reduce CH₄ production either by competing with hydrogenotrophic methanogenic archaea for H₂ as well as formate or by increasing succinate (Asanuma, Iwamoto, & Hino, 1999; Mamuad et al., 2012; Mamuad et al., 2014). Therefore, cellulosytic and fumarate reducing bacteria are extensively studied symbiotic anaerobes for interspecies H₂ transfer of MFI. Comparative metabolic analysis shows that 237 metabolic enzymes are shared across the MFI, *F. succinogens* and *R. albus* and 210 enzymes are common between *F. succinogens* and *R. albus* (Fig. 2). MFI has 126 unique enzymes across *F. succinogens* and *R. albus*, 38 enzymes shared with *R. albus* and 44 enzymes with *F. succinogens*. Hence, studying metabolic symbiosis of these genomes is important to comprehend the gut physiology and metabolic disorders of veterinary animals.

3. Systems biology paradigm

The biochemical function of individual genes and proteins of microorganisms has been investigated by traditional molecular approaches. A complexity of microbial symbiosis and metabolic crosstalk has been reconciled by recent quantitative systems biology advances. Genome-scale metabolic models are being as the promising computational platforms for studying intracellular metabolism and interspecies interactions of microbial communities and for hypothesis testing (Liu, Agren, Bordel, & Nielsen, 2010).

3.1. Genome-scale reconstructions for methanogenic archaea

Systematic analysis of methanogenic archaea and mutualistic anaerobic bacteria provides an opportunity to capture growth parameters and bacterial community composition (Durmus, Cakir, Ozgür, & Guthke, 2015; Stolyar, Van Dien, Hillesland, & Pinel, 2007). *In silico* models for *M. barkeri* (IAF692; IMG746) (Feist, Scholten, Palsson, Brockman, & Ideker, 2006; Donnet, Feist, Metcalf, & Price, 2013), *M. acetivorans* (iMB745; iVS941) (Benedict, Gao, Zhao, & Huang, 2014; Sadhukhan & Raghunathan, 2014; Shoaie Gao, Zhao, & Huang, 2014; Shoaie et al., 2013; Shoaie et al., 2013; Shoaie et al., 2013; Shoaie et al., 2013) and *Methanococcus maripaludis* (iMM518) (Gao, Widiastuti, Karimi, & Zhou, 2014) have been previously developed for studying their metabolic behaviors on different growth substrates. In addition to that, a metabolic flux model has been reconstructed for understanding a microbial mutualism between *M. maripaludis* (Goyal et al., 2014) and *Desulfovibrio vulgaris* (Stolyar et al., 2007).

3.2. Genome-scale reconstructions for gut-microbe interactions

Several genome-scale models have been developed for evaluating the mechanistic details of gut-microbe interactions (Ding et al., 2016; Gao, Zhao, & Huang, 2014; Sadhukhan & Raghunathan, 2014; Shoaiie and Nielsen, 2014; Shoaiie et al., 2013; Shoaiie et al., 2013; Shoaiie et al., 2013; Shoaiie et al., 2013). GHIDIAS (Xiang, Tian, & He, 2007), HPDB (Kumar & Nanduri, 2010), PHISTO (Durmus et al., 2013), PATRIC (Wattam, Gabbard, Shukla, & Sobral, 2014), PHI-base (Urban, Irvine, Cuzick, & Hammond-Kosack, 2015), CASINO (Shoaiie et al., 2015), HMI™ module (Marzorati et al., 2014), and NetCooperate (Levy, Carr, Kreimer, Freilich, & Borenstein, 2015) are web-based tools and databases accessible for studying the microbiome, diet-microbe and microbe-host interactions. GeoSymbio (Franklin, Stat, Pochon, Putnam, & Gates, 2012) and SymbioGenomesDB (Reyes-Prieto, Vargas-Chávez, Latorre, & Moya, 2015) are specialized computational resources developed for learning the host-microbiome interactions and microbial symbiosis (Table 3).

4. CH₄ mitigation interventions

CH₄ mitigation stratagies should ideally target features that are conserved across all rumen methanogenic archaea. Consequently, other beneficial anaerobes continue their normal digestive functions in the ruminants (Gottlieb, Wacher, Silman, & Pimentel, 2016; Weimer, Stevenson, Mertens, & Thomas, 2008). Several CH₄ mitigation
interventions have been investigated such as change in dietary composition like use of fatty acids (Agarwal, Kamra, Chatterjee, Ravindra, & Chaudhary, 2008), tannin (Kumar et al., 2009), monensin (Weimer et al., 2008), plant extracts (Goel, Makkar, & Becker, 2008; Sirohi et al., 2012), fumarate and chemical inhibitors (Chidthaisong & Conrad, 2000; Miller & Wolin, 2001; Ungerfeld, Rust, Boone, & Liu, 2004), and anti-methanogenic vaccines (Wedlock, Janssen, Leahy, Shu, & Buddle, 2013; Williams, Popovski, & Rea, 2009). So far, only a small percentage of CH4 mitigation has been successfully implemented by dietary changing. Some chemical inhibitors have been investigated to destroy the pathogenic bacteria, and those inhibitors may be beneficial to the host, which in turn affects the rumen microbiota. Thus, it is important to access the effect of methanogenic inhibitors on the stability of rumen healthy microbiota and also to discover new chemogenomic targets for CH4 mitigation.

4.1. Methanogenic antibiotics and inhibitors

Chemical inhibitors or enzymes targeting essential functions of methanogenic archaea are delivered via slow-release capsules administered to the rumen. Neomycin, pseudomonic acid (Boccuzzi, Zhang, & Metcalf, 2000; Jenal, Rechsteiner, & Tan, 1991), puromycin (Gernhardt, Possot, & Foglino, 1990), 8-aza-2, 6-diaminopurine (Pritchett, Zhang, & Metcalf, 2004) and 8-aza-hypoxanthine (Moore & Leigh, 2005) are methanogenic antibiotics, and inhibitors are presently used against M. maripaludis and M.arkeri. Ethyl 2-butynoate, lovatatin, mevastatin, fluoroacetate, chloroform, 2-bromoethanesulfonate, and 2-nitroethanol are potential methanogenic inhibitors investigated to inhibit the methanogenesis of Methanobrevibacter and Methanobacterium (Chidthaisong & Conrad, 2000; Miller & Wolin, 2001; Ungerfeld et al., 2004). The growth of methanogenic archaea and persistence of 2-bromoethanesulfonate resistance increased with administration of it in bovine (Van Nevel & Demeyer, 1996). M. ruminantium, M. mazeli and M. mobile found to be resistant to 3-bromopropanesulfonate up to 250 μmol/L in pure cultures (Ungerfeld et al., 2004). Therefore, it is consistent with the limited efficacy of 2-bromoethanesulfonate and 3-bromopropanesulfonate in lowering CH4 production by rumen microbiome (Karnati, Sylvester, Ribeiro, Gilligan, & Firkins, 2009; Patra, Park, Kim, & Yu, 2017).

Monensin inhibits the methanogenesis from formate, but not from H2–CO2 in ruminants (Dellinger & Ferry, 1984). Bovine somatotrophin, monensin and lasalocid have been extensively used in beef and cattle farming to improve growth rates (Abrar, Kondo, Kitamura, Ban-Tokuda, & Matsui, 2016; Appuhamy et al., 2013; Etherton, 2013). Monensin affects electrolyte transport of methanogenic and propionate-producing bacteria. It also inhibits some bacteria responsible for proteolysis and deamination. A long term supplementation of monensin does not have an implementation in CH4 reduction efficacy (Hook, Northwood, Wright, & McBride, 2009). Interestingly, Thermoplasma archaea are methylotrophic (methylamine degrading) methanogens found in bovine rumen, which are able to mitigate methane emissions from lactating cows upon dietary supplementation with rapeseed oil (Poulsen et al., 2013). Thus, methanogenic inhibitors have been investigated in regard to their affect on the total population of Methanobacterium, Methanobrevibacter, Methanosphaera and Thermoplasmata making unbalanced microbial ecosystem in the rumen gut (Witzig, Zeder, & Rodehuts cord, 2018; Zhou, Meng, & Yu, 2011; Zhu et al., 2017).

4.2. Chemogenomic targets

Generally, chemogenetic antibiotics and inhibitors that target the key enzymes involved in the biosynthesis of cell wall, protein, vitamins and cofactors of MFI. Hydroxymethylglutaryl-SCoA reductase, aconitate, coenzyme M are common targets for many methanogenic inhibitors (Chidthaisong & Conrad, 2000; Miller & Wolin, 2001; Ungerfeld et al., 2004) (Table 4; Supplementary). Some of the methanogenic inhibitors have shown to decrease the proton gradient across the membrane, loss of digestible energy for ruminants, regulation of formate and H2 oxidation, carbohydrate-fermentation and acetate metabolism (Chen & Wolin, 1979; Chidthaisong & Conrad, 2000; Liu, 2005).
Table 5
Chemogenomic and vaccine targets identified in *M. formicicum* formate/methane mitigation.

| Metabolism                        | Gene/Locus tag | EC       | Molecular function                                      |
|-----------------------------------|----------------|----------|---------------------------------------------------------|
| Amino acid metabolism             | RS11100        | 4.2.3.4  | 3-Dehydroquinate synthase                               |
|                                   | RS02945        | 2.5.1.19 | 3-Phosphoshikimate 1-carboxyvinyltransferase            |
|                                   | RS01470        | 4.1.1.20 | Diaminopimelate decarboxylase                           |
| Cell cycle                        | RS05130| RS05135 | 5.99.1.3 | DNA topoisomerase VI subunit AB                         |
| Cell envelop                      | glmU           | 2.3.1.157| Glucosamine-1-phosphate N-acetyltransferase            |
| Central carbon metabolism         | sdhA/Rs12465   | 2.3.1.101| Formylmethanofuran–tetrahydromethanopterinformyltransferase |
|                                   | RS07380        | 2.8.4.1  | Coenzyme-B sulfselenothioltransferase                   |
|                                   | RS01770        | 2.1.1.86 | Tetrahydromethanopterin S-methyltransferase subunit ABCDEFGH |
|                                   | RS09605        | 1.2.99.5 | Formylmethanofuran dehydrogenase subunit E              |
|                                   | RS09415| RS09420| RS09430| RS09435| RS07735| RS00350| RS00355| RS00360| RS00365 | 3.5.4.27 | N(5),N(10)-methyltetrahydromethanopterin cyclohydrolase |
|                                   | RS09460| RS09465| RS09470| RS09475 | 2.5.4.14 | 3.6.3.14* | ATP synthase subunit ABCDFK |
| Lipid metabolism                  | ubaA/Rs02925   | 2.5.1.1  | Dimethylallyltransferase                                |
|                                   | RS05485        | 5.3.3.2  | Isopentenyl pyrophosphate isomerase                     |
| Protein biosynthesis              | RS01925        | 6.3.5.6  | Asparaginyl-tRNA synthase subunit CDE                   |
|                                   | RS04105        | 6.3.5.7  | Glutaminyl-tRNA synthase subunit ABCDE                  |
| Vitamins and cofactors            | RS01035        | 2.4.2.52 | Triphosphoribosyl-diphospho-CoA synthase                |
| Energy metabolism                 | RS06810        | 2.7.9.1  | Selenide, water dikinase                                |
|                                   | rsbA, berA, berC, berD | 1.3.7.8  | Benzoyl-CoA reductase                                   |
| Protein fate                      | RS09460| RS09465| RS09470| RS09475 | 2.1.1.86 | 3.4.2.3.43* | Tetrahydromethanopterin S-methyltransferase subunit ABCDEFGH |
4.3. Veterinary vaccination

Immunization is one of the novel CH\textsubscript{4} mitigation strategies in which the animals acquire immunity against a particular rumen methanogenic archaea (Iqbal, Cheng, Zhu, & Zeshan, 2008; Mitsumori & Sun, 2008; Ulyatt, Lassey, Shelton, & Walker, 2002). When animals are vaccinated, salivary antibodies are produced in the animal against rumen methanogenic archaea. A vaccine developed against Streptococcus bovis and Lactobacillus species causes a lactic acidosis, which elicits an immune response against rumen methanogenic archaea (Gill, Shu, & Leng, 2000; Shu et al., 2000). Using VF3 and VF7 antigens, anti-methanogenic vaccines have been investigated for the reduction of CH\textsubscript{4} emission from enteric fermentation (Williams et al., 2009; Wright et al., 2004). Vaccination of sheep with methanogenic archaeal fractions has been developed for effective CH\textsubscript{4} mitigation (Wedlock et al., 2010). Genome sequence of *M. ruminantium* M1 was compared with closely related methanogenic archaea to identify conserved methanogen surface proteins as suitable candidates for the development of vaccines (Leahy et al., 2010). Energy metabolism (EC 2.1.1.86, 3.6.3.14), protein fate (EC 3.4.23.43) and adhesion/cell surface proteins are identified as vaccine targets for MFI (Table 5; Supplementary). Yet, new CH\textsubscript{4} mitigation interventions should be addressed in the development of alternative veterinary vaccines against MFI. Any veterinary vaccine should be targeted methanogen-specific proteins and should not affect the growth of other beneficial microorganisms, which can be resolved by systems-biology approach.

5. Conclusions

Grazing ruminant animals are important contributors to the CH\textsubscript{4} pool and account for 25% of greenhouse gas emission in the world. A genome-scale metabolic network of MFI could be reconstructed to elucidate its metabolic symbiosis across the gut microbiota and host. The mechanisms underlying syntrophic and competitive behaviors of this microorganism can be explored with experiment-driven molecular hypotheses. Metabolic modeling process may serve as a platform to discover and prioritize the potential chemoogenic and vaccine targets from MFI for CH\textsubscript{4} mitigation interventions. To resolve this issue effectively at the systems-scale, we should address the following questions; 1. What are the key metabolites to be produced from MFI to ensure its growth and methanogenesis in a rumen ecosystem? 2. How does the core metabolic network of MFI determined its symbiotic behavior across the physiologically distinct anaerobes? and 3. How is MFI interacting with gut microbiota via metabolic crosstalk in response to microbial symbiosis, drugs and nutrients?

The development of CH\textsubscript{4} mitigation interventions is a great concern for improving animal production efficiency because of the demand for increased meat and milk products. The H\textsubscript{2} scavenging action of MFI is an essential function, since it prevents accumulation of H\textsubscript{2} produced as a result of enteric fermentation. Moreover, it is imperative for us to know that if the rumen methanogenic archaea are inhibited, what would be the alternate ways in which to reduce the accumulation of H\textsubscript{2}. Metabolic symbiosis of MFI in response to different environmental stimuli has resulted from the action of syntrophic and competitive bacteria in the ruminants, and it is important to understand its growth physiology in the gut environment. CH\textsubscript{4} mitigation strategies should be developed, but without affecting the beneficial rumen microbiome and microbiota, and without compromise to the digestive function of ruminants. Methanogenic antibiotics, inhibitors and vaccines have been narrow spectrum and species-specific activity, reflecting the demand for the potential target discovery for wide-range of methanogenic archaea.

Conflict of interest

The authors declare that this article’s content has no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jvas.2018.09.001.

References

Abir, A., Kondo, M., Kitamura, T., Ban-Tokuda, T., & Matsui, H. (2016). Effect of sup- plementation of rice bran and furamone alone or in combination on in vitro rumen fermentation, methanogenesis and methanogens. *Animal Science Journal*, 87, 398–404.

Agarwal, N., Kamra, D. N., Chatterjee, P. N., Ravindra, K., & Chaudhary, L. C. (2008). In vitro methanogenesis, microbial profile and fermentation of green forages with buffalo rumen liquor as influenced by 2-bromoethanesulfonic acid. *Asian-Australasian Journal of Animal Sciences*, 21, 818–823.

Andrade-Montemayor, H., Gasca, T. G., & Kawas, J. (2009). Ruminal fermentation modification of protein and carbohydrate by means of roasted and estimation of microbial protein synthesis. *Revista Brasileira de Zootecnia*, 38.

Appuhamy, J. A., Strathe, A. B., Jayasundara, S., Wagner-Riddle, C., Dijkstra, J., France, J., et al. (2013). Anti-methanogenic effects of monensin in dairy and beef cattle: A meta-analysis. *Journal of Dairy Science*, 96, 5161–5173.

Azanuma, N., Iwanoto, M., & Hino, T. (1999). Effect of the addition of furamone on methane production by ruminal microorganisms by ruminal microorganisms in vitro. *Journal of Dairy Science*, 82, 780–787.

Balch, W. E., Fox, G. E., & Magurn, L. J. (1979). Methanogens: Reevaluation of a unique biological group. *Microbiological Reviews*, 43, 260–296.

Bath, C., Morrison, M., Ross, E. M., Hayes, B. J., & Cocks, B. G. (2012). The symbiotic rumen microbiome and cattle performance: A brief review. *Animal Production Science*, 52, 876–881.

Battumur, U., Yoon, Y. M., & Kim, C. H. (2016). Isolation and characterization of a new *Methanobacterium formicicum* KOR-1 from an anaerobic digestor using pig slurry. *Asian-Australasian Journal of Animal Sciences*, 29, 586–593.

Benedict, M. N., Gnssnerman, M. C., Metcalf, W. W., & Price, N. D. (2012). Genome-scale metabolic reconstruction and hypothesis testing in the methanogenic archaeon *Methanosarcina acetivorans* C2A. *Journal of Bacteriology*, 194, 855–865.

Bharathi, M., & Chellapandi, P. (2017). Intergeneric evolution and metabolic cross-talk between rumen and thermophilic autotrophic methanogenic archaea. *Molecular Phylogenetics and Evolution*, 107, 293–304.

Bocazzi, P., Zhang, J. K., & Metcalf, W. W. (2000). Generation of dominant selectable markers for resistance to pseudomonac acid by cloning and mutagenesis of the ileS gene from the archaeon *Methanosarcina Barkeri*. *Journal of Bacteriology*, 182, 2611–2618.

Broers, C. A., Meijers, H. H., Symens, J. C., Stumm, C. K., Vogels, G. D., & Brugerolle, G. (1993). Symbiotic association of *Psalterionomas vulgaris* n. sp. with *Methanobacterium formicum*. *European Journal of Protistology*, 29, 98–105.

Bryant, M. P., & Boone, D. R. (1987). Isolation and characterization of *Methanobacterium formicicum* MF. *International Journal of Systematic and Evolutionary*, 37, 171.

Burecl, R. (2010). Regulation of metabolism: A cross-talk between gut microbiota and its human host. *Physiology*, 27, 300–307.

Cani, P. D., & Delzenne, N. M. (2009). The role of the gut microbiota in energy meta- bolism and metabolic disease. *Current Pharmaceutical Design*, 15, 1546–1558.

Chaucheysras-Durand, F., Massiglia, S., Fonzy, G., & Forano, E. (2010). Influence of the composition of the cellulolytic flora on the development of hydrogenotrophic microorganisms, hydrogen utilization, and methane production in the rumens of nong- tobolioclytically reared lambs. *Applied and Environmental Microbiology*, 76, 7931–7937.

Chellapandi, P., Prabaharan, D., & Uma, L. (2010). Evaluation of methanogenic activity of biogas plant slurry for monitoring codigestion of osein factory wastes and cyanobacteria biomass. *Applied Biochemistry and Biotechnology*, 162, 524–535.

Chen, M., & Wolin, M. J. (1979). Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria. *Applied and Environmental Microbiology*, 38, 72–77.

Chidhaisong, A., & Conrad, R. (2000). Pattern of non-methanogenic and methanogenic degradation of cellulose in anoxic rice field soil. *FEMS Microbiology Ecology*, 31, 87–94.

Darcy, T. J., Sandman, K., & Reeve, J. N. (1995). *Methanobacterium formicicum*, a meso- philic methanogen, contains three H\textsubscript{2}o histones. *Journal of Bacteriology*, 177, 858–860.

Dellinger, C. A., & Ferry, J. G. (1984). Effect of monensin on growth and methanogenesis of *Methanobacterium formicicum*. *Applied and Environmental Microbiology*, 48, 680–682.
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