# GENOME SCALE METABOLIC RECONSTRUCTION AND HYPOTHESIS TESTING IN THE METHANOGENIC ARCHAEON METHANOSARCINA ACETIVORANS C2A

BY

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# Abstract

*Methanosarcina acetivorans* strain C2A is a marine methanogenic archaeon notable for its substrate utilization, genetic tractability, and novel energy conservation mechanisms. To help probe the implications of this organism's unique metabolism, we have constructed and manually curated a genome-scale metabolic model, iMB744, accounting for 744 of the 4540 predicted protein coding genes (16%) in the *M. acetivorans* genome. The reconstruction effort has identified key knowledge gaps and differences in the peripheral metabolism and central metabolism between methanogenic species. Using flux balance analysis, the model quantitatively predicts wild type phenotypes and is 96% accurate in knockout lethality predictions compared to currently available experimental data. Flux balance analysis was used to probe the mechanisms and energetics of byproduct formation and growth on carbon monoxide, and the nature of the reaction catalyzed by the soluble heterodisulfide reductase HdrABC in *M. acetivorans.* This work highlights the great utility of constraint-based modeling for identifying feasible solutions to biological questions and provides insights into the workings of the cell at the genome scale.

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# Introduction

Methanogenic archaea are unique in their ability to grow on low energy substrates such as acetic acid by splitting them into methane and other byproducts. Methanogens are a critical part of the global carbon cycle, consuming byproducts of other natural bioprocesses that would otherwise be recalcitrant in sulfate poor, anaerobic environments (11). They also play an important role in global warming, since methane is a greenhouse gas twenty times as potent as carbon dioxide (44) and methanogenesis is the primary mechanism for methane emission into the atmosphere (1).

*Methanosarcina* is the only known genus of methanogens with members that can utilize all of the known methanogenic pathways (acetoclastic, methylotrophic, hydrogenotrophic, and methyl reduction) (82). This metabolic diversity makes these species relatively permissive to metabolic and genetic manipulations compared to other methanogens. To help capitalize on this, the genomes of three *Methanosarcina* species have been sequenced (14, 19, 39). In addition, genetic manipulation tools have been developed for several of these species, including directed mutagenesis and constitutive promoters in *Methanosarcina acetivorans* (2, 34, 85, 87).

The constraint-based reconstruction and analysis (COBRA) strategy is a powerful paradigm for consolidating large amounts of metabolic knowledge and synthesizing that knowledge into quantitative phenotypic predictions (48, 55). To perform constraint-based analysis, it is necessary to reconstruct the metabolic network from the bottom up, beginning with a sequenced and

annotated genome and ending with a network of reactions and reaction-gene associations. Many metabolic reconstructions have been curated by hand and used to make useful predictions such as identification of putative drug targets and the design of novel strains for enhanced biofuel production (48, 77).

In recent years, there have been strong advances towards automating much of the reconstruction process (29), which are needed to continue the exponential increase in the number of genome-scale metabolic models (45, 48). However, for study of methanogens, automated reconstructions are problematic, because their predictions tend to be overly homogenized and incomplete for organisms with highly specialized metabolism. Methanogens are well known for their unusual metabolic capabilities, which are in part derived from their unique ecological niche (13). Automatically predicted networks are also dependent on the completeness of reaction databases, which is more limited for archaea than it is for other domains of life. Hence, manual curation is necessary to obtain reliable predictions from metabolic models of these organisms.

*M. acetivorans* is notable for its substrate utilization. Unlike most other methanogens, it can grow and produce methane using methylated substrates, carbon monoxide or acetate, but it cannot grow with hydrogen as its primary energy source (66). Also unlike most methanogens, *M. acetivorans* is genetically tractable. Therefore, this organism offers opportunities to learn about novel energy conservation mechanisms. An independent reconstruction for *M. acetivorans* strain C2A has recently been reported (63). However, the previously reported reconstruction was primarily curated using an automated curation pipeline including the GapFind, GapFill, and

GrowMatch algorithms (33, 62). As a result, the published reconstruction maintains many of the disadvantages of other automated reconstructions mentioned previously. We present iMB744, a quantitative, genome-scale metabolic model of *M. acetivorans* resulting from a more literature-centric approach to model curation, as detailed in published protocols (77). As many literature sources as possible were integrated to generate a highly accurate list of metabolic reactions. Using constraint-based modeling, we have predicted growth phenotypes and probed possible hypotheses for the workings of incompletely understood parts of the *M. acetivorans* metabolic network. The analysis thus represents a successful application of the hypothesis-driven modeling approach.

# Materials and Methods

### Model Reconstruction

An initial list of potential reaction-gene associations in *Methanosarcina acetivorans* str. C2A was generated based on a union of data in the Kyoto Encyclopedia of Genes and Genomes (KEGG) (32), MetaCyc (12), the Model SEED reconstruction (29), the Transport Protein Analysis Database (TransportDB) (58), and UniProt (76). Reactions from the existing *Methanosarcina barkeri* str. Fusaro reconstruction (16) and the BiGG database (65) were added if there was sufficient evidence for their inclusion, based on sequence homology and/or literature-based curation, or to fill gaps in the annotation. Some gene suggestions from EFICAZ, which includes evidence from other bioinformatics tools like PFAM, were also incorporated (3). Gene associations were verified whenever possible using bidirectional BLASTP against archaeal protein products with experimentally verified functions (10). In case of conflicts, metabolic functions suggested from literature were chosen over those suggested in the databases, and inconsistent reactions were removed from the model.

Logical gene protein relationships (GPR) were constructed manually based on literature or database evidence. For example, genes annotated or characterized to be separate subunits of a complex were given an "AND" relationship. If there was no evidence of a protein complex catalyzing a reaction with multiple genes, the genes were all assigned an OR relationship.

All intracellular and transport reactions were computationally mass and charge balanced at a pH of 7 based on charges and formulas computed with ACD/Labs software (Version 12; Advanced Chemistry Development, Inc.). Charges and formulas are available in appendix B.

**Construction of the Biomass Reaction**

The biomass reaction is a sink on essential cell components that represents the consumption of molecular building blocks (such as amino acids and nucleotides) required for cell division. The biomass reaction for *Methanosarcina acetivorans* str. C2A was modified from the closest relative for which a biomass reaction had previously been built, *Methanosarcina barkeri* str. Fusaro (16). This biomass objective function was first expanded by incorporating more detailed carbohydrate data from *M. barkeri* (31) and adding methanofuran-B to the list of required cofactors (41) . Then, coefficients for lipids were modified based on available data on the unique lipid composition of *M. acetivorans* (69). Nucleotide and amino acid coefficients specific to *M. acetivorans* were calculated based the published genome sequence according to established procedures (77). The coefficients of trace elements were assumed to be the same as those in the *M. barkeri* biomass equation.

Growth-associated maintenance was included in the biomass equation and was set to 65 mmol/gDW, similar to the *M. barkeri* and *E. coli* FBA models (15, 16), to account for energy costs for growth (such as production of macromolecules from biomass components). See appendix B for a listing of biomass components and ratios.

**Flux Balance Analysis (FBA)**

Exponential growth phenotypes were predicted using flux balance analysis (FBA), which has been previously reviewed (52). All reactions in the model were represented in a stoichiometric matrix, **S**, in which each column represented a reaction and each row a metabolite. Hence, the entry (*i,j*) of **S** contained the stoichiometric coefficient of metabolite *i* in reaction *j*.

If metabolite concentrations are assumed to be constant (steady state), conservation of mass requires that:

$$\mathbf{S}v = 0$$

where v is the vector of reaction fluxes (reaction rates). Because there were more reactions than metabolites in the model, multiple possible flux distributions were possible that all satisfied the mass balance.

Reaction fluxes were also constrained by setting minimum and maximum fluxes. In the current study, the reversibility of each reaction was determined based on literature, database evidence, and thermodynamic calculations. The flux through reversible reactions was unconstrained, while that of irreversible reactions was set to have a $\mathbf{v}_{min}$=0. Substrate uptake rates were set to experimentally measured values for purposes of simulations (see Appendix C for values and references). The reaction rate through the ATP maintenance reaction (ATPM) was set to 2.5 mmol/gDW/hr to account for upkeep energy costs. This value is somewhat lower than the

experimental value of 8.39 mmol/gDW/hr used in the current *E. coli* model, but it is close to that in the published *M. barkeri* model (16). Experimental data in *Methanosarcina mazei* suggests that it is a low value (57), which could be a reflection of its dependence on low-energy substrates for growth.

Under the assumption that the cell seeks to maximize its growth potential, the specific growth rate was predicted by maximizing the flux through the biomass reaction subject to the aforementioned constraints:

$$\text{Max } v_{biomass}$$

Subject to:

$$\mathbf{S}v = 0$$

$$\mathbf{v}_{min} < \mathbf{v} < \mathbf{v}_{max}$$

Reaction fluxes were predicted in mmol/gDW/hr, and growth rates were predicted in $hr^{-1}$. FBA problems were solved using the COBRA toolbox in MATLAB (4) linked to the GLPK linear program solver.

Defined high salt (HS) media without vitamin supplement was used for all simulations. The media composition is listed in appendix B and was defined from Sowers *et al.* (67).

**Knockout lethality studies**

To perform knockout lethality studies, every gene except those knocked out was assigned a

Boolean value of 1 (TRUE), and knocked out genes were assigned a value of 0 (FALSE). The Boolean gene protein relationships were evaluated for every reaction, and reactions with a GPR evaluating to FALSE were removed from the model. After modifying the network in this way, FBA to make a growth-no growth decision (growth was defined as a predicted $v_{biomass}>10^{-5}$ $hr^{-1}$). Lethality predictions were compared to published gene knockout phenotype data (see Appendix C for references). For substrates with an unknown uptake rate (such as monomethylamine), the uptake rate was assumed to be 15 mmol/gDW/hr, similar to the calculated rate for growth on methanol (73), for purposes of FBA simulations.

**Calculation of Potential ATP Yield**

To calculate potential ATP yield during growth on CO, a linear programming problem identical to flux balance analysis was solved, but the flux through the non-growth associated maintenance (ATPM) reaction was maximized instead of the biomass equation. To force flux through a particular pathway, reactions involved with other ATP-generating pathways were constrained to have zero flux. For example, to calculate the ATP yield for acetogenesis, the HDR reaction involved in methanogenesis was constrained to have zero flux. The potential ATP yield was calculated by dividing the maximum flux through the ATP maintenance reaction by the CO uptake rate.

# Results

### Model Reconstruction

The metabolic network of *Methanosarcina acetivorans* was curated and validated as described in the methods and in Figure 1. After curation, the reconstruction has become a valuable knowledge base for the metabolism of this organism. In addition to reactions included in the model, reactions that were specifically excluded from the model due to literature or modeling evidence were also recorded. Complete lists of reactions included and gene-protein relationships can be found in Appendix B.

The metabolic network of *M. acetivorans* consists mostly of reactions required for synthesis of amino acids, nucleotides, and cofactors (Figure 1D). This was not surprising given relatively low nutritional requirements of this organism. There are still significant gaps in the knowledge of these pathways. For example, no homologues to currently known IMP dehydrogenase genes could be found in the genome of *M. acetivorans*, but the reaction catalyzed by this enzyme is predicted to be essential for nucleic acid synthesis. We have identified a number of other metabolic gaps that serve as potential targets for future experimentation (see Appendix A).

*M. acetivorans* is the second organism within the genus *Methanosarcina* to have a curated genome-scale metabolic model, after *M. barkeri* (16). Many of the differences between the published metabolic model of *M. barkeri* and the presented model of *M. acetivorans* are due to

new literature sources for novel metabolic paths unique to the archaea. For example, the published model of *M. barkeri* included the pentose phosphate pathway for synthesis of five-carbon sugars. However, the genes encoding for ribulose-5-phosphate 3-epimerase, transaldolase, and the two transketolase reactions in that pathway are apparently absent in many methanogens, including *M. barkeri* and *M. acetivorans*. Recently, an alternative pathway for synthesis of ribulose-5-phosphate was characterized in *Methanocaldococcus jannaschii* (25). The genes involved in that pathway had strong homology to genes in *M. acetivorans*. Therefore, the reactions in the pentose phosphate pathway were excluded from the metabolic model of *M. acetivorans,* and the new pathway was added to the model.

The *M. acetivorans* metabolic model accounts for key differences in methanogenesis pathways between *M. acetivorans* and *M. barkeri* (Figure 1B). These differences account for much of the variation in growth and secretion rates observed between these two species. Most notably, unlike *M. barkeri*, *M. acetivorans* cannot grow on $H_2$ and $CO_2$ and grows on CO using a completely different pathway that involves secretion of acetate, methylsulfides and formate (50, 60). *M. acetivorans* is also able to grow on dimethylsulfide, whereas *M. barkeri* can only perform methanogenesis from that substrate (74). The published model includes pathways to perform all of these functions.

The reconstructed network includes pathways for synthesizing most of the cofactors unique to methanogens (the exception is methanophenazine, which to the authors' knowledge has no proposed synthesis pathway in any organism). The coenzyme M, methanopterin, and $F_{420}$

biosynthesis pathways in *M. acetivorans* all appear to diverge from the pathways identified in *Methanocaldococcus*, based on the lack of sequence identity with biochemically verified gene products in that organism (Figure 2). For example, the gene responsible for synthesis of (S)-2-hydroxyglutaric acid in the *M. jannaschii* methanopterin biosynthesis pathway (MJ_1425) has no identity with any genes in *M. acetivorans*. An alternative pathway for coenzyme M synthesis has already been characterized in *M. acetivorans* and is included in the present model (21). Since no alternative pathways have yet been proposed for methanopterin or $F_{420}$ synthesis, the pathways from *Methanocaldococcus* were tentatively included and are likely targets for future model updates.

**F420 Regeneration during Growth on Carbon Monoxide**

Both *M. acetivorans* and *M. barkeri* grow on carbon monoxide by oxidizing it to $CO_2$ and subsequently reducing $CO_2$ to methane (17). In methanogens, the reduction of carbon dioxide to methane requires oxidation of two equivalents of coenzyme $F_{420}$. Therefore, to simulate growth on carbon monoxide, it was necessary to include a feasible mechanism for re-reducing coenzyme $F_{420}$. In CO-grown *M. barkeri,* reduced $F_{420}$ is probably regenerated by generation of molecular hydrogen via the reverse action of Ech hydrogenase, followed by hydrogenation of oxidized $F_{420}$ via the $F_{420}$-reducing hydrogenase, Frh (43, 47). Ech hydrogenase is not present in the *M. acetivorans* genome, and although an *frh* operon is present, it does not encode a functional enzyme (26). The mechanism for $F_{420}$ regeneration in *M. acetivorans* remains unknown (17), but since *M. acetivorans* is viable on CO, such a mechanism must exist.

The current model postulates that $F_{420}$ is regenerated by the combined action of $F_{420}$ dehydrogenase (Fpo) and the Rnf complex (Figure 3A). In the proposed pathway, Rnf would reduce methanophenazine with ferredoxin, and subsequently, Fpo would run in reverse to reduce $F_{420}$. This hypothesis is consistent with the high levels of Fpo protein and transcript measured during growth on CO (35). The net generation of a proton gradient during regeneration of $F_{420}$ (1 $H^+/F_{420}$ regenerated) is reasonable since *M. barkeri* also generates a proton gradient during $F_{420}$ regeneration through the action of Ech (83). Reverse action of Fpo has not been observed experimentally, but it is thermodynamically feasible in an environment containing excesses of oxidized $F_{420}$ and reduced methanophenazine.

As an alternative hypothesis, we also tried to implement a $F_{420}$-ferredoxin oxioreductase reaction for the purposes of regenerating coenzyme $F_{420}$ during growth on CO (7). In the presence of such a reaction, growth on CO was successfully predicted (data not shown). However, the presence of such a reaction was predicted to make a Δ*rnf* mutant viable on acetate, contrary to experimental evidence (18). According to the model, a Δ*rnf* mutant growing on acetate could survive with a lower growth rate by reducing coenzyme $F_{420}$ with the putative oxioreductase and then conserving energy with Fpo and heterodisulfide reductase (Hdr). In reality, this alternative path might not produce enough ATP to make the cell viable on acetate, since Fpo probably does not pump as many proton equivalents across the cell membrane as Rnf. The wild type ATP yield during growth on acetate is only about 1 ATP/acetate (5).

**Byproduct Formation Mechanisms and Energetics during Growth on CO**

*M. acetivorans* produces acetate, formate, methane, and methylsulfides as byproducts when grown on carbon monoxide (in addition to $CO_2$) (50, 60). It is known that *M. acetivorans* produces acetate through the action of phosphoacetyltransferase (Pta) and acetate kinase (Ack) (60), but the mechanisms of formate and methylsulfide production are less clear. To probe the energetics of byproduct formation, it was necessary to hypothesize likely mechanisms for how they are produced.

It is currently unknown how or why *M. acetivorans* generates formate during growth on CO. One possibility is that it is produced as a byproduct of carbon monoxide dehydrogenase during growth on CO to prevent toxic CO accumulation in the cell (50, 61). The CO dehydrogenase enzyme from *Rhodospirillum rubrum* has been shown to create formate as a byproduct, and formate may formed by a similar mechanism in *M. acetivorans* (30, 61), although the physiological substrate for the reaction is still unknown. In order to investigate formate production, the following reaction was tentatively included in the model:

$$\text{CODH3\_SIDERXN: } CO + H_2O \rightarrow \text{FORMATE} + H^+$$

This reaction implies that formate production does not yield ATP.

Methylsulfides are only produced in small amounts during growth on CO (50). Therefore, it is unlikely that the path to produce them is a major source of ATP for the cell. The recently-characterized Mts enzymes may be partly responsible for production of dimethylsulfide in *M.*

*acetivorans,* although due to the very low ratio of dimethylsulfide production rate to the transcription level of these enzymes, the *in vivo* function of these enzymes remains unclear (49). Due to the similar structure of sulfide ($HS^-$) and coenzyme M ($C_2H_5O_3S_2$), it is reasonable to hypothesize that methylsulfide ($CH_4S$) is formed from a reaction of Mtr. Under this hypothesis, the following reaction was added to the model for production of methylsulfide:

$$\text{MSS: } MH_4SPT + HS^- + H^+ + 2\ Na^+ \rightarrow CH_4S + H_4SPT + 2\ Na^+(e)$$

Here, $H_4SPT$ is tetrahydrosarcinopterin and $MH_4SPT$ is its methylated version.

Under optimal growth conditions, flux balance analysis predicted that only methane would be produced. To investigate the cause of this prediction, the ATP potential was calculated for production of each byproduct per mole of CO consumed, as described in the methods. The ATP yield from methanogenesis (0.56 ATP/CO) was calculated to be significantly higher than that for acetogenesis (0.38 ATP/CO), methylsulfide production (0.33 ATP/CO), or formate generation (0 ATP/CO), which explains why FBA predicts only methanogenesis. It does not explain why all of these byproducts are produced or why acetogenesis is mandatory for growth on CO – these questions are explored in following sections.

**Regulation of CO Levels in the *M. acetivorans* Cell**

*M. acetivorans* encodes at least two complete carbon monoxide dehydrogenase (CODH) operons, and their relative expression during growth on CO may depend on the concentration of CO in the media (61). Since formate production may be a result of a side reaction of CODH, it is

tempting to speculate that the level of carbon monoxide in the cell is regulated by the balance of the levels of these proteins, one of which produces formate as a byproduct and one which does not. Flux balance analysis predicts that if the proposed mechanism for formate production is correct, reducing the flux through the primary reaction catalyzed by CODH leads to production of formate (Figure 3C). This indicates that such a balance could be a feasible mechanism for controlling CO toxicity in the cell.

**Analysis of Acetogenesis during Growth on CO**

The sodium-pumping methyltransferrase Mtr catalyzes the reversible transfer of methyl from methyl-tetrahydrosarcinopterin to methyl-Coenzyme M. Although this reaction is typically considered an essential part of the methanogenesis pathway, it is strongly down-regulated during growth on CO compared to other substrates (61). The observation of methanogenesis from CO despite the diminutive role of Mtr has inspired the hypothesized existence of a Mtr "bypass" reaction that performs the same reaction but does not generate a sodium gradient (17). Such a reaction could help balance the increased ATP potential with Mtr with a possible greater kinetic capacity without the sodium pump, hence permitting tolerance to a greater range of environmental CO concentrations (17). To test the effects of the bypass reaction on metabolism, the bypass was added to the network. Subsequently, flux balance analysis was used to predict whether acetogenesis would occur in a Δ*mtr* strain during growth on CO. Only methanogenesis was predicted in the Δ*mtr* strain, because the theoretical ATP yield of methanogenesis in a Δ*mtr* strain was still predicted to be higher than that of acetogenesis (0.44 ATP/CO and 0.38 ATP/CO,

respectively). Therefore, even without Mtr, methanogenesis is more energetically favorable than acetogenesis.

It is unclear if inhibition of methanogenesis is the true cause of acetogenesis in *M. acetivorans* during growth on CO. Physiological evidence exists both supporting (60) and refuting (50) this hypothesis. If methanogenesis is indeed inhibited, it is unknown which reactions are inhibited. To probe possible causes of acetogenesis in *M. acetivorans*, flux balance analysis was used to predict acetate secretion rates after individually limiting each reaction's rate to within 80% of the wild type flux. The inhibition of certain methanogenic reactions (catalyzed by Hdr, Mcr, and Mrp) or the membrane-bound ATP synthase was predicted to lead to significant levels of acetate secretion (> 0.1 mmol/gDW/hr). However, inhibition of purine synthesis could also lead to acetogenesis (Figure 3B). This remained true at different levels of inhibition as well (data not shown). The identification of this potential explanation for acetogenesis during growth on CO highlights the power of a systems approach for generating hypotheses, which could be tested using genetic manipulations. A study based solely on the methanogenesis pathways would be less likely to identify this possibility.

**Comparison of Predicted Growth Phenotypes to Experimental Data**

Flux balance analysis was used to predict growth phenotypes for wild type strains of *Methanosarcina acetivorans* growing on acetate, methanol, and carbon monoxide, the three substrates for which growth and substrate uptake data are available (60, 68, 73). Predicted

growth rates were highly dependent on substrate uptake rates, which varied up to 100% depending on the data set used to perform the calculation (see Appendix C). It was possible to pick uptake rates within the experimentally feasible ranges for each substrate that matched the observed growth rates and growth yields within 20% (Table 1).

The rate of methanogenesis was predicted to be much lower during growth on methanol compared to experiment, which was partly because the predicted growth rate was lower than the experimentally determined values. However, the ratios of products are consistent with experimental data. When maximizing ATP yield during growth on methanol, the predicted ratio of methane to $CO_2$ produced was exactly 3:1, as would be expected to balance redox potentials in the cell (7). However, when optimizing for growth, the actual ratio of methane to $CO_2$ secreted was predicted in the model to be 3.8:1, because the carbon dioxide-fixing activity of carbon monoxide dehydrogenase/acetyl CoA synthase reduced the net secretion of carbon dioxide.

Comparison of knockout lethality predictions to available data indicates that the model correctly predicts the growth/no growth phenotypes of 60/63 knockout mutants correctly (Table 2). All of the incorrect predictions were cases in which genes were experimentally shown to be lethal but predicted to be nonlethal. A Δ*mch* knockout strain was predicted to be viable on acetate, but this knockout is known to be lethal on that substrate (27). The *mch* gene is essential due to the need for *M. acetivorans* to reduce $F_{420}$ for use in anabolic reactions such as the $F_{420}$-dependent glutamate synthase (56). However, another mechanism for reducing $F_{420}$ is necessary for growth on CO, and flux balance analysis predicts that such a mechanism could also be used to reduce

$F_{420}$ during growth on acetate, therefore making *mch* nonessential. Further study is needed to resolve the tension between these experimental observations.

Two of the incorrect lethality predictions involved acetogenesis during growth on carbon monoxide. The genes encoding Pta and Ack are essential for growth on CO (60), presumably because without acetogenesis, the ATP generation capabilities are insufficient for growth. Flux balance analysis incorrectly predicts that a Δ*pta*Δ*ack* mutant inhibited in methanogenesis can still grow by producing methylsulfides. The predicted pathway has a potential ATP yield of 0.33 ATP/CO. It is possible that the production of methylsulfides does not actually result in generation of a sodium gradient. In this case the ATP yield would only be 0.17 ATP/CO, insufficient to overcome the ATP maintenance requirement. It is also possible that the hypothesized mechanism is correct but kinetically limited. Only small quantities of methylsulfides are produced during growth on CO, despite the high level of *mts* genes presumably responsible for their production on that substrate (49).

Knockout lethality data enabled the exclusion of certain reactions whose genes had sequence similarity with genes in other organisms, but which catalyzed reactions that were inconsistent with physiological data in *M. acetivorans* itself. For example, *M. acetivorans* uses Ack and Pta to activate acetate to acetyl-CoA during acetoclastic methanogenesis, and cannot grow on acetate without the encoding genes (60). However, the *M. acetivorans* genome also encodes genes (MA3168 and MA3602) with high sequence identity to a complex in *Methanocaldococcus jannaschii* that catalyzes an alternative pathway for activating acetate (46). Including this

reaction would make Pta and Ack nonessential for growth on acetate. On this basis, the genes involved with the alternative pathway were assumed to be nonfunctional, and the reactions in the alternative pathway were excluded from the model.

**Exploration of an Alternate Heterodisulfide Reductase (hdrABC) on Methanol**

HdrABC is a soluble heterodisulfide reductase typically found in methanogens without cytochromes (70). Most methanogens with cytochromes, including *Methanosarcina* species, use a membrane-bound heterodisulfide reductase HdrDE instead of the soluble HdrABC to couple methanogenesis to ATP production (75). Therefore, the discovery that *M. acetivorans* encodes and uses both types of heterodisulfide reductase during growth on methyltrophic substrates was a surprise (7). Since the HdrABC complex is not a sodium or proton pump, it is unclear if the activity of this complex is coupled to ATP synthesis in *M. acetivorans*.

In *Methanothermobacter marburgensis,* a methanogen without cytochromes, HdrABC is coupled to ATP synthesis through its interaction with the MvhADG hydrogenase complex (70). The HdrABC/MvhADG complex in *M. marburgensis* uses an electron bifurcation mechanism, in which the electrons from two equivalents of molecular hydrogen are donated to ferredoxin and to the heterodisulfide (70). The reduced ferredoxin can then be used to generate ATP. *M. acetivorans* lacks the genes encoding the MvhADG complex, so it is likely that the HdrABC complex in that organism has a separate function. It has been suggested that *M. acetivorans* HdrABC may also use an electron bifurcation mechanism, splitting the electrons of two fully-

reduced ferredoxins between heterodisulfide and coenzyme $F_{420}$ (7). Alternatively, the HdrABC may simply reduce heterodisulfide with ferredoxin, acting as a sink for excess ferredoxin produced during oxidation of methanol to $CO_2$.

To test the electron bifurcation hypothesis in a genome-scale context, the phenotype of *M. acetivorans* was simulated with and without electron bifurcation in HdrABC. Two additional constraints were necessary to obtain reasonable predictions. Reactions catalyzed by Pta and Ack were disabled to prevent acetate secretion, which has not been observed during growth on methanol (36), and the flux through pyruvate-acetyl CoA oxioreductase was set to be equal to the wild-type value to prevent secretion of formate and other unobserved byproducts during growth on methanol (68). In the presence of Rnf, the simulations did not predict utilization of HdrABC regardless of mechanism. However, a Δ*rnf* mutant was predicted to utilize HdrABC to oxidize ferredoxin. The mutant was predicted to grow 35% slower than the wild type without bifurcation and 20% slower with bifurcation (Figure 4B). A Δ*rnf* mutant actually grows about 25% slower on methanol than the wild type (William Metcalf, unpublished data), so within experimental error it is difficult to tell which mechanism is correct. Further experiments could help elucidate the true mechanism.

# Discussion

We have built and manually curated a computable genome-scale model of metabolism in *M. acetivorans*, only the third methanogen species to be reconstructed (after *M. barkeri* (16) and *M. jannaschii* (78)) and the second in the genus *Methanosarcina*. We have used flux balance analysis to probe incompletely understood pathways for ATP generation and product formation using carbon monoxide as a substrate. The simulations lend support to the hypothesis that the generation of both acetate and formate could come about as a result of the inhibitory effects of carbon monoxide in the cell. In particular, acetogenesis could be favored kinetically due to the relatively low potential of methanogenesis or purine synthesis under CO inhibition, while formate generation could result from regulation the relative transcription levels of multiple carbon monoxide dehydrogenase isozymes present in the cell. Therefore, flux balance analysis was useful to probe the effects of both metabolic and regulatory constraints on the metabolic network.

Flux balance analysis has also proven useful for testing alternate hypotheses in our study of the soluble heterodisulfide reductase. The possibility of electron bifurcation as a more likely mechanism for action of this enzyme opens up many interesting experimental questions, such as how the complex could have evolved to use different substrates in *M. acetivorans* than it does in *M. marburgensis*, a distantly related methanogen, or whether the HdrABC complex in *M. acetivorans* interacts with other metabolic complexes in the methanogenic pathways in a similar

way that *M. marburgensis* interacts with Mvh (70). Much work remains to be done to answer these questions and identify for sure whether the bifurcation mechanism is at work or not.

The reconstruction endeavor has led to significant insight resulting from the combination of data in numerous studies in the literature. It has also identified the presence and impact of gaps in the knowledge of this organism, helping to focus the continued experimental efforts towards understanding metabolism in this species. Due to the incomplete knowledge of this organism, the metabolic reconstruction necessarily involved several assumptions, such as the mechanism of regeneration for coenzyme $F_{420}$ on CO, which may prove to be incorrect. As experimental data continues become available, the model will be continuously compared against experiment and provide novel hypothesis in an iterative process that lies at the heart of systems biology.

# Figures and Tables

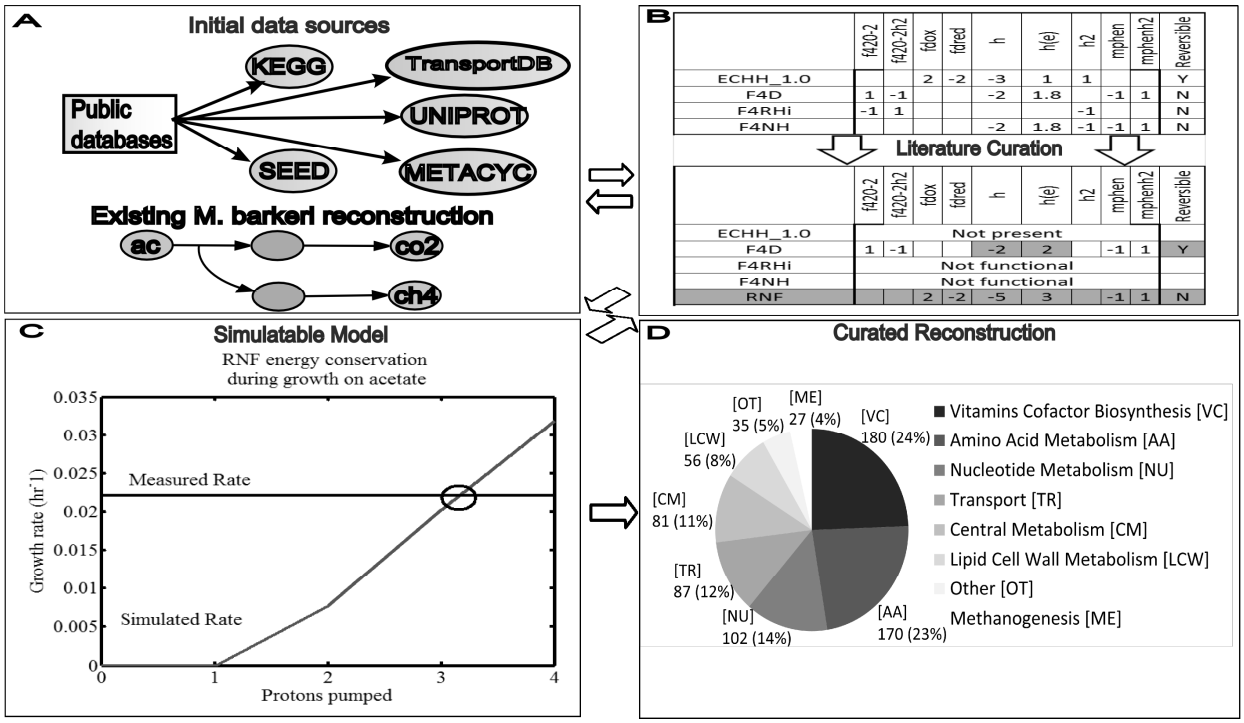


Figure 1. Overview of the reconstruction process for *Methanosarcina acetivorans* str. C2A. (A): Possible reactions and gene associations were pulled from the existing reconstruction of *Methanosarcina barkeri* str. Fusaro, a closely related methanogen, and several online databases, including Metacyc, BIGG, KEGG, the SEED, and Uniprot. (B): Reactions were added and removed based on a thorough literature review covering 289 of the reactions in the model. Shown are some key differences in energy conservation reactions between *M. acetivorans* and *M. barkeri*. (C) Growth was simulated using flux balance analysis, which seeks the physically realizable flux distribution that would yield the highest growth rate. (D) The final reconstruction contained reactions related to synthesis of essential biomass components, cell wall components, and methanogenesis, among others.

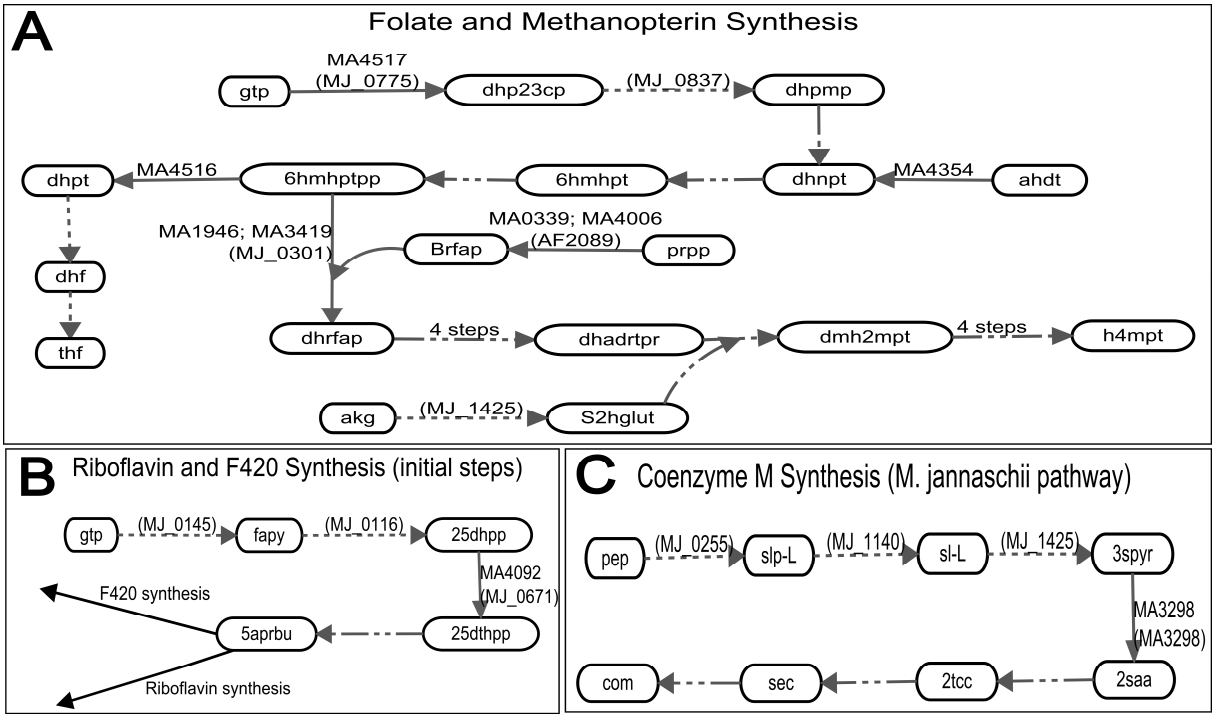


Figure 2. Apparent divergence of Methanopterin (A), Riboflavin/Coenzyme $F_{420}$ (B), and Coenzyme M (C) synthesis between *M. acetivorans* and other archaea. Metabolites are shown in circles with the ID given in the model (see supplemental material for complete names). Genes shown in parenthesis are biochemically verified, while the MA genes not in parentheses are the predicted *M. acetivorans* gene homologues, if any are present. Solid arrows (⟶) represent reactions with gene association in *M. acetivorans*, partially broken arrows (⟶) represent reactions biochemical evidence in other archaea (not in *M. acetivorans*) and no known gene association, and dotted arrows (⟶) represent reactions with verified gene associations in another organism, but no homologous genes are present in *M. acetivorans*. MA: *M. acetivorans*; MJ: *Methanocaldococcus jannaschii*; AF: *Archaeoglobus fulgidus*.

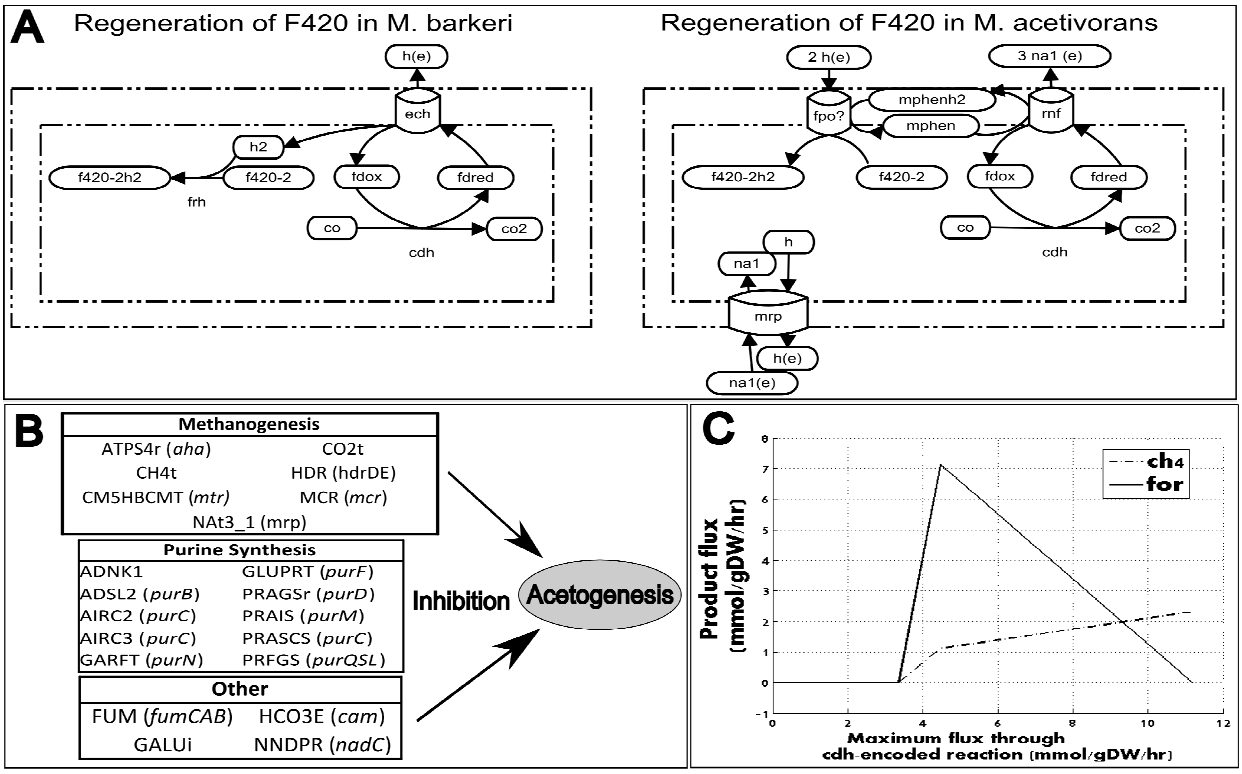


Figure 3. Analysis of growth of *M. acetivorans* on carbon monoxide. (A) Regeneration of coenzyme $F_{420}$ during growth on carbon monoxide for both *M. barkeri* (left) and proposed pathway for *M. acetivorans* (right). cdh: Carbon monoxide dehydrogenase; ech: ech hydrogenase; f420-2: Oxidized coenzyme $F_{420}$; f420-2h2: Reduced coenzyme $F_{420}$; fdox/fdred: oxidized and reduced ferredoxin; fpo: $F_{420}$ dehydrogenase; frh: F420-reducing hydrogenase; mphen/mphenh2: oxidized and reduced methanophenazine; mrp: Multiple resistance protein (Na+/H+ pump); rnf: *Rhodobacter* nitrogen fixation complex. (B) Inhibition of a range of reactions leads to predictions of acetogenesis during growth on CO, including reactions in late methanogenesis and reactions involved in purine biosynthesis. Reactions IDs are shown (with gene names in parenthesis if available) that led to greater than 0.01 mmol/gDW/hr production of acetate after being restricted to <80% of the optimal flux. (C) Flux balance analysis predicts that limitation of CO dehydrogenase activity leads to formate production.

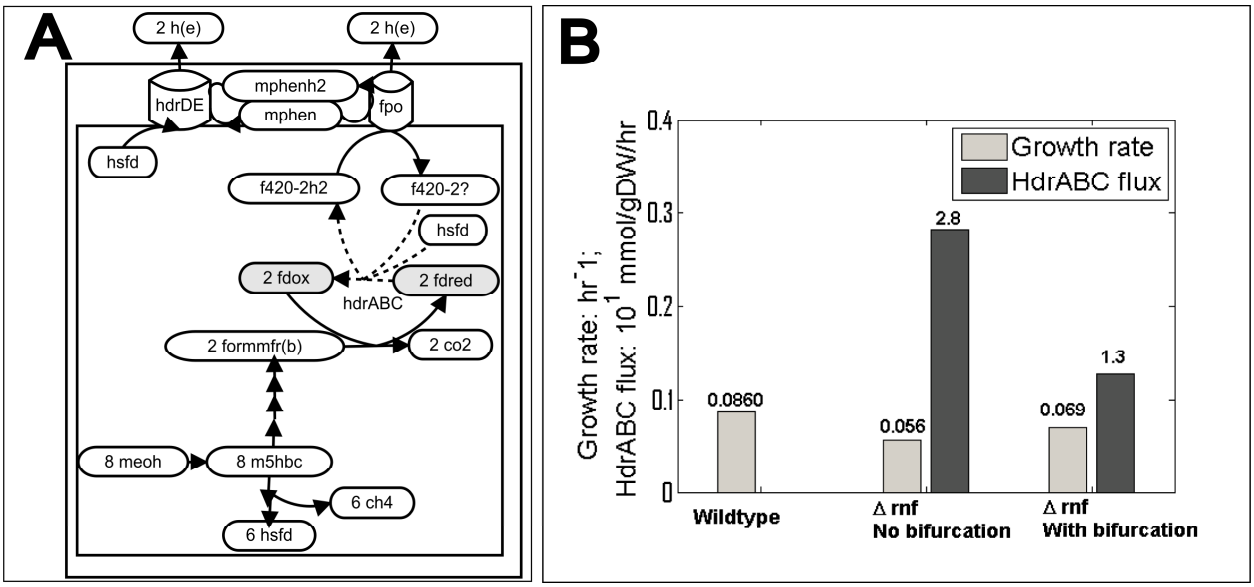


Figure 4: (A) Hypothesized bifurcation mechanism of the soluble heterodisulfide reductase HdrABC during growth on methylotrophic substrates in *M. acetivorans*. (B) Flux balance analysis predicts that HdrABC is not used when Rnf is available, but a Δ*rnf* mutant is predicted to carry flux through HdrABC. The growth rate for the Δ*rnf* was predicted to be about 20% less than wild type with bifurcation and 35% less without.

Table 1: Growth and secretion rates and yields of *M. acetivorans* on methanol, acetate, and carbon monoxide using experimentally feasible uptake rates (60, 68, 73). For simulations on CO, no additional constraints to Hdr or Cdh were assigned. All measured values are averaged across literature sources.

| | | **Growth ($hr^{-1}$)** | | **Growth yield (gDW/mmol)** | | **CH4 rate (mmol/gDW/hr)** | |
|---|---|---|---|---|---|---|---|
| **Substrate** | **Measured** | **Measured** | **Predicted** | **Measured** | **Predicted** | **Measured** | **Predicted** |
| Acetate | 7 | 0.023 | 0.021 | 2.4 | 3.0 | 4.9 | 6.6 |
| Methanol | 20 | 0.098 | 0.086 | 5.2 | 4 | 22 | 13.3 |
| CO | 11.6 | 0.029 | 0.030 | 2.5 | 2.6 | 0.4 | 2.3 |

Table 2: Knockout lethality predictions from FBA (L = lethal and N = nonlethal) and agreement with experimental results. (C): correct prediction; [X]: incorrect prediction. No marking means no experimental data is available for that knockout under those conditions. AC: acetate; DMA: dimethylamine; DMS: dimethylsulfide; MeOH: Methanol; MMA: monomethylamine; TMA: trimethylamine. See supplemental material for knockout data references.

| **Genotype** | **AC** | **CO** | **DMA** | **DMS** | **MeOH** | **MMA** | **TMA** |
|---|---|---|---|---|---|---|---|
| Δ*ack*Δ*pta* | L (C) | **N [X]** | N | N | N (C) | N | N |
| Δ*atpDCIXBEFAG* | N (C) | N | N | N | N (C) | N (C) | N |
| Δ*cooS1F* | N | N (C) | N | N | N | N | N |
| Δ*cooS2* | N | N (C) | N | N | N | N | N |
| Δ*hdrABC* | N (C) | N (C) | N | N | N (C) | N | N |
| Δ*hdrED* | L (C) | N | L | L | L (C) | L | L (C) |
| Δ*mch* | **N [X]** | L | L | L | L (C) | L | L |
| Δ*mtaA1* | N (C) | N | N (C) | N | L (C) | N (C) | N (C) |
| Δ*mtaB1C1*Δ*mtaB2C2*Δ*mtaB3C3* | N (C) | N | N (C) | N | L (C) | N (C) | N (C) |
| Δ*mtaA1*Δ*mtaB1C1*Δ*mtaB2C2*Δ*mtaB3C3* | **N [X]** | N | N (C) | N | L (C) | N (C) | N (C) |
| Δ*mtbA* | N (C) | N | L (C) | N | N (C) | L (C) | N (C) |
| Δ*mtsD*Δ*mtsF*Δ*mtsH* | N (C) | N (C) | N | L (C) | N (C) | N | N (C) |
| Δ*mtsX*Δ*mtsY, X and Y two mts genes* | N (C) | N (C) | N | N (C) | N (C) | N | N (C) |
| Δ*rnfXCDGEABY* | L (C) | L | N (C) | N (C) | N (C) | N (C) | N (C) |
| Δ*lysK* | N | N | N (C) | N | N (C) | N (C) | N (C) |
| Δ*lysS* | N | N | N (C) | N | N (C) | N (C) | N (C) |
| Δ*mtr* | L (C) | N | L | L | L (C) | L | L (C) |
| TOTAL CORRECT | 11/13 | 5/6 | 7/7 | 3/3 | 15/15 | 8/8 | 11/11 |

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# Appendix A. Analysis of Model Gaps

Like any metabolic reconstruction, the presented reconstruction of *Methanosarcina acetivorans* contains gaps in the genome annotation due to an incomplete knowledge about its metabolism. Many of the pathways in the Bacteria domain differ from those in the Archaea, and much progress has been made illuminating those differences. However, as this supplemental shows, there is clearly more work to be done to completely understand metabolism in the Archaea, and particularly in *M. acetivorans*.

Presented here is a list of gaps in central metabolism of *M. acetivorans* as of the time of publication. The metabolites are all given the ID found in the metabolic model. Some of the pathways described here have other biochemical evidence such as carbon labeling, but the evidence was described in other organisms and no genes have been identified to carry out the proposed functions. Other pathways or reactions have known genes in other organisms but they have no significant sequence homology with any genes in *M. acetivorans*.

The genome of *Methanosarcina acetivorans* contains over 1000 ORFs annotated as hypothetical proteins. It is our hope that this list will be useful for assigning functions to some of them and furthering the general knowledge of archaeal metabolic functions.

**Figure Key**

The key to the figures in this section is shown in table A.1 below. If a gene product in another organism has been verified to perform the reaction, the gene locus is shown in parenthesis (e.g. (MJ_0001) ). Any genes in *M. acetivorans* predicted to perform the same reaction are shown adjacent (no *M. acetivorans* gene is shown if there was no sequence similarity with the verified genes).

Table A.1. Key to model gap figures.

| Color | Pattern | Meaning |
|---|---|---|
| **Red** |  | Genetic evidence suggests the genes needed to perform the reaction are **not** present in *M. acetivorans*. |
| **Blue** |  | No genes associated with the reaction are known, but there is evidence aside from genetic evidence for the pathway's existence in other organisms |
| **Green** |  | At least one gene associated with the reaction is predicted or known to be present in *M. acetivorans* |

**4-aminobenzoate Biosynthesis**

4-aminobenzoate (4abz) is an intermediate required for synthesis of both folates and tetrahydromethanopterin according to the proposed pathways for each. *Methanosarcina barkeri* has to be supplemented with this compound to grow, but *M. acetivorans* does not. This could be because *M. acetivorans* does not require folates, while *M. barkeri* does (9). It could also be due to the ability of *M. acetivorans* but not *M. barkeri* to synthesize 4abz *de novo*.

The genes for the canonical pathway for 4abz synthesis in *E. coli* are apparently missing in

*Methanosarcina*. However, an alternative pathway has been proposed in *Methanococcus maripalidus* (which also lacks the canonical pathway) based on carbon labeling (54). No genes in this proposed pathway have been identified yet, so it is unknown if the pathway could exist in *M. acetivorans*. It has been added based on the lack of need to uptake 4abz from the medium.

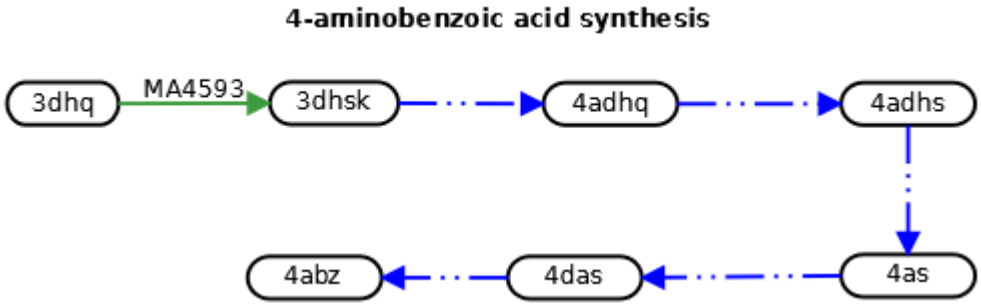


Figure A.1. Synthesis pathway for 4-aminobenzoic acid included in the *M. acetivorans* metabolic model.

**Biotin Biosynthesis**

Unlike in *Methanocaldococcus jannaschii*, none of the genes in the canonical pathway for biotin synthesis appear to be present in *M. acetivorans*. Except for the first reaction, suggested in a review by Robert White (84), the reactions listed here are all from KEGG. Note that many archaea do not possess these genes, and it has been suggested that *M. jannaschii* acquired them by lateral gene transfer from the bacteria (72).

Only the biotin synthase has (weak) homology with a *M. acetivorans* gene, and that gene has been suggested to have a role in pyrrolysine synthesis rather than biotin synthesis (38). It is

unknown if *M. acetivorans* actually synthesizes biotin - the pathway has been included under the assumption that it does.

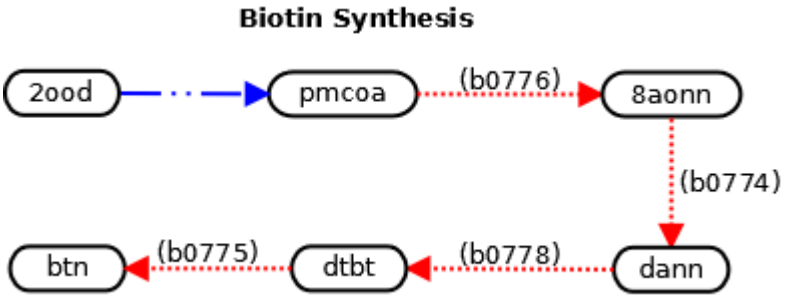


Figure A.2. Synthesis pathway for biotin included in the *M. acetivorans* metabolic model.

**Coenzyme A Biosynthesis**

The canonical pathway for coenzyme A biosynthesis is different in the archaea than in the bacterial domain. Some work has already been done to unravel the pathway in the archaea (MM2281 and TK1686 encode novel archaeal reactions) (86). However, if *M. acetivorans* can synthesize (R)-pantothenate *de novo*, the pathway for doing so remains unknown, for the canonical genes for both of the known pathways seem to be missing (one pathway shown). The gene responsible for catalyzing the final step in the synthesis is also unknown.

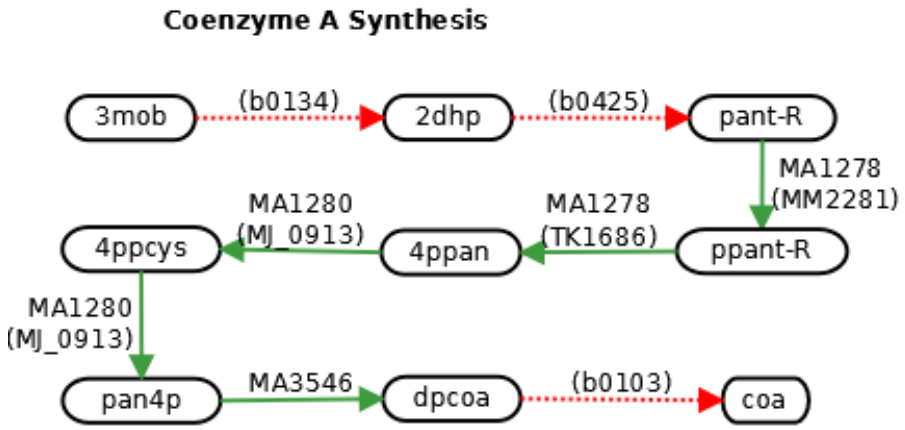


Figure A.3. Synthesis pathway for coenzyme A included in the *M. acetivorans* metabolic model.

### Coenzyme $F_{390}$ Metabolism

Coenzyme $F_{390}$ is a derivative of Coenzyme $F_{420}$ that appears to function in a redox-sensing mechanism in methanogenic archaea (80). The coenzyme $F_{390}$ synthetase has been characterized in *M. thermoautotrophicum* str. Marburg and has a strong homologue in *M. acetivorans* (79). However, although the Coenzyme $F_{390}$ hydrolase enzyme has been purified (80), the gene encoding this enzyme is still unknown.

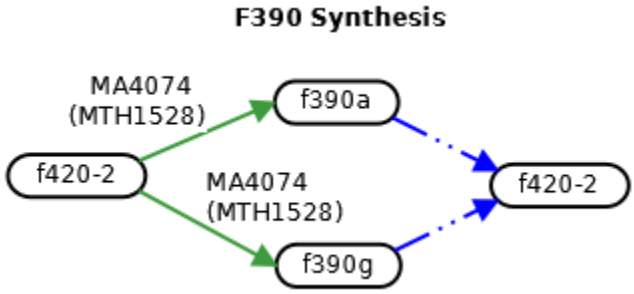


Figure A.4. Synthesis and degradation pathway for coenzyme $F_{390}$ included in the *M. acetivorans* metabolic model.

**Coenzyme $F_{420}$ and Riboflavin Biosynthesis**

Both the canonical beginning of the pathway for riboflavin synthesis and the alternate pathway characterized in *M. jannaschii* (24) appear to be missing in *M. acetivorans*, based on lack of homology with characterized genes. The third step has a characterized gene in *M. jannaschii* and is homologous to a gene in *M. acetivorans* (E ~ 1E-40), and the fourth step has no known gene.

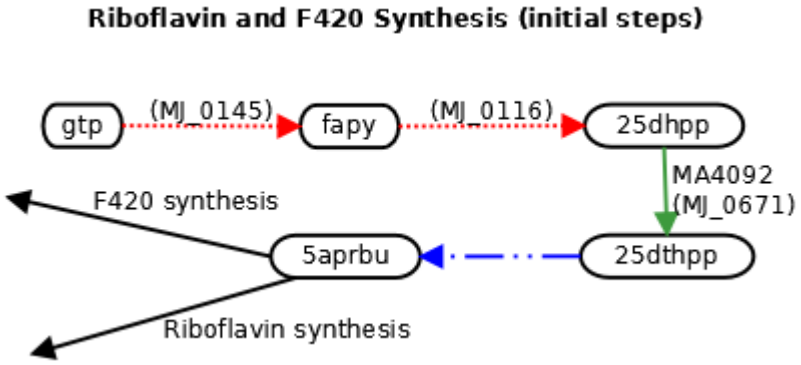


Figure A.5. The initial reactions involved in the synthesis pathways for coenzyme $F_{420}$ and riboflavin included in the *M. acetivorans* metabolic model.

**Coenzyme $F_{430}$ Biosynthesis**

Coenzyme $F_{430}$ is a nickel-containing coenzyme essential in some of the energy-conserving steps in methanogenesis (53). Coenzyme $F_{430}$ synthesis has been studied relatively little compared to pathways for synthesis of the other methanogenic cofactors, but a potential pathway has been proposed for its synthesis from dihydrosirohydrochlorin in five steps (53). No genes were identified. The exact chemical structure of most of the intermediates is unknown except for 15,17$^3$-seco-F430-17$^3$-acid (sf430a), which was identified in *Methanobrevibacter arboriphilus* (53).

It has been suggested that genes annotated as magnesium chelatases may in fact be nickel chelatases involved in this pathway (81). This is especially possible since the heme synthesis pathway in the *Methanosarcina* is likely to be different from the canonical pathway (see "Heme biosynthesis"), and therefore Protoporphyrin IX, the usual precursor for magnesium chelatase, would not necessarily be synthesized.

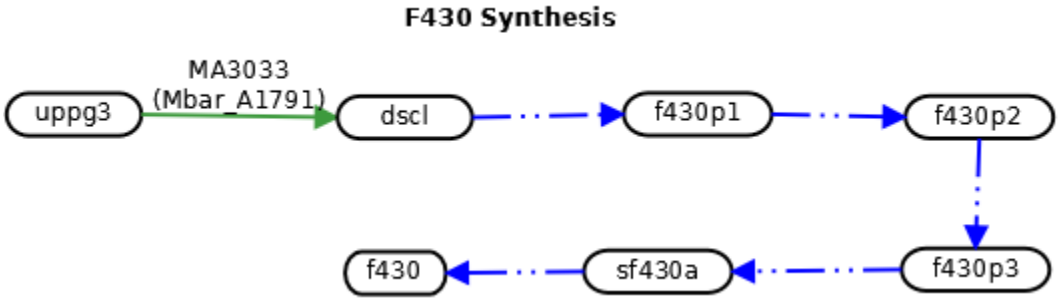


Figure A.6. Pathway for synthesis of coenzyme $F_{430}$ included in the *M. acetivorans* metabolic model.

**Coenzyme M Biosynthesis**

Coenzyme M is one of the methyl carriers in the methanogenesis pathway. A coenzyme M synthesis pathway has been identified in *Methanocaldococcus jannaschii*, and the genes in the first part of the pathway have been characterized (20, 22, 23). The first part of this pathway seems to be missing from the *Methanosarcina*, and indeed, an alternative pathway has been characterized in *M. acetivorans*, and the genes involved have been identified (21). However, the genes responsible for the last steps in the pathway have not yet been identified. The main text displays the pathway as it exists in *M. jannaschii*. The pathways in *M. jannaschii* and in *M. acetivorans* are shown below.

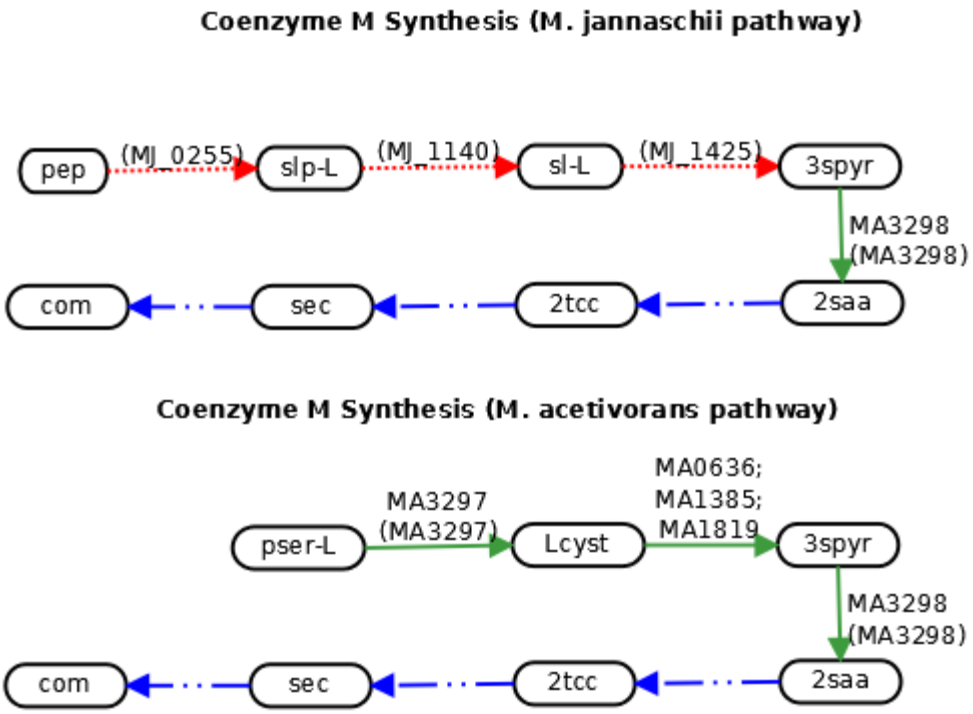


Figure A.7. Comparison of the coenzyme M biosynthesis pathway in *Methanocaldococcus jannaschii* and in *Methanosarcina acetivorans*. Only the *M. acetivorans* pathway was included in the metabolic model.

**Folate and Methanopterin Biosynthesis**

Most of the steps in folate and methanopterin biosynthesis have no characterized genes in the archaea. As discussed in the main text, even some of the genes that have been identified for these functions in *M. jannaschii* have no homologues in *Methanosarcina*. It is not clear how *Methanosarcina* synthesizes folates, but it is known that *Methanosarcina barkeri* possesses enzymes that depend on tetrahydrofolate for activity (9), and the same is assumed true of *M. acetivorans*. Therefore, there are likely novel enzymes and possibly a novel pathway to be discovered in this genus.

Most of the genes in the pathway have no gene association so it is difficult to tell the extent of the possible differences between the *Methanocaldococcus* and *Methanosarcina* synthesis pathways. However, even from those genes that are known, there are clear differences. The central part of the pathway seems to be the same but the initial synthesis and the addition of the hydroxyglutarate moiety to methanopterin apparently differ from the mechanisms in *Methanocaldococcus*. An alternate reaction for synthesis of dihydroneopterin (dhnpt) was suggested from KEGG, but the precursor metabolite for this reaction (ahdt) has no clear synthesis pathway in *M. acetivorans*, so this path seems more unlikely than that from *Methanocaldococcus*. It has been shown for completeness.

Previous work has suggested that there may be two pathways for synthesis of methanopterin in *M. barkeri,* one of which does not depend on synthesis of 4-aminobenzoate (4abz). However, the genetic evidence indicates that even if *M. acetivorans* has multiple synthesis pathways, they may *both* be different from that found in *M. jannaschii*. The nature of the differences between the pathways will become clearer as more genes in the pathway are identified and characterized.

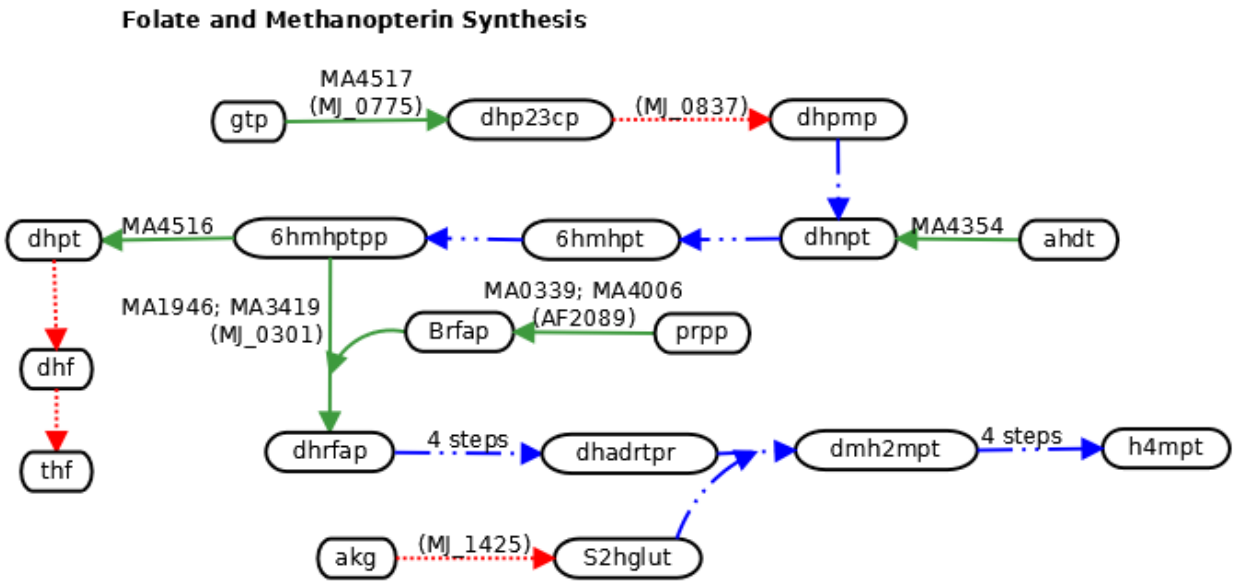


Figure A.8. Synthesis pathways for folate and methanopterin included in the *M. acetivorans* metabolic model.

**Heme Biosynthesis**

Since *Methanosarcina* is a genus of methanogens with cytochromes (28), it must have a way to synthesize heme. Many genes in the later part of the canonical protoheme synthesis pathway (from uroporphyrinogen III to protoheme) are missing. The one gene that KEGG suggests to be present in *M. acetivorans* (HemG) would encode a reaction requiring oxygen for function, although *M. acetivorans* is strictly anaerobic. Therefore, it is highly likely an alternative pathway exists for synthesis of heme in *Methanosarcina* and other cytochrome-containing methanogens. Although it is not fully characterized, an alternate pathway for heme (protoheme) synthesis has been suggested and partially characterized in *Methanosarcina barkeri*. The alternate pathway branches from precorrin 2 and involves SAM proteins (8). The only characterized genes which may be involved in the pathway are already annotated for their correct functions (71). Two different starts to the pathway have been suggested. In one possible pathway, precorrin-2 is

oxidized to sirohydrochlorin with PC2-DH and then proceeds to protoheme by an unknown set of reactions (71). In another, precorrin 2 is decarboxylated and synthesis of protoheme proceeds from there (8). The latter pathway is shown below.

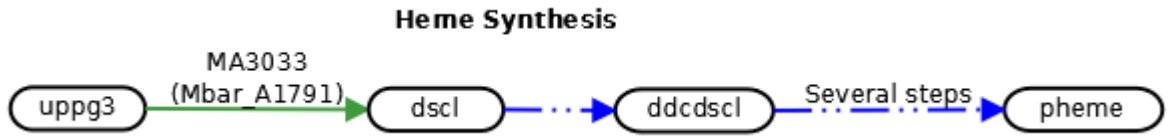


Figure A.9. Protoheme biosynthesis pathway included in the *M. acetivorans* metabolic model.

**Lysine Biosynthesis**

There are at least four known pathways for *de novo* lysine synthesis (37). The known genes involved in the pathways from thdp to 26dap-M appear to be missing in *M. acetivorans*. It has recently been reported that *M. jannaschii* utilizes the DapL pathway for lysine synthesis (37). One of the genes in this pathway has strong homology to a *M. acetivorans* gene, but one of them has no genes with significant sequence identity.

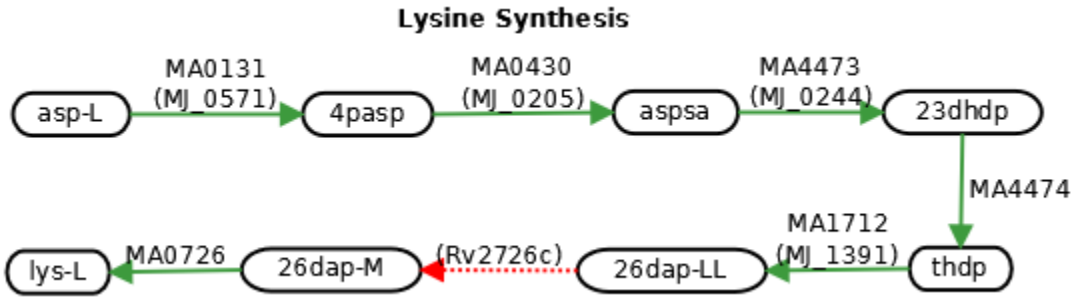


Figure A.10. Lysine biosynthesis pathway included in the *M. acetivorans* metabolic model.

**Methanofuran Biosynthesis**

The methanofuran biosynthesis pathway, as reported in metacyc, was incorporated in the model with no genetic evidence, except for the reaction TYRCBOX. The gene responsible for that reaction was characterized in *M. jannaschii* and has a strong sequence homology with *M. acetivorans* gene MA0006. The remainder are hypothetical reactions based on chemical identification of some of the intermediates in *M. jannaschii* (84).

Note that *M. barkeri* (and probably *M. acetivorans*) uses methanofuran (b), while Methanococci use methanofuran (a). According to the difference in these structures, *M. acetivorans* probably does not need to synthesize hexane-1,3,4,6-tetracarboxylate as an intermediate in methanofuran synthesis. Instead, it appears that the final steps would be the addition of two additional glutamate residues to 4-[N-gamma-L-glutamyl-gamma-L-glutamyl-)-p-(ß-aminoethyl)phenoxy-methyl]-2-(aminomethyl)furan .

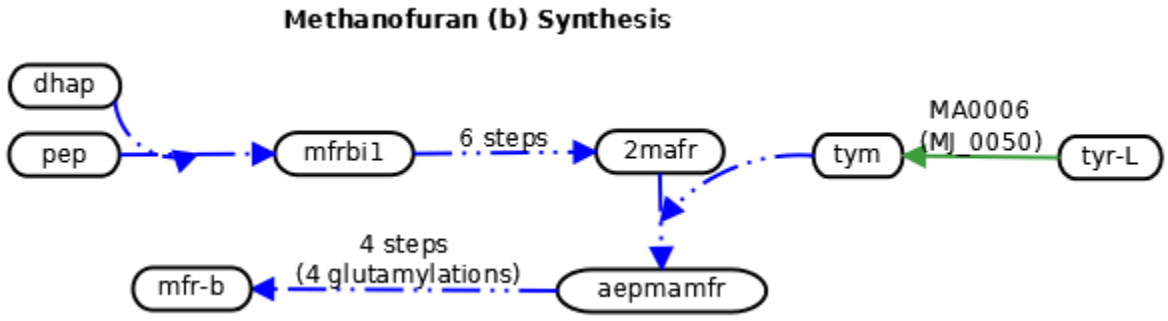


Figure A.11. The biosynthesis pathway for methanofuran(b) included in the *M. acetivorans* metabolic model.

**Methanophenazine Biosynthesis**

Unlike the other methanogenic cofactors, the authors are not aware of any proposed pathways for synthesis of methanophenazine in any organisms, including *M. acetivorans*. Even though quinones have not been found in methanogens, there are 14 genes annotated for functions in ubiquinone synthesis in *M. acetivorans*. On the other hand, *M. jannaschii*, which does not use methanophenazine in its methanogenic pathways, contains no genes annotated for ubiquinone synthesis functions (KEGG predicts three through clustering analysis, but none of them agrees with the annotation). It is possible that some of these genes in *M. acetivorans* are involved in the synthesis of methanophenazine, which performs a similar function to quinones in that organism. Phenazines are synthesized as antibiotics in many Bacteria and some genes are known (42), but no sequence homology was identified (with the exception of *phzF*, MA3532). Biochemical experimentation will be necessary to identify the genes and pathways actually involved.

**Purine Synthesis**

Although most of the genes for synthesis of purines are present, two critical enzymes seem to be missing in *Methanosarcina*: IMP dehydrogenase, and GMP kinase. Both of these enzymes are predicted to be present in *Methanocaldococcus* based on sequence homology. They have been included in the model because they perform critical functions in nucleic acid synthesis.

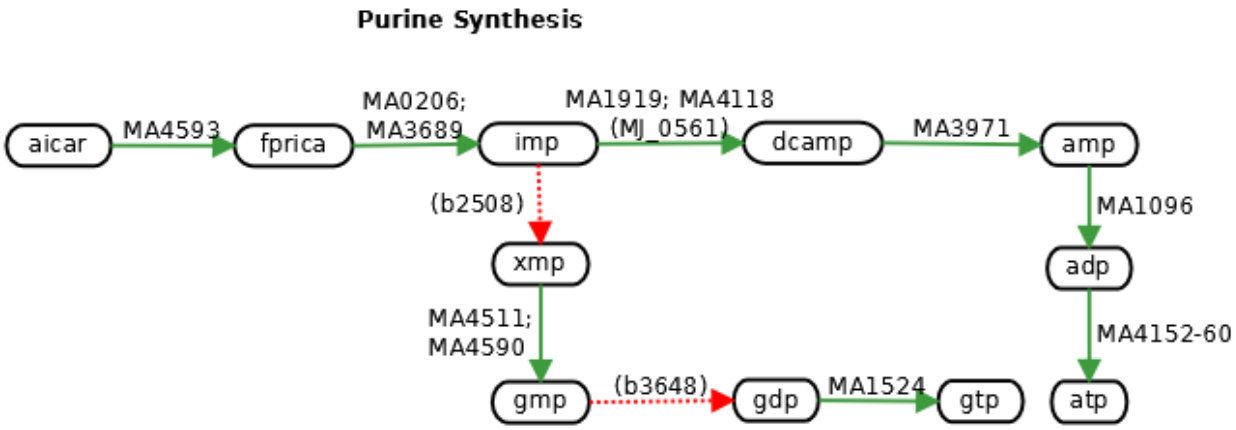


Figure A.12. Biosynthesis pathways for guanosine and adenosine triphosphates included in the *M. acetivorans* metabolic model.

# Appendix B. Model Details

This appendix contains lists of metabolites (Table B.1.), reactions (Table B.2.), media components used to perform FBA simulations (Table B.3.), and the complete composition of the biomass equation used as an objective function in FBA simulations (Table B.4.).

Table B.1. List of metabolites in the *M. acetivorans* metabolic model.

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| 10fthf | 10-Formyltetrahydrofolate | C20H22N7O7 | -1 |
| 13dpg | 3-Phospho-D-glyceroyl phosphate | C3H4O10P2 | -4 |
| 1pyr4m5c | 1-Pyrroline-4-methyl-5-carboxylate | C6H8NO2 | -1 |
| 1pyr5c | 1-Pyrroline-5-carboxylate | C5H6NO2 | -1 |
| 23dhdp | 2,3-Dihydrodipicolinate | C7H6NO4 | -1 |
| 23dhmb | (R)-2,3-Dihydroxy-3-methylbutanoate | C5H9O4 | -1 |
| 23dhmp | (R)-2,3-Dihydroxy-3-methylpentanoate | C6H11O4 | -1 |
| 24sf | 2,4-substituted-furan | C6H4O7P | -3 |
| 25aics | (S)-2-[5-Amino-1-(5-phospho-D-ribosyl)imidazole-4-carboxamido]succinate | C13H15N4O12P | -4 |
| 25dhpp | 2,5-Diamino-6-hydroxy-4-(5'-phosphoribosylamino)-pyrimidine | C9H14N5O8P | -2 |
| 25dthpp | 2,5-diamino-6-ribitylamino-4(3H)-pyrimidinone 5'-phosphate | C9H16N5O8P | -2 |
| 26dap-LL | LL-2,6-Diaminoheptanedioate | C7H14N2O4 | 0 |
| 26dap-M | meso-2,6-Diaminoheptanedioate | C7H14N2O4 | 0 |
| 2ahbut | (S)-2-Aceto-2-hydroxybutanoate | C6H9O4 | -1 |
| 2c25dho | 2-Carboxy-2,5-dihydro-5-oxofuran-2-acetate | C7H4O6 | -2 |
| 2cpr5p | 1-(2-Carboxyphenylamino)-1-deoxy-D-ribulose 5-phosphate | C12H13NO9P | -3 |
| 2dhp | 2-Dehydropantoate | C6H9O4 | -1 |
| 2dr1p | 2-Deoxy-D-ribose 1-phosphate | C5H9O7P | -2 |
| 2dr5p | 2-Deoxy-D-ribose 5-phosphate | C5H9O7P | -2 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| 2frald | 2-furaldehyde | C6H5O6P | -2 |
| 2h3moa | 3-Hydroxy-3-methyl-2-oxobutanoic acid | C5H7O4 | -1 |
| 2ins | 2-Inosose | C6H10O6 | 0 |
| 2ippm | 2-Isopropylmaleate | C7H8O4 | -2 |
| 2mafr | 2-methylamine-furan | C6H9NO5P | -1 |
| 2mahmp | 2-Methyl-4-amino-5-hydroxymethylpyrimidine diphosphate | C6H8N3O7P2 | -3 |
| 2mop | 2-Methyl-3-oxopropanoate | C4H5O3 | -1 |
| 2obut | 2-Oxobutanoate | C4H5O3 | -1 |
| 2ood | 2-oxooctanedioic acid | C8H10O5 | -2 |
| 2pg | D-Glycerate 2-phosphate | C3H4O7P | -3 |
| 2pglyc | 2-Phosphoglycolate | C2H2O6P | -3 |
| 2plac-L | 2-phospho-L-lactate | C3H4O6P | -3 |
| 2ppoh | 2-Propanol | C3H8O | 0 |
| 2saa | 2-sulfoacetaldehyde | C2H3O4S | -1 |
| 2tcc | 2-(sulfomethyl)thiazolidine-4-carboxylic acid | C5H8NO5S2 | -1 |
| 34hpp | 3-(4-Hydroxyphenyl)pyruvate | C9H7O4 | -1 |
| 35cgmp | 3',5'-Cyclic GMP | C10H11N5O7P | -1 |
| 36dahx | (3S)-3,6-Diaminohexanoate | C6H15N2O2 | 1 |
| 3c2hmp | 3-Carboxy-2-hydroxy-4-methylpentanoate | C7H10O5 | -2 |
| 3c3hmp | 3-Carboxy-3-hydroxy-4-methylpentanoate | C7H10O5 | -2 |
| 3c4mop | 3-Carboxy-4-methyl-2-oxopentanoate | C7H8O5 | -2 |
| 3dhq | 3-Dehydroquinate | C7H9O6 | -1 |
| 3dhsk | 3-Dehydroshikimate | C7H7O5 | -1 |
| 3h3mop | (R)-3-Hydroxy-3-methyl-2-oxopentanoate | C6H9O4 | -1 |
| 3hcdgggp | CDP-2-O-(3'-hydroxy)geranyl-3-O-geranyl-sn-glycerol | C52H85N3O14P2 | -2 |
| 3hdgggp | 2-O-(3'-hydroxy)geranyl-3-O-geranyl-sn-glycerol | C43H73O7P1 | -2 |
| 3hdgggps | 2-O-(3'-hydroxy)geranyl-3-O-geranyl-sn-glyceroserine | C46H79N1O9P1 | -1 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| 3hdggpg | 2-O-(3'-hydroxy)geranyl-3-O-geranyl-sn-glycerol-1-phospho-3'-sn-glycerol | C46H80O9P1 | -1 |
| 3hdggpgp | 2-O-(3'-hydroxy)geranyl-3-O-geranyl-sn-glycerol-1-phospho-3'-sn-glycerol phosphate | C46H79O12P2 | -3 |
| 3hdggpi | 2-O-(3'hydroxy)geranyl-3-O-geranyl-sn-glycero-1-phospho-myo-inositol | C49H84O12P1 | -1 |
| 3hdpgpe | 2-O-(3'-hydroxy)phytanyl-3-O-phytanyl-sn-glycero-l-phosphoethanolamine | C45H94O7N1P1 | 0 |
| 3hdpgpg | 2-O-(3'-hydroxy)phytanyl-3-O-phytanyl-sn-glycerol-1-phospho-3'-sn-glycerol | C46H94O9P1 | -1 |
| 3hdpgpi | 2-O-(3'-hydroxyl)phytanyl-3-O-phytanyl-sn-glycero-1-phospho-myo-inositol | C49H98O12P1 | -1 |
| 3hdpgps | 2-O-(3'-hydroxy)phytanyl-3-O-phytanyl-sn-glycero-l-phosphoserine | C46H93N1O9P1 | -1 |
| 3hfrdp | 3-hydroxy-farnesyl diphosphate | C15H27O8P2 | -3 |
| 3hggdp | 3-hydroxy-geranylgeranyl diphosphate | C20H35O8P2 | -3 |
| 3hgrdp | 3-hydroxy-geranyl diphsophate | C10H19O8P2 | -3 |
| 3hmp | 3-Hydroxy-2-methylpropanoate | C4H7O3 | -1 |
| 3ig3p | C'-(3-Indolyl)-glycerol 3-phosphate | C11H12NO6P | -2 |
| 3mob | 3-Methyl-2-oxobutanoate | C5H7O3 | -1 |
| 3mop | (S)-3-Methyl-2-oxopentanoate | C6H9O3 | -1 |
| 3ophb | 3-Octaprenyl-4-hydroxybenzoate | C47H69O3 | -1 |
| 3pg | 3-Phospho-D-glycerate | C3H4O7P | -3 |
| 3php | 3-Phosphohydroxypyruvate | C3H2O7P | -3 |
| 3psme | 5-O-(1-Carboxyvinyl)-3-phosphoshikimate | C10H9O10P | -4 |
| 3spyr | 3-Sulfopyruvate | C3H2O6S | -2 |
| 3uib | 3-Ureidoisobutyrate | C5H9N2O3 | -1 |
| 4abut | 4-Aminobutanoate | C4H9NO2 | 0 |
| 4abutn | 4-Aminobutanal | C4H10NO | 1 |
| 4abz | 4-Aminobenzoate | C7H6NO2 | -1 |
| 4adhq | 4-amino-3-dehydroquinic acid | C7H11NO5 | 0 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| 4adhs | 4-amino-3-dehydroshikimate | C7H8NO4 | -1 |
| 4ahmmp | 4-Amino-5-hydroxymethyl-2-methylpyrimidine | C6H10N3O | 1 |
| 4ampm | 4-Amino-2-methyl-5-phosphomethylpyrimidine | C6H8N3O4P | -2 |
| 4as | 4-aminoshikimate | C7H11NO4 | 0 |
| 4das | 4-amino-3-dehydroxycyclohexa-1,5-diene-1-carboxylate | C7H9NO3 | 0 |
| 4gaepmamfr | 4-[N-γ-L-glutamyl-)-p-(β-aminoethyl)phenoxy-methyl]-2-(aminomethyl)furan | C19H26N3O5 | 1 |
| 4ggaepmamfr | 4-[N-γ-L-glutamyl-γ-L-glutamyl-)-p-(β-aminoethyl)phenoxy-methyl]-2-(aminomethyl)furan | C24H32N4O8 | 0 |
| 4gggaepmamfr | 4-[N-γ-L-glutamyl-γ-L-glutamyl-γ-L-glutamyl-)-p-(β-aminoethyl)phenoxy-methyl]-2-(aminomethyl)furan | C29H38N5O11 | -1 |
| 4hba | 4-Hydroxy-benzyl alcohol | C7H8O2 | 0 |
| 4hbz | 4-Hydroxybenzoate | C7H5O3 | -1 |
| 4hphac | 4-Hydroxyphenylacetate | C8H7O3 | -1 |
| 4mhetz | 4-Methyl-5-(2-hydroxyethyl)-thiazole | C6H9NOS | 0 |
| 4mop | 4-Methyl-2-oxopentanoate | C6H9O3 | -1 |
| 4mpetz | 4-Methyl-5-(2-phosphoethyl)-thiazole | C6H8NO4PS | -2 |
| 4pasp | 4-Phospho-L-aspartate | C4H6NO7P | -2 |
| 4ppan | D-4'-Phosphopantothenate | C9H15NO8P | -3 |
| 4ppcys | N-((R)-4-Phosphopantothenoyl)-L-cysteine | C12H20N2O9PS | -3 |
| 4r5au | 4-(1-D-Ribitylamino)-5-aminouracil | C9H16N4O6 | 0 |
| 56dthm | 5,6-Dihydrothymine | C5H8N2O2 | 0 |
| 56dura | 5,6-dihydrouracil | C4H6N2O2 | 0 |
| 5aizc | 5-amino-1-(5-phospho-D-ribosyl)imidazole-4-carboxylate | C9H11N3O9P | -3 |
| 5aop | 5-Amino-4-oxopentanoate | C5H9NO3 | 0 |
| 5aprbu | 5-Amino-6-(5'-phosphoribitylamino)uracil | C9H15N4O9P | -2 |
| 5c5pdriaz | 5-Carboxyamino-1-(5-phospho-D-ribosyl)imidazole | C9H11N3O9P | -3 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| 5caiz | 5-phosphoribosyl-5-carboxyaminoimidazole | C9H11N3O9P | -3 |
| 5hbc | 5-Hydroxybenzimidazolylcob(II)amide | C60H84CoN13O15P | 0 |
| 5hbc_red | 5-Hydroxybenzimidazolylcob(I)amide | C60H84CoN13O15P | -1 |
| 5hbzid | 5-hydroxybenzimidazole | C7H6N2O | 0 |
| 5mcyt | 5-Methylcytosine | C5H7N3O | 0 |
| 5mdr1p | 5-Methylthio-5-deoxy-D-ribose 1-phosphate | C6H11O7PS | -2 |
| 5mdru1p | 5-Methylthio-5-deoxy-D-ribulose 1-phosphate | C6H11O7PS | -2 |
| 5mta | 5-Methylthioadenosine | C11H15N5O3S | 0 |
| 5mthf | 5-Methyltetrahydrofolate | C20H24N7O6 | -1 |
| 5odhf2a | 5-Oxo-4,5-dihydrofuran-2-acetate | C6H5O4 | -1 |
| 5oxpro | 5-Oxoproline | C5H6NO3 | -1 |
| 5pmev | (R)-5-Phosphomevalonate | C6H10O7P | -3 |
| 5pr5hbz | N1-(5-Phospho-alpha-D-ribosyl)-5-hydroxybenzimidazole | C12H13N2O8P | -2 |
| 6ax | 6-Aminohexanoate | C6H13NO2 | 0 |
| 6ax6ax | N-(6-Aminohexanoyl)-6-aminohexanoate | C12H24N2O3 | 0 |
| 6hmhpt | 6-hydroxymethyl dihydropterin | C7H9N5O2 | 0 |
| 6hmhptpp | 6-hydroxymethyl-dihydropterin pyrophosphate | C7H8N5O8P2 | -3 |
| 6pthp | 6-Pyruvoyl-5,6,7,8-tetrahydropterin | C9H12N5O3 | 1 |
| 7mhp | 7-mercaptoheptanoic acid | C7H13O2S | -1 |
| 7mht | 7-mercaptoheptanoylthreonine | C11H20NO4S | -1 |
| 7ohp | 7-oxoheptanoic acid | C7H11O3 | -1 |
| 8aonn | 8-Amino-7-oxononanoate | C9H17NO3 | 0 |
| aacald | Aminoacetaldehyde | C2H6NO | 1 |
| aacoa | Acetoacetyl-CoA | C25H36N7O18P3S | -4 |
| ac | Acetate | C2H3O2 | -1 |
| acald | Acetaldehyde | C2H4O | 0 |
| accoa | Acetyl-CoA | C23H34N7O17P3S | -4 |
| acetol | Acetol | C3H6O2 | 0 |
| acetone | Acetone | C3H6O | 0 |
| acg5p | N-Acetyl-L-glutamyl 5-phosphate | C7H9NO8P | -3 |
| acg5sa | N-Acetyl-L-glutamate 5-semialdehyde | C7H10NO4 | -1 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| acgam1p | N-Acetyl-D-glucosamine 1-phosphate | C8H14NO9P | -2 |
| acglu | N-Acetyl-L-glutamate | C7H9NO5 | -2 |
| achms | O-Acetyl-L-homoserine | C6H11NO4 | 0 |
| acmana | N-Acetyl-D-mannosamine | C8H15NO6 | 0 |
| acnam | N-Acetylneuraminate | C11H18NO9 | -1 |
| acon-C | cis-Aconitate | C6H3O6 | -3 |
| aconm | E-3-carboxy-2-pentenedioate 6-methyl ester | C7H6O6 | -2 |
| acon-T | trans-Aconitate | C6H3O6 | -3 |
| acorn | N2-Acetyl-L-ornithine | C7H14N2O3 | 0 |
| acser | O-Acetyl-L-serine | C5H9NO4 | 0 |
| actn-R | (R)-Acetoin | C4H8O2 | 0 |
| actp | Acetyl phosphate | C2H3O5P | -2 |
| adcobdam | Adenosyl cobyrinate diamide | C55H68CoN11O15 | -4 |
| adcobhex | adenosyl-cobyric acid | C55H76CoN15O11 | 0 |
| ade | Adenine | C5H5N5 | 0 |
| adn | Adenosine | C10H13N5O4 | 0 |
| adocbi | Adenosyl cobinamide | C58H84CoN16O11 | 1 |
| adocbip | Adenosyl cobinamide phosphate | C58H83CoN16O14P | -1 |
| adocblhbi | Adenosylcobalamin-HBI | C70H96CoN18O18P | 0 |
| adp | ADP | C10H12N5O10P2 | -3 |
| adprib | ADP-ribose | C15H21N5O14P2 | -2 |
| aepmamfr | p-(β-aminoethyl)phenoxy-methyl-2-(aminomethyl)furan | C14H20N2O2 | 2 |
| agdpcbi | Adenosine-GDP-cobinamide | C68H95CoN21O21P2 | -1 |
| agdpgpi | N-Acetyl-D-glucosaminyl archaetidylinositol | C57H111N1O16P1 | -1 |
| agm | Agmatine | C5H16N4 | 2 |
| ah6p-D | D-arabino-Hex-3-ulose 6-phosphate; | C6H11O9P | -2 |
| ahcys | S-Adenosyl-L-homocysteine | C14H20N6O5S | 0 |
| ahdt | 2-Amino-4-hydroxy-6-(erythro-1,2,3-trihydroxypropyl)dihydropteridine triphosphate | C9H13N5O13P3 | -3 |
| aicar | 5-Amino-1-(5-Phospho-D-ribosyl)imidazole-4-carboxamide | C9H13N4O8P | -2 |
| air | 5-amino-1-(5-phospho-D-ribosyl)imidazole | C8H12N3O7P | -2 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| akg | 2-Oxoglutarate | C5H4O5 | -2 |
| ala-B | beta-Alanine | C3H7NO2 | 0 |
| alac-S | (S)-2-Acetolactate | C5H7O4 | -1 |
| ala-L | L-Alanine | C3H7NO2 | 0 |
| alatrna | L-Alanyl-tRNA(Ala) | C3H6NOR | 1 |
| amet | S-Adenosyl-L-methionine | C15H23N6O5S | 1 |
| amob | S-Adenosyl-4-methylthio-2-oxobutanoate | C15H19N5O6S | 0 |
| amp | AMP | C10H12N5O7P | -2 |
| anth | Anthranilate | C7H6NO2 | -1 |
| appl | 1-Aminopropan-2-ol | C3H10NO | 1 |
| applp | D-1-Aminopropan-2-ol O-phosphate | C3H9NO4P | -1 |
| aps | Adenosine 5'-phosphosulfate | C10H12N5O10PS | -2 |
| arg-L | L-Arginine | C6H15N4O2 | 1 |
| argsuc | N(omega)-(L-Arginino)succinate | C10H17N4O6 | -1 |
| argtrna | L-Arginyl-tRNA(Arg) | C6H14N4OR | 2 |
| asn-L | L-Asparagine | C4H8N2O3 | 0 |
| asntrna | asparagine-tRNA(asn) | C4H7N2O2R | 1 |
| asntrna(asp) | aspartate-tRNA(asn) | C4H5NO3R | 0 |
| asp-L | L-Aspartate | C4H6NO4 | -1 |
| aspsa | L-Aspartate 4-semialdehyde | C4H7NO3 | 0 |
| asptrna | L-Aspartyl-tRNA(Asp) | C4H5NO3R | 0 |
| athr-L | L-Allo-threonine | C4H9NO3 | 0 |
| atp | ATP | C10H12N5O13P3 | -4 |
| atrz | Atrazine | C8H14ClN5 | 0 |
| bamppald | beta-Aminopropion aldehyde | C3H8NO | 1 |
| biomass_met | Biomass | | |
| Brfap | 4-(B-D-ribofuranosyl)aminobenzene 5'-phosphate | C11H14NO7P | -2 |
| btamp | Biotinyl-5'-AMP | C20H27N7O9PS | -1 |
| btn | Biotin | C10H15N2O3S | -1 |
| btnp | Biotin synthesis protein (uncharged) | R | 0 |
| btnp-s2 | Biotin synthesis protein (charged) | Fe2S2R | 2 |
| ca2 | Calcium | Ca | 2 |
| cala | N-Carbamoyl-beta-alanine | C4H7N2O3 | -1 |
| camp | cAMP | C10H11N5O6P | -1 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| caphis | 2-(3-Carboxy-3-aminopropyl)-L-histidine | C10H16N4O4 | 0 |
| carn | L-Carnosine | C9H15N4O3 | 1 |
| cbasp | N-Carbamoyl-L-aspartate | C5H6N2O5 | -2 |
| cbi | Cobinamide | C48H72CoN11O8 | 0 |
| cbl1 | Cob(I)alamin | C62H88CoN13O14P | -1 |
| cbl1hbi | Cob(I)alamin-HBI | C60H84CoN13O15P | -1 |
| cbp | Carbamoyl phosphate | CH2NO5P | -2 |
| cd2 | Cadmium | Cd | 2 |
| cdgggp | CDP-2,3-digeranylgeranyl-sn-glycerol | C52H83N3O13P2 | -2 |
| cdp | CDP | C9H12N3O11P2 | -3 |
| cgly | Cys-Gly | C5H10N2O3S | 0 |
| ch4 | methane | CH4 | 0 |
| ch4s | Methanethiol | CH4S | 0 |
| chor | chorismate | C10H8O6 | -2 |
| cit | Citrate | C6H5O7 | -3 |
| citrac | Citraconic acid | C5H4O4 | -2 |
| citr-L | L-Citrulline | C6H13N3O3 | 0 |
| cl | Chloride | Cl | -1 |
| cmaphis | 2-[3-Carboxy-3-(methylammonio)propyl]-L-histidine | C11H18N4O4 | 0 |
| cmp | CMP | C9H12N3O8P | -2 |
| cmpacna | CMP-N-acetylneuraminate | C20H29N4O16P | -2 |
| co | Carbon monoxide | CO | 0 |
| co1dam | Cob(I)yrinate a,c diamide | C45H56CoN6O12 | -5 |
| co2 | CO2 | CO2 | 0 |
| co2dam | Cob(II)yrinate a,c diamide | C45H56CoN6O12 | -4 |
| coa | Coenzyme A | C21H32N7O16P3S | -4 |
| cob | coenzyme b | C11H19N1O7P1S1 | -3 |
| cobalt2 | Co2+ | Co | 2 |
| cobya | Cobyrinate | C45H52CoN4O14 | -6 |
| codhpre6 | cobalt-dihydro-precorrin 6 | C44H47CoN4O16 | -7 |
| com | coenzyme m | C2H5O3S2 | -1 |
| copre2 | cobalt-precorrin 2 | C42H38N4O16Co | -8 |
| copre3 | cobalt-precorrin 3 | C43H40CoN4O16 | -8 |
| copre4 | cobalt-precorrin 4 | C44H43CoN4O16 | -7 |
| copre5 | cobalt-precorrin 5 | C45H45CoN4O16 | -7 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| copre6 | cobalt-precorrin 6 | C44H44CoN4O16 | -8 |
| copre8 | cobalt-precorrin 8 | C45H51CoN4O14 | -7 |
| csn | Cytosine | C4H5N3O | 0 |
| ctp | CTP | C9H12N3O14P3 | -4 |
| cu2 | Cu2+ | Cu | 2 |
| cys-L | L-Cysteine | C3H7NO2S | 0 |
| cystrna | L-Cysteinyl-tRNA(Cys) | C3H6NOSR | 1 |
| cystrna(pser) | O-Phosphoseryl-tRNA(Cys) | C3H5NO5PR | -1 |
| cytd | Cytidine | C9H13N3O5 | 0 |
| dad-2 | Deoxyadenosine | C10H13N5O3 | 0 |
| dadp | dADP | C10H12N5O9P2 | -3 |
| damp | dAMP | C10H12N5O6P | -2 |
| dann | 7,8-Diaminononanoate | C9H21N2O2 | 1 |
| datp | dATP | C10H12N5O12P3 | -4 |
| db4p | 3,4-dihydroxy-2-butanone 4-phosphate | C4H7O6P | -2 |
| dcamp | N6-(1,2-Dicarboxyethyl)-AMP | C14H14N5O11P | -4 |
| dcdp | dCDP | C9H12N3O10P2 | -3 |
| dcmp | dCMP | C9H12N3O7P | -2 |
| dctp | dCTP | C9H12N3O13P3 | -4 |
| dcyt | Deoxycytidine | C9H13N3O4 | 0 |
| ddcdscl | 12,18-didecarboxyprecorrin-2 | C40H43N4O12 | -5 |
| ddhrb | 7,8-didemethyl-8-hydroxy-5-deazariboflavin | C16H16N3O7 | -1 |
| dgdp | dGDP | C10H12N5O10P2 | -3 |
| dgggp | digeranylgeranylglyceryl phosphate | C43H71O6P1 | -2 |
| dgggps | 2,3-di-O-geranylgeranyl-sn-glyceroserine | C46H77N1O8P1 | -1 |
| dggpg | 2,3-di-O-geranyl-sn-glycerol-1-phospho-3'-sn-glycerol | C46H78O8P1 | -1 |
| dggpgp | 2,3-di-O-geranyl-sn-glycerol-1-phospho-3'-sn-glycerol phosphate | C46H77O11P2 | -3 |
| dggpi | 2,3-di-O-geranyl-sn-glycero-1-phospho-myo-inositol | C49H82O11P1 | -1 |
| dgmp | dGMP | C10H12N5O7P | -2 |
| dgsn | Deoxyguanosine | C10H13N5O4 | 0 |
| dgtp | dGTP | C10H12N5O13P3 | -4 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| dha | Dihydroxyacetone | C3H6O3 | 0 |
| dhadr | 7,8-dihydropterin-6-ylmethyl-1-(4-aminophenyl)-1-deoxy-D-ribitol | C18H25N6O5 | 1 |
| dhadrdpr | 7,8-dihydropterin-6-ylmethyl-1-(4-aminophenyl)-1-deoxy-5-[1-A-D-ribofuranosyl 5'-diphosphate]-D-ribitol | C23H32N6O15P2 | -2 |
| dhadrp | 7,8-dihydropterin-6-ylmethyl-1-(4-aminophenyl)-1-deoxy-D-ribitol 5'-phosphate | C18H24N6O8P | -1 |
| dhadrpr | 7,8-dihydropterin-6-ylmethyl-1-(4-aminophenyl)-1-deoxy-5-[1-A-D-ribofuranosyl 5'-phosphate]-D-ribitol | C23H32N6O12P | -1 |
| dhadrtpr | 7,8-dihydropterin-6-ylmethyl-1-(4-aminophenyl)-1-deoxy-5-[1-A-D-ribofuranosyl 5'-triphosphate]-D-ribitol | C23H32N6O18P3 | -3 |
| dhap | Dihydroxyacetone phosphate | C3H5O6P | -2 |
| dhf | 7,8-Dihydrofolate | C19H20N7O6 | -1 |
| dhlpro | Dihydrolipolprotein | C8H16NOS2R | 0 |
| dhnpt | Dihydroneopterin | C9H14N5O4 | 1 |
| dhor-S | (S)-Dihydroorotate | C5H5N2O4 | -1 |
| dhp23cp | 7,8-dihydronepterin 2' :3'-cyclicphosphate | C9H12N5O6P | 0 |
| dhpmp | Dihydroneopterin monophosphate | C9H13N5O7P | -1 |
| dhpt | Dihydropteroate | C14H14N6O3 | 0 |
| dhrfap | 7,8-dihydropterin-6-ylmethyl-4-(B-D-ribofuranosyl) aminobenzene 5'-phosphate | C18H22N6O8P | -1 |
| didp | dIDP | C10H11N4O10P2 | -3 |
| ditp | dITP | C10H11N4O13P3 | -4 |
| dkfp | 6-deoxy-5-ketofructose 1-phosphate | C6H9O8P | -2 |
| dkmp | 6-deoxy-5-ketomannitol 1-phosphate | C6H11O8P | -2 |
| dma | dimethylamine | C2H8N | 1 |
| dmh2mpt | didemethylated 7,8-dihydromethanopterin | C28H37N6O16P | -2 |
| dmlz | 6,7-Dimethyl-8-(1-D-ribityl)lumazine | C13H18N4O6 | 0 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| dmpp | Dimethylallyl diphosphate | C5H9O7P2 | -3 |
| dms | Dimethyl sulfide | C2H6S | 0 |
| dna_met | Biomass – DNA component | | |
| dnad | Deamino-NAD+ | C21H24N6O15P2 | -2 |
| dohau | 3,7-dideoxy-D-threo-hepto-2-amino-6-ulosonate | C7H13O5N | 0 |
| dohdu | 3,7-dideoxy-D-threo-hepto-2,6-diulosonate | C7H9O6 | -1 |
| dpcoa | Dephospho-CoA | C21H33N7O13P2S | -2 |
| dpgpe | 2,3-O-phytanyl-sn-glycero-1-phosphoethanolamine | C45H94O6N1P1 | 0 |
| dpgpg | 2,3-di-O-phytanyl-sn-glycerol-1-phospho-3'-sn-glycerol | C46H94O8P1 | -1 |
| dpgpi | 2,3-O-phytanyl-sn-glycero-1-phospho-myo-inositol | C49H98O11P1 | -1 |
| dpgps | 2,3-O-phytanyl-sn-glycero-1-phosphoserine | C46H93N1O8P1 | -1 |
| drib | Deoxyribose | C5H10O4 | 0 |
| dscl | dihydrosirohydrochlorin (precorrin 2) | C42H41N4O16 | -7 |
| dtbt | Dethiobiotin | C10H17N2O3 | -1 |
| dtdp | dTDP | C10H13N2O11P2 | -3 |
| dtdp4d6dg | dTDP-4-dehydro-6-deoxy-D-glucose | C16H22N2O15P2 | -2 |
| dtdp4d6dm | dTDP-4-dehydro-6-deoxy-L-mannose | C16H22N2O15P2 | -2 |
| dtdpglu | dTDPglucose | C16H24N2O16P2 | -2 |
| dtdprmn | dTDP-L-rhamnose | C16H24N2O15P2 | -2 |
| dtmp | dTMP | C10H13N2O8P | -2 |
| dttp | dTTP | C10H13N2O14P3 | -4 |
| dudp | dUDP | C9H11N2O11P2 | -3 |
| dump | dUMP | C9H11N2O8P | -2 |
| duri | Deoxyuridine | C9H12N2O5 | 0 |
| dutp | dUTP | C9H11N2O14P3 | -4 |
| dxyl5p | 1-deoxy-D-xylulose 5-phosphate | C5H9O7P | -2 |
| e4p | D-Erythrose 4-phosphate | C4H7O7P | -2 |
| eig3p | D-erythro-1-(Imidazol-4-yl)glycerol 3-phosphate | C6H9N2O6P | -2 |
| etha | Ethanolamine | C2H8NO | 1 |
| etoh | Ethanol | C2H6O | 0 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| f1p | D-Fructose 1-phosphate | C6H11O9P | -2 |
| f390a | coenzyme F390 (adenosine) | C39H43N10O24P2 | -5 |
| f390g | coenzyme F390 (guanosine) | C39H42N10O25P2 | -6 |
| f420-0 | coenzyme ferredoxin 420-0 | C19H19N3O12P | -3 |
| f420-1 | coenzyme ferredoxin 420-1 | C24H25N4O15P | -4 |
| f420-2 | coenzyme ferredoxin 420-2 (oxidized) | C29H31N5O18P | -5 |
| f420-2h2 | coenzyme ferredoxin 420-2 (reduced) | C29H34O18N5P1 | -4 |
| f420-3 | coenzyme ferredoxin 420-3 | C34H37N6O21P | -6 |
| f420-4 | coenzyme ferredoxin 420-4 | C39H43N7O24P | -7 |
| f420-5 | coenzyme ferredoxin 420-5 | C44H49N8O27P | -8 |
| f420-6 | coenzyme ferredoxin 420-6 | C49H55N9O30P | -9 |
| f420-7 | coenzyme ferredoxin 420-7 | C54H61N10O33P | -10 |
| f430 | coenzyme F430 | C42H46N6NiO13 | -4 |
| f430p1 | coenzyme 430 precursor 1 | C42H43N6NiO14 | -5 |
| f430p2 | coenzyme F430 precursor 2 | C42H45N6NiO14 | -5 |
| f430p3 | coenzyme f430 precursor 3 | C42H47N6NiO14 | -5 |
| f6p | D-Fructose 6-phosphate | C6H11O9P | -2 |
| fad | Flavin adenine dinucleotide oxidized | C27H31N9O15P2 | -2 |
| fald | Formaldehyde | CH2O | 0 |
| fapy | 2-amino-5-formylamino-6-ribosylamino-4(3H)-pyrimidinone 5'-monophosphate (FAPy) | C10H14N5O9P | -2 |
| fc1p | L-Fuculose 1-phosphate | C6H11O8P | -2 |
| fcd | Flavin cytosine dinucleotide oxidized | C26H31N7O16P2 | -2 |
| fdox | ferredoxin (oxidized) 2[4Fe-4S] | Fe8S8X | 3 |
| fdp | D-Fructose 1,6-bisphosphate | C6H10O12P2 | -4 |
| fdred | ferredoxin (reduced) 2[4Fe-4S] | Fe8S8X | 2 |
| fe2 | Fe2+ | Fe | 2 |
| fe3 | Fe3+ | Fe | 3 |
| fgam | N2-Formyl-N1-(5-phospho-D-ribosyl)glycinamide | C8H13N2O9P | -2 |
| fgd | Flavin guanosine dinucleotide oxidized | C27H31N9O16P2 | -2 |
| fmettrna | N-Formylmethionyl-tRNA | C6H9NO2SR | 0 |
| fmn | FMN | C17H19N4O9P | -2 |
| fol | Folate | C19H17N7O6 | -2 |
| for | Formate | CH1O2 | -1 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| formh4spt | formyltetrahydrosarcinapterin | C36H49N7O20P1 | -3 |
| formmfr(b) | formylmethanofuran b | C35H43N6O15 | -3 |
| fpram | 2-(Formamido)-N1-(5-phospho-D-ribosyl)acetamidine | C8H15N3O8P | -1 |
| fprica | 5-Formamido-1-(5-phospho-D-ribosyl)imidazole-4-carboxamide | C10H13N4O9P | -2 |
| frdp | Farnesyl diphosphate | C15H25O7P2 | -3 |
| fru | D-Fructose | C6H12O6 | 0 |
| fum | Fumarate | C4H2O4 | -2 |
| g1p | D-Glucose 1-phosphate | C6H11O9P | -2 |
| g3p | Glyceraldehyde 3-phosphate | C3H5O6P | -2 |
| g6p | D-Glucose 6-phosphate | C6H11O9P | -2 |
| gal1p | alpha-D-Galactose 1-phosphate | C6H11O9P | -2 |
| galactan | Galactan (poly-glycogen) | C6H10O5 | 0 |
| gald | Glyceraldehyde | C3H6O3 | 0 |
| gam1p | D-Glucosamine 1-phosphate | C6H13NO8P | -1 |
| gam6p | D-Glucosamine 6-phosphate | C6H13NO8P | -1 |
| gar | N1-(5-Phospho-D-ribosyl)glycinamide | C7H14N2O8P | -1 |
| gcald | Glycolaldehyde | C2H4O2 | 0 |
| gdp | GDP | C10H12N5O11P2 | -3 |
| gdpddman | GDP-4-dehydro-6-deoxy-D-mannose | C16H21N5O15P2 | -2 |
| gdpfuc | GDP-L-fucose | C16H23N5O15P2 | -2 |
| gdpgpi | glucosaminyl archaetidyl-myo-inositol | C55H110N1O15P1 | 0 |
| gdpmann | GDP-D-mannose | C16H23N5O16P2 | -2 |
| gdpofuc | GDP-4-oxo-L-fucose | C16H21N5O15P2 | -2 |
| ggdp | Geranylgeranyl diphosphate | C20H33O7P2 | -3 |
| gggp | (S)-3-O-geranylgeranylglyceryl phosphate | C23H39O6P1 | -2 |
| glc-D | D-Glucose | C6H12O6 | 0 |
| glcn | D-Gluconate | C6H11O7 | -1 |
| glnam | N-Glycoloyl-neuraminate; | C11H18NO10 | -1 |
| glnamcmp | CMP-N-glycoloylneuraminate; | C20H29N4O17P | -2 |
| gln-L | L-Glutamine | C5H10N2O3 | 0 |
| glntrna | L-Glutaminyl-tRNA | C5H9N2O2R | 1 |
| glu1sa | L-Glutamate 1-semialdehyde | C5H10NO3 | 1 |
| glu5p | L-Glutamate 5-phosphate | C5H8NO7P | -2 |
| glu5sa | L-Glutamate 5-semialdehyde | C5H9NO3 | 0 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| glu-L | L-Glutamate | C5H8NO4 | -1 |
| glutrna | L-Glutamyl-tRNA(Glu) | C5H7NO3R | 0 |
| glutrna(gln) | L-glutaminyl-tRNA(gln) | C5H7NO3R | 0 |
| glx | Glyoxylate | C2H1O3 | -1 |
| gly | Glycine | C2H5NO2 | 0 |
| glyald | D-Glyceraldehyde | C3H6O3 | 0 |
| glyb | Glycine betaine | C5H11NO2 | 0 |
| glyc | Glycerol | C3H8O3 | 0 |
| glyc1p | Glycerol 1-phosphate | C3H7O6P | -2 |
| glyclt | Glycolate | C2H3O3 | -1 |
| glycogen | glycogen | C6H10O5 | 0 |
| glyc-R | (R)-Glycerate | C3H5O4 | -1 |
| glytrna | Glycyl-tRNA(Gly) | C2H4NOR | 1 |
| gmp | GMP | C10H12N5O8P | -2 |
| grdp | Geranyl diphosphate | C10H17O7P2 | -3 |
| gsn | Guanosine | C10H13N5O5 | 0 |
| gthox | Oxidized glutathione | C20H30N6O12S2 | -2 |
| gthrd | Reduced glutathione | C10H16N3O6S | -1 |
| gtp | GTP | C10H12N5O14P3 | -4 |
| gua | Guanine | C5H5N5O | 0 |
| h | H+ | H | 1 |
| h2 | H2 | H2 | 0 |
| h2acon-C | cis-(homo)2aconitate | C8H7O6 | -3 |
| h2mpt | 7,8-dihydromethanopterin | C30H41N6O16P | -2 |
| h2o | H2O | H2O | 0 |
| h2o2 | Hydrogen peroxide | H2O2 | 0 |
| h2s | Hydrogen sulfide | H2S | 0 |
| h3acon-C | cis-(homo)3aconitate | C9H9O6 | -3 |
| h4mpt | 5,6,7,8-tetrahydromethanopterin | C30H43N6O16P | -2 |
| h4spt | tetrahydrosarcinapterin | C35H49N7O19P1 | -3 |
| hacon-C | cis-homoaconitate | C7H5O6 | -3 |
| hacon-T | trans-homoaconitate | C7H5O6 | -3 |
| hatrz | Hydroxyatrazine | C8H15N5O | 0 |
| hcarn | Homocarnosine | C10H17N4O3 | 1 |
| hcit | 2-Hydroxybutane-1,2,4-tricarboxylate | C7H7O7 | -3 |
| hco3 | Bicarbonate | CHO3 | -1 |
| hcys-L | L-Homocysteine | C4H9NO2S | 0 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| he2thpp | 2-(alpha-Hydroxyethyl)thiamine diphosphate; | C14H20N4O8P2S | -2 |
| hepdp | all-trans-Heptaprenyl diphosphate | C35H57O7P2 | -3 |
| hgbam | Hydrogenobyrinate a,c diamide | C45H59N6O12 | -3 |
| his-L | L-Histidine | C6H9N3O2 | 0 |
| hisp | L-Histidinol phosphate | C6H11N3O4P | -1 |
| hista | Histamine | C5H10N3 | 1 |
| histd | L-Histidinol | C6H12N3O | 1 |
| histrna | L-Histidyl-tRNA(His) | C6H8N3OR | 1 |
| hmbil | Hydroxymethylbilane | C40H38N4O17 | -8 |
| hmgcoa | Hydroxymethylglutaryl-CoA | C27H39N7O20P3S | -5 |
| hom-L | L-Homoserine | C4H9NO3 | 0 |
| hpglu | Tetrahydropteroyltri-L-glutamate | C24H34N8O12 | 0 |
| hphaccoa | 4-Hydroxyphenylacetyl-CoA | C29H38N7O18P3S | -4 |
| hpyr | Hydroxypyruvate | C3H3O4 | -1 |
| hsfd | heterodisulfide | C13H22N1O10P1S3 | -4 |
| hspmd | sym-Homospermidine | C8H24N3 | 3 |
| hxan | Hypoxanthine | C5H4N4O | 0 |
| iasp | Iminoaspartate | C4H4NO4 | -1 |
| ibcoa | Isobutyryl-CoA | C25H38N7O17P3S | -4 |
| icit | Isocitrate | C6H5O7 | -3 |
| id3acald | Indole-3-acetaldehyde | C10H9NO | 0 |
| idp | IDP | C10H11N4O11P2 | -3 |
| ihcit-T | threo-isohomocitrate | C7H7O7 | -3 |
| ile-L | L-Isoleucine | C6H13NO2 | 0 |
| iletrna | L-Isoleucyl-tRNA(Ile) | C6H12NOR | 1 |
| imacp | 3-(Imidazol-4-yl)-2-oxopropyl phosphate | C6H7N2O5P | -2 |
| imp | IMP | C10H11N4O8P | -2 |
| ind3ac | Indole-3-acetate | C10H8NO2 | -1 |
| indaccoa | S-2-(indol-3-yl)acetyl-CoA | C31H39N8O17P3S | -4 |
| indole | Indole | C8H7N | 0 |
| indpyr | Indolepyruvate | C11H8NO3 | -1 |
| inost | myo-Inositol | C6H12O6 | 0 |
| ins | Inosine | C10H12N4O5 | 0 |
| ipdp | Isopentenyl diphosphate | C5H9O7P2 | -3 |
| ipp | isopentyl phosphate | C5H9O4P | -2 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| itp | ITP | C10H11N4O14P3 | -4 |
| k | potassium | K | 1 |
| lac-D | D-Lactate | C3H5O3 | -1 |
| lac-L | L-Lactate | C3H5O3 | -1 |
| lald-L | L-Lactaldehyde | C3H6O2 | 0 |
| Lcyst | L-cysteate | C3H6NO5S | -1 |
| leu-L | L-Leucine | C6H13NO2 | 0 |
| leutrna | L-Leucyl-tRNA(Leu) | C6H12NOR | 1 |
| lgt-S | (R)-S-Lactoylglutathione | C13H20N3O8S | -1 |
| lipid_met | Biomass – lipid component | | |
| lppg | lactyl-(2)-diphospho-(5')-guanosine | C13H16N5O13P2 | -3 |
| lpro | Lipoylprotein | C8H14NOS2R | 0 |
| lys-L | L-Lysine | C6H15N2O2 | 1 |
| lystrna | L-Lysine-tRNA (Lys) | C6H14N2OR | 2 |
| m3hdp | methyl-3-hydroxyl diphosphate | C5H11O8P2 | -3 |
| m5hbc | Co-Methyl-Co-5-hydroxybenzimidazolylcobamide | C61H87CoN13O15P | 0 |
| mal-L | L-Malate | C4H4O5 | -2 |
| man1p | D-Mannose 1-phosphate | C6H11O9P | -2 |
| man6p | D-Mannose 6-phosphate | C6H11O9P | -2 |
| mcom | methylcoenzyme m | C3O3S2H7 | -1 |
| menylh4spt | methenyl-tetrahydrosarcinapterin | C36H48N7O19P1 | -2 |
| meoh | Methanol | CH4O1 | 0 |
| mercppyr | Mercaptopyruvate | C3H3O3S | -1 |
| methf | 5,10-Methenyltetrahydrofolate | C20H20N7O6 | -1 |
| met-L | L-Methionine | C5H11NO2S | 0 |
| mettrna | L-Methionyl-tRNA (Met) | C5H10NOSR | 1 |
| mev-R | (R)-Mevalonate | C6H11O4 | -1 |
| mfr(b) | Methanofuran b | C34H44N6O14 | -2 |
| mfrbi1 | methanofuran intermediate 1 | C6H9O13P2 | -5 |
| mfrbi2 | methanofuran intermediate 2 | C6H8O9P | -3 |
| mfrbi3 | methanofuran intermediate 3 | C6H6O8P | -3 |
| mfrbi4 | phosphate ester of dihydrofuran : methanofuran biosynthesis intermediate 4 | C6H6O8P | -3 |
| mg2 | magnesium | Mg | 2 |
| mh4spt | N5-methyl-tetrahydrosarcinapterin | C36H51N7O19P1 | -3 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| mhpglu | 5-Methyltetrahydropteroyltri-L-glutamate | C25H36N8O12 | 0 |
| mi3p-D | 1D-myo-Inositol 3-phosphate | C6H11O9P | -2 |
| mleneh4spt | N5,N10-methylene-5,6,7,8-tetrahydromethanopterin | C36H49N7O19P1 | -3 |
| mlthf | 5,10-Methylenetetrahydrofolate | C20H22N7O6 | -1 |
| mma | Methylamine | C1H6N | 1 |
| mmal | Methylmalonate | C4H4O4 | -2 |
| mmh2mpt | monomethylated 7,8-dihydromethanoterin | C29H39N6O16P | -2 |
| mn2 | Mn2+ | Mn | 2 |
| mobd | Molybdate | MoO4 | -2 |
| mphen | methanophenazine (oxidized) | C37N2O1H50 | 0 |
| mphenh2 | methanophenazine (reduced) | C37N2O1H52 | 0 |
| msa | Malonate semialdehyde | C3H3O3 | -1 |
| mthgxl | Methylglyoxal | C3H4O2 | 0 |
| n2 | nitrogen | N2 | 0 |
| na1 | Sodium | Na | 1 |
| nabl | N-epsilon-acetyl-beta-lysine | C8H16N2O3 | 0 |
| nac | Nicotinate | C6H4NO2 | -1 |
| nad | Nicotinamide adenine dinucleotide | C21H26N7O14P2 | -1 |
| nadh | Nicotinamide adenine dinucleotide - reduced | C21H27N7O14P2 | -2 |
| nadp | Nicotinamide adenine dinucleotide phosphate | C21H25N7O17P3 | -3 |
| nadph | Nicotinamide adenine dinucleotide phosphate - reduced | C21H26N7O17P3 | -4 |
| nh4 | Ammonium | H4N | 1 |
| ni2 | nickel | Ni | 2 |
| nicrns | Nicotinate D-ribonucleoside | C11H13NO6 | 0 |
| nicrnt | Nicotinate D-ribonucleotide | C11H12NO9P | -2 |
| nmn | NMN | C11H14N2O8P | -1 |
| no2 | Nitrite | NO2 | -1 |
| o2 | O2 | O2 | 0 |
| o2- | Oxygen radical | O2 | -1 |
| oaa | Oxaloacetate | C4H2O5 | -2 |
| octdp | all-trans-Octaprenyl diphosphate | C40H65O7P2 | -3 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| ohepa | 2-oxoheptanedioic acid | C7H8O5 | -2 |
| ohexa | 2-oxohexanedioic acid | C6H6O5 | -2 |
| orn | Ornithine | C5H13N2O2 | 1 |
| orot | Orotate | C5H3N2O4 | -1 |
| orot5p | Orotidine 5'-phosphate | C10H10N2O11P | -3 |
| osuc | Oxalosuccinate | C6H3O7 | -3 |
| oxa | Oxalate | C2O4 | -2 |
| pac | Phenylacetic acid | C8H7O2 | -1 |
| pan4p | Pantetheine 4'-phosphate | C11H21N2O7PS | -2 |
| pant-R | (R)-Pantoate | C6H11O4 | -1 |
| pap | Adenosine 3',5'-bisphosphate | C10H11N5O10P2 | -4 |
| paps | 3'-Phosphoadenylyl sulfate | C10H11N5O13P2S | -4 |
| pep | Phosphoenolpyruvate | C3H2O6P | -3 |
| phaccoa | Phenylacetyl-CoA | C29H38N7O17P3S | -4 |
| phe-L | L-Phenylalanine | C9H11NO2 | 0 |
| pheme | protoheme | C34H30FeN4O4 | -2 |
| phetrna | L-Phenylalanyl-tRNA(Phe) | C9H10NOR | 1 |
| phom | O-Phospho-L-homoserine | C4H8NO6P | -2 |
| phpyr | Phylloquinone | C9H7O3 | -1 |
| phydp | Phytyl diphosphate | C20H39O7P2 | -3 |
| pi | Phosphate | HO4P | -2 |
| pmcoa | Pimeloyl-CoA | C28H41N7O19P3S | -5 |
| pnto-R | (R)-Pantothenate | C9H16NO5 | -1 |
| polyacgal | Poly-N-acetylgalactosamine | C8H13NO5 | 0 |
| polyglcur | Poly-D-glucuronate | C6H7O6 | -1 |
| ppa | Propionate (n-C3:0) | C3H5O2 | -1 |
| ppant-R | 4-phosphopantoate | C6H10O7P | -3 |
| ppap | Propanoyl phosphate | C3H5O5P | -2 |
| ppbng | Porphobilinogen | C10H13N2O4 | -1 |
| ppcoa | Propanoyl-CoA | C24H36N7O17P3S | -4 |
| pphn | Prephenate | C10H8O6 | -2 |
| ppi | Diphosphate | HO7P2 | -3 |
| pppi | Inorganic triphosphate | HO10P3 | -4 |
| pram | 5-Phospho-beta-D-ribosylamine | C5H11NO7P | -1 |
| pran | N-(5-Phospho-D-ribosyl)anthranilate | C12H13NO9P | -3 |
| prbamp | 1-(5-Phosphoribosyl)-AMP | C15H19N5O14P2 | -4 |
| prbatp | 1-(5-Phosphoribosyl)-ATP | C15H19N5O20P4 | -6 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| prfp | 1-(5-Phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide | C15H21N5O15P2 | -4 |
| prlp | 5-[(5-phospho-1-deoxyribulos-1-ylamino)methylideneamino]-1-(5-phosphoribosyl)imidazole-4-carboxamide | C15H21N5O15P2 | -4 |
| pro-L | L-Proline | C5H9NO2 | 0 |
| protein_met | Biomass – protein component | | |
| protrna | L-Prolyl-tRNA(Pro) | C5H8NOR | 1 |
| prpp | 5-Phospho-alpha-D-ribose 1-diphosphate | C5H8O14P3 | -5 |
| psd5p | Pseudouridine 5'-phosphate | C9H11N2O9P | -2 |
| pser-L | O-Phospho-L-serine | C3H6NO6P | -2 |
| ptrc | Putrescine | C4H14N2 | 2 |
| pydx5p | Pyridoxal 5'-phosphate | C8H8NO6P | -2 |
| pyr | Pyruvate | C3H3O3 | -1 |
| pyr-L | L-pyrrolysine | C12H21N3O3 | 0 |
| pyrtrna | trna(pyr-L) | C12H20N3O2R | 1 |
| quln | Quinolinate | C7H3NO4 | -2 |
| r15bp | D-Ribose 1,5-bisphosphate | C5H8O11P2 | -4 |
| r2mmal | D-Citramalate | C5H6O5 | -2 |
| r5hbzi | N1-(alpha-D-ribosyl)-5-hydroxybenzimidazole | C12H14N2O5 | 0 |
| r5p | alpha-D-Ribose 5-phosphate | C5H9O8P | -2 |
| rb15bp | D-Ribulose 1,5-bisphosphate | C5H8O11P2 | -4 |
| Rh2cit | (R)-(homo)2citrate | C8H9O7 | -3 |
| Rh3cit | (R)-(homo)3citrate | C9H11O7 | -3 |
| rib-D | D-Ribose | C5H10O5 | 0 |
| ribflv | Riboflavin | C17H20N4O6 | 0 |
| rna_met | Biomass – RNA component | | |
| rnam | N-Ribosylnicotinamide | C11H15N2O5 | 1 |
| ru5p-D | D-Ribulose 5-phosphate | C5H9O8P | -2 |
| S2hglut | (S)-2-Hydroxyglutarate | C5H6O5 | -2 |
| scl | sirohydrochlorin | C42H40N4O16 | -6 |
| sec | sulfoethylcysteine | C5H10NO5S2 | -1 |
| ser-L | L-Serine | C3H7NO3 | 0 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| sertrna | L-Seryl-tRNA(Ser) | C3H6NO2R | 1 |
| sf430a | 15,17-seco-F430-17-acid | C42H47N6NiO14 | -5 |
| Shcit | S-homocitrate | C7H7O7 | -3 |
| sheme | Siroheme | C42H36FeN4O16 | -8 |
| skm | Shikimate | C7H9O5 | -1 |
| skm5p | Shikimate 5-phosphate | C7H8O8P | -3 |
| sl26da | N-Succinyl-LL-2,6-diaminoheptanedioate | C11H16N2O7 | -2 |
| sl2a6o | N-Succinyl-2-L-amino-6-oxoheptanedioate | C11H12NO8 | -3 |
| so3 | Sulfite | O3S | -2 |
| so4 | Sulfate | O4S | -2 |
| succ | Succinate | C4H4O4 | -2 |
| succoa | Succinyl-CoA | C25H35N7O19P3S | -5 |
| suchms | O-Succinyl-L-homoserine | C8H12NO6 | -1 |
| sucsal | Succinic semialdehyde | C4H5O3 | -1 |
| thdp | 2,3,4,5-Tetrahydrodipicolinate | C7H8NO4 | -1 |
| thf | 5,6,7,8-Tetrahydrofolate | C19H22N7O6 | -1 |
| thm | Thiamin | C12H17N4OS | 1 |
| thmmp | Thiamin monophosphate | C12H16N4O4PS | -1 |
| thmpp | Thiamine diphosphate | C12H16N4O7P2S | -2 |
| thr-L | L-Threonine | C4H9NO3 | 0 |
| thrp | L-Threonine O-3-phosphate | C4H8NO6P | -2 |
| thrtrna | L-Threonyl-tRNA(Thr) | C4H8NO2R | 1 |
| thym | Thymine | C5H6N2O2 | 0 |
| thymd | Thymidine | C10H14N2O5 | 0 |
| tih2cit | (-)threo-iso(homo)2citrate | C8H9O7 | -3 |
| tih3cit | (-)threo-iso(homo)3citrate | C9H11O7 | -3 |
| tma | trimethylamine | C3H10N | 1 |
| trace_met | Biomass – trace component | | |
| trdox | Oxidized thioredoxin | X | 0 |
| trdrd | Reduced thioredoxin | XH2 | 0 |
| trnaala | tRNA(Ala) | R | 0 |
| trnaarg | tRNA(Arg) | R | 0 |
| trnaasn | tRNA(Asn) | R | 0 |
| trnaasp | tRNA(Asp) | R | 0 |
| trnacys | tRNA(Cys) | R | 0 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| trnagln | tRNA(Gln) | R | 0 |
| trnaglu | tRNA (Glu) | R | 0 |
| trnagly | tRNA(Gly) | R | 0 |
| trnahis | tRNA(His) | R | 0 |
| trnaile | tRNA(Ile) | R | 0 |
| trnaleu | tRNA(Leu) | R | 0 |
| trnalys | tRNA(Lys) | R | 0 |
| trnamet | tRNA(Met) | R | 0 |
| trnaphe | tRNA(Phe) | R | 0 |
| trnapro | tRNA(Pro) | R | 0 |
| trnaser | tRNA(Ser) | R | 0 |
| trnathr | tRNA(Thr) | R | 0 |
| trnatrp | tRNA(Trp) | R | 0 |
| trnatyr | tRNA(Tyr) | R | 0 |
| trnaval | tRNA(Val) | R | 0 |
| trp-L | L-Tryptophan | C11H12N2O2 | 0 |
| trptrna | L-Tryptophanyl-tRNA(Trp) | C11H11N2OR | 1 |
| tsul | Thiosulfate | O3S2 | -2 |
| tym | Tyramine | C8H12NO | 1 |
| tyr-L | L-Tyrosine | C9H11NO3 | 0 |
| tyrtrna | L-Tyrosyl-tRNA(Tyr) | C9H10NO2R | 1 |
| uaccg | UDP-N-acetyl-3-O-(1-carboxyvinyl)-D-glucosamine | C20H26N3O19P2 | -3 |
| uacgam | UDP-N-acetyl-D-glucosamine | C17H25N3O17P2 | -2 |
| uacmam | UDP-N-acetyl-D-mannosamine | C17H25N3O17P2 | -2 |
| uamr | UDP-N-acetylmuramate | C20H28N3O19P2 | -3 |
| udp | UDP | C9H11N2O12P2 | -3 |
| udpacgal | UDP-N-acetyl-D-galactosamine | C17H25N3O17P2 | -2 |
| udpg | UDPglucose | C15H22N2O17P2 | -2 |
| udpgal | UDPgalactose | C15H22N2O17P2 | -2 |
| udpglcur | UDP-D-glucuronate | C15H19N2O18P2 | -3 |
| ump | UMP | C9H11N2O9P | -2 |
| unknown_cbl1deg | unknown degradation product of cbl1 for adocbl-HBI synthesis | C14H16N2O6P1 | -1 |
| unknown_rbfdeg | unknown riboflavin degradation product | C10H14N2O5 | 0 |
| uppg3 | Uroporphyrinogen III | C40H36N4O16 | -8 |

Table B.1. (continued)

| Model ID | Met name | Charged Formula | Charge |
|---|---|---|---|
| ura | Uracil | C4H4N2O2 | 0 |
| urea | Urea | CH4N2O | 0 |
| uri | Uridine | C9H12N2O6 | 0 |
| utp | UTP | C9H11N2O15P3 | -4 |
| val-L | L-Valine | C5H11NO2 | 0 |
| valtrna | L-Valyl-tRNA(Val) | C5H10NOR | 1 |
| wo4 | Tungstenate | WO4 | -2 |
| xan | Xanthine | C5H4N4O2 | 0 |
| xmp | Xanthosine 5'-phosphate | C10H11N4O9P | -2 |
| xtp | XTP | C10H11N4O15P3 | -4 |
| xtsn | Xanthosine | C10H12N4O6 | 0 |
| xu5p-D | D-Xylulose 5-phosphate | C5H9O8P | -2 |
| zn2 | Zinc | Zn | 2 |

Table B.2. List of reactions and gene associations (GPR) in the *M. acetivorans* metabolic model.

| Reaction ID | Reaction | GPR |
|---|---|---|
| 2H3MOAOX | 23dhmb[c] + nadp[c] <=> h[c] + nadph[c] + 2h3moa[c] | MA3790 |
| 2MOPRED | 2mop[c] + h2o[c] + nad[c] -> (2) h[c] + mmal[c] + nadh[c] | MA2860 |
| 2OBUTHE2THPPTRX | 2obut[c] + he2thpp[c] -> 2ahbut[c] + thmpp[c] | (MA1354 and MA1958) |
| 2PLS | gtp[c] + lac-L[c] -> 2plac-L[c] + gdp[c] + h[c] | |
| 4ABZt2r | 4abz[e] + h[e] <==> 4abz[c] + h[c] | |
| 4ASD | 4as[c] --> h2o[c] + 4das[c] | |
| 4DHSD | 4adhs[c] + (2) h[c] + nadh[c] --> nad[c] + 4as[c] | |
| 4DHSS | 4adhq[c] --> 4adhs[c] + h2o[c] + h[c] | |
| 5HBCOX | (2) h[c] + (2) 5hbc_red[c] -> (2) 5hbc[c] + h2[c] | |
| 5HBCR | atp[c] + fdred[c] + h2o[c] + 5hbc[c] -> adp[c] + fdox[c] + h[c] + pi[c] + 5hbc_red[c] | (MA0150 or MA0849 or MA4360) |
| 5HBZIDS | ribflv[c] -> unknown_rbfdeg[c] + 5hbzid[c] | |
| 7MHS | 7ohp[c] + cys-L[c] + h[c] + nadh[c] -> 7mhp[c] + nad[c] + ser-L[c] | |
| 7MHTS | 7mhp[c] + atp[c] + thr-L[c] -> 7mht[c] + adp[c] + h[c] + pi[c] | |
| ABTA | 4abut[c] + akg[c] <=> glu-L[c] + sucsal[c] | MA2859 |
| ACACT1 | (2) accoa[c] <=> aacoa[c] + coa[c] | MA4042 |
| ACBIPGT | adocbip[c] + gtp[c] + h[c] -> agdpcbi[c] + ppi[c] | MA0938 |
| ACCOAL2r | atp[c] + coa[c] + ppa[c] <=> adp[c] + pi[c] + ppcoa[c] | (MA3168 and MA3602) |
| ACGK | acglu[c] + atp[c] -> acg5p[c] + adp[c] | MA4515 |
| ACGS | accoa[c] + glu-L[c] -> acglu[c] + coa[c] + h[c] | (MA3564 or MA4339) |
| ACKr | ac[c] + atp[c] <=> actp[c] + adp[c] | MA3606 |
| ACLDC | alac-S[c] + h[c] -> actn-R[c] + co2[c] | MA3744 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| ACLS | h[c] + (2) pyr[c] -> alac-S[c] + co2[c] | ( MA1354 or MA1958 or MA3792 ) and ( MA3791 or MA3854 ) |
| ACNPLYS | acnam[c] + pi[c] <=> h2o[c] + pep[c] + acmana[c] | MA3767 |
| ACONMT | amet[c] + acon-T[c] -> ahcys[c] + aconm[c] | MA1801 |
| ACONTa | cit[c] <=> acon-C[c] + h2o[c] | MA0250 |
| ACONTb | icit[c] <=> acon-C[c] + h2o[c] | MA0250 |
| ACOTA | acg5sa[c] + glu-L[c] -> acorn[c] + akg[c] | MA0119 |
| ACP1(FMN) | fmn[c] + h2o[c] -> pi[c] + ribflv[c] | MA2649 |
| ACSERHS | acser[c] + trdrd[c] + tsul[c] -> ac[c] + cys-L[c] + h[c] + so3[c] + trdox[c] | (MA2715 or MA2720) |
| ACt2r | ac[e] + h[e] <==> ac[c] + h[c] | MA4008 |
| ACTNt2r | actn-R[e] + h[e] <==> actn-R[c] + h[c] | |
| ACYP | 13dpg[c] + h2o[c] -> 3pg[c] + h[c] + pi[c] | MA3367 |
| ACYP_2 | actp[c] + h2o[c] -> ac[c] + h[c] + pi[c] | MA3367 |
| ADCBHBIR | adocblhbi[c] + h2o[c] -> adn[c] + h[c] + 5hbc_red[c] | |
| ADCL2 | 4das[c] --> h2o[c] + 4abz[c] + h[c] | |
| ADCPS1 | adcobhex[c] + appl[c] + atp[c] -> adocbi[c] + adp[c] + h[c] + pi[c] | MA0941 |
| ADCPS2 | adcobhex[c] + applp[c] + atp[c] -> adocbip[c] + adp[c] + h[c] + pi[c] | MA0941 |
| ADCYRS | adcobdam[c] + (4) atp[c] + (4) gln-L[c] + (4) h2o[c] -> adcobhex[c] + (4) adp[c] + (4) glu-L[c] + (4) h[c] + (4) pi[c] | MA3250 |
| ADD | ade[c] + h[c] + h2o[c] -> hxan[c] + nh4[c] | MA2310 |
| ADK1 | amp[c] + atp[c] <=> (2) adp[c] | MA1096 |
| ADK2 | amp[c] + pppi[c] <=> adp[c] + ppi[c] | MA1096 |
| ADK3 | amp[c] + gtp[c] <=> adp[c] + gdp[c] | MA1096 |
| ADK4 | amp[c] + itp[c] <=> adp[c] + idp[c] | MA1096 |
| ADKd | damp[c] + datp[c] <==> (2) dadp[c] | MA1096 |
| ADNCYC | atp[c] -> camp[c] + ppi[c] | MA4044 |
| ADNK1 | adn[c] + atp[c] -> adp[c] + amp[c] + h[c] | MA1373 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| ADOCBIAH | adocbi[c] + h2o[c] -> adcobhex[c] + appl[c] | MA1623 |
| ADOCBLS2 | agdpcbi[c] + r5hbzi[c] -> adocblhbi[c] + gmp[c] + h[c] | MA0939 |
| ADPRDP | adprib[c] + h2o[c] --> amp[c] + (2) h[c] + r5p[c] | MA0113 |
| ADPT | amp[c] + ppi[c] <=> ade[c] + prpp[c] | MA0717 |
| ADSK | atp[c] + aps[c] -> adp[c] + h[c] + paps[c] | MA4246 |
| ADSL1 | dcamp[c] -> amp[c] + fum[c] | MA3971 |
| ADSL2 | 25aics[c] <=> aicar[c] + fum[c] | MA3971 |
| ADSS | asp-L[c] + gtp[c] + imp[c] -> dcamp[c] + gdp[c] + (2) h[c] + pi[c] | (MA1919 or MA4118) |
| AGAIAGT | dpgpi[c] + uacgam[c] -> agdpgpi[c] + h[c] + udp[c] | MA0798 |
| AGAID | agdpgpi[c] + h2o[c] -> ac[c] + gdpgpi[c] | MA0990 |
| AGMT | agm[c] + h2o[c] -> ptrc[c] + urea[c] | MA3986 |
| AGPR | acg5p[c] + h[c] + nadph[c] -> acg5sa[c] + nadp[c] + pi[c] | MA3566 |
| AH6PI | ah6p-D[c] <=> f6p[c] | MA1384 |
| AHC | ahcys[c] + h2o[c] -> adn[c] + hcys-L[c] | MA1275 |
| AHGDx | S2hglut[c] + nad[c] <=> akg[c] + h[c] + nadh[c] | |
| AHMMPS | air[c] + nadh[c] + (2) h[c] -> 4ahmmp[c] + gcald[c] + pi[c] + nad[c] | (MA0261 or MA1790 or MA4329) |
| AHSERL2 | achms[c] + h2s[c] -> ac[c] + h[c] + hcys-L[c] | MA2715 |
| AICART | 10fthf[c] + aicar[c] <=> fprica[c] + thf[c] | MA4012 |
| AIRCr | air[c] + co2[c] <==> 5aizc[c] + h[c] | MA1376 |
| AIRC2 | air[c] + atp[c] + hco3[c] -> adp[c] + h[c] + pi[c] + 5caiz[c] | (MA0428 or MA1376 or MA4063) |
| AIRC3 | 5aizc[c] <=> 5caiz[c] | (MA0428 or MA1376 or MA4063) |
| AKACAL | accoa[c] + h2o[c] + ohexa[c] -> Rh2cit[c] + coa[c] + h[c] | MA3342 |
| AKGCAL | accoa[c] + akg[c] -> coa[c] + h[c] + hacon-T[c] | MA3342 |
| AKP1 | ahdt[c] + (3) h2o[c] -> dhnpt[c] + (2) h[c] + (3) pi[c] | MA4354 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| AKPCAL | accoa[c] + h2o[c] + ohepa[c] -> Rh3cit[c] + coa[c] + h[c] | MA3342 |
| AKSCAL | 2ood[c] + accoa[c] + h2o[c] -> glyc-R[c] + pmcoa[c] | |
| AKSDC | 2ood[c] + h[c] -> 7ohp[c] + co2[c] | |
| ALACCBX | he2thpp[c] + pyr[c] -> alac-S[c] + thmpp[c] | (MA1354 and MA1958) |
| ALACt2r | alac-S[e] + h[e] <==> alac-S[c] + h[c] | |
| ALAt4r | ala-L[e] + na1[e] <==> ala-L[c] + na1[c] | MA2837 |
| ALATA_L | akg[c] + ala-L[c] <=> glu-L[c] + pyr[c] | MA0636 |
| ALATRS | ala-L[c] + atp[c] + trnaala[c] -> amp[c] + ppi[c] + alatrna[c] | MA0194 |
| ALCD19x | glyald[c] + h[c] + nadph[c] -> glyc[c] + nadp[c] | MA2630 |
| ALCD19y | glyald[c] + h[c] + nadph[c] -> glyc[c] + nadp[c] | (MA0403 or MA1901) |
| ALCD20x | nad[c] + 2ppoh[c] <=> h[c] + nadh[c] + acetone[c] | MA2630 |
| ALCD20y | nadp[c] + 2ppoh[c] <=> h[c] + nadph[c] + acetone[c] | (MA0403 or MA1901) |
| ALCD22_L_f420_ | lald-L[c] + f420-2[c] + h[c] <=> mthgxl[c] + f420-2h2[c] | |
| ALCD2x | etoh[c] + nad[c] <=> acald[c] + h[c] + nadh[c] | MA2630 |
| ALCD2y | etoh[c] + nadp[c] <=> acald[c] + h[c] + nadph[c] | (MA0403 or MA1901) |
| ALDD1 | fald[c] + h2o[c] + nad[c] -> for[c] + (2) h[c] + nadh[c] | MA0417 |
| ALDD20x | h2o[c] + id3acald[c] + nad[c] -> (2) h[c] + ind3ac[c] + nadh[c] | MA2860 |
| ALDD2x | acald[c] + h2o[c] + nad[c] <=> ac[c] + (2) h[c] + nadh[c] | MA2860 |
| ALDD31 | aacald[c] + h2o[c] + nad[c] --> nadh[c] + gly[c] + (2) h[c] | MA2860 |
| ALKP | dhap[c] + h2o[c] -> dha[c] + pi[c] | MA4354 |
| ALR2_f420_ | mthgxl[c] + f420-2h2[c] -> f420-2[c] + acetol[c] + h[c] | (MA0422 or MA2291 or MA2292 or MA2756) |
| AMAOTr | amet[c] + 8aonn[c] <=> amob[c] + dann[c] | |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| AMPMS2 | air[c] + h2o[c] + nad[c] -> 4ampm[c] + (2) for[c] + (3) h[c] + nadh[c] | MA1790 |
| AMPTASECG | cgly[c] + h2o[c] -> cys-L[c] + gly[c] | (MA1605 or MA2653) |
| ANPRT | ppi[c] + pran[c] <=> anth[c] + prpp[c] | MA2989 |
| ANS | chor[c] + gln-L[c] -> anth[c] + glu-L[c] + h[c] + pyr[c] | (MA2986 and MA2987) |
| ANS2 | chor[c] + nh4[c] -> anth[c] + h[c] + h2o[c] + pyr[c] | (MA2986 and MA2987) |
| AOXSr | ala-L[c] + h[c] + pmcoa[c] <=> co2[c] + coa[c] + 8aonn[c] | |
| APAT2 | akg[c] + ala-B[c] <=> glu-L[c] + msa[c] | MA2859 |
| APPLP | appl[c] + atp[c] -> adp[c] + applp[c] + h[c] | MA0940 |
| ARGDC | arg-L[c] + h[c] -> agm[c] + co2[c] | (MA3012 or MA3496) |
| ARGDr | arg-L[c] + h2o[c] <=> citr-L[c] + nh4[c] | MA0792 |
| ARGSL | argsuc[c] <=> arg-L[c] + fum[c] | MA1318 |
| ARGSS | asp-L[c] + atp[c] + citr-L[c] -> amp[c] + argsuc[c] + h[c] + ppi[c] | MA2142 |
| ARGTRS | arg-L[c] + atp[c] + trnaarg[c] -> amp[c] + ppi[c] + argtrna[c] | MA0043 |
| ASADi | 4pasp[c] + h[c] + nadph[c] -> aspsa[c] + nadp[c] + pi[c] | MA0430 |
| ASD | dpgps[c] + h[c] -> co2[c] + dpgpe[c] | MA0115 |
| ASNN | asn-L[c] + h2o[c] -> asp-L[c] + nh4[c] | MA1317 |
| ASNS1 | asp-L[c] + atp[c] + gln-L[c] + h2o[c] <=> amp[c] + asn-L[c] + glu-L[c] + h[c] + ppi[c] | (MA0051 or MA1966) |
| ASNTRS2-1 | trnaasn[c] + atp[c] + asp-L[c] --> amp[c] + ppi[c] + asntrna(asp)[c] | MA1684 |
| ASNTRS2-2 | asntrna(asp)[c] + gln-L[c] + atp[c] + h2o[c] --> adp[c] + pi[c] + h[c] + glu-L[c] + asntrna[c] | (MA4522 and MA4523 and MA4524) |
| ASP1DC | asp-L[c] + h[c] -> ala-B[c] + co2[c] | MA1949 |
| ASPCT | asp-L[c] + cbp[c] -> cbasp[c] + h[c] + pi[c] | (MA4501 and MA4502) |
| ASPKi | asp-L[c] + atp[c] -> 4pasp[c] + adp[c] | MA0131 |
| ASPO2x | asp-L[c] + nad[c] -> h[c] + iasp[c] + nadh[c] | MA0958 |
| ASPO2y | asp-L[c] + nadp[c] -> h[c] + iasp[c] + nadph[c] | MA0958 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| ASPTA | akg[c] + asp-L[c] <=> glu-L[c] + oaa[c] | (MA0636 or MA1385 or MA1819) |
| ASPTRS | asp-L[c] + atp[c] + trnaasp[c] -> amp[c] + ppi[c] + asptrna[c] | MA1684 |
| ATGH | dggpg[c] + (8) h[c] + (8) nadph[c] -> dpgpg[c] + (8) nadp[c] | (MA0691 or MA0692 or MA1484) |
| ATIH | dggpi[c] + (8) h[c] + (8) nadph[c] -> dpgpi[c] + (8) nadp[c] | (MA0691 or MA0692 or MA1484) |
| ATPFORTRX | aicar[c] + atp[c] + for[c] -> adp[c] + fprica[c] + pi[c] | (MA0206 or MA3689) |
| ATPHs | atp[c] + h[c] + h2o[c] -> itp[c] + nh4[c] | |
| ATPM | atp[c] + h2o[c] -> adp[c] + h[c] + pi[c] | MA3706 |
| ATPPRT | ppi[c] + prbatp[c] <=> atp[c] + prpp[c] | MA0217 |
| ATPS1 | atp[c] + h[c] + h2o[c] --> adp[c] + (2) h[e] + pi[c] | (MA1678 or MA2833) |
| ATPS4r | (4) h[e] + adp[c] + pi[c] <=> (3) h[c] + atp[c] + h2o[c] | (MA4152 and MA4153 and MA4154 and MA4155 and MA4156 and MA4157 and MA4158 and MA4159 and MA4160) |
| ATSH | dgggps[c] + (8) h[c] + (8) nadph[c] -> dpgps[c] + (8) nadp[c] | (MA0691 or MA0692 or MA1484) |
| BACCL | atp[c] + btn[c] + h[c] -> btamp[c] + ppi[c] | MA0676 |
| BAMPPALDOX | bamppald[c] + h2o[c] + nad[c] -> ala-B[c] + (2) h[c] + nadh[c] | MA2860 |
| BLAT | 36dahx[c] + accoa[c] -> coa[c] + h[c] + nabl[c] | MA3978 |
| BMt | biomass_met[c] --> biomass_met[e] | |
| BRFAPS | 4abz[c] + h[c] + prpp[c] -> Brfap[c] + co2[c] + ppi[c] | (MA0339 or MA4006) |
| BSPRR | (2) h2s[c] + (2) fe2[c] + (2) fdox[c] + btnp[c] -> (2) fdred[c] + (4) h[c] + btnp-s2[c] | |
| BTNabc | btn[e] + atp[c] + h2o[c] --> btn[c] + adp[c] + pi[c] + h[c] | ( MA4340 and ( MA4341 or MA4342 ) and MA4343 ) |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| BTNt2i | btn[e] + h[e] --> btn[c] + h[c] | MA4340 |
| BTS5 | dtbt[c] + btnp-s2[c] + (2) fdox[c] -> btn[c] + (2) fe2[c] + h2s[c] + btnp[c] + (2) fdred[c] | |
| CA2abc | atp[c] + ca2[e] + h2o[c] --> adp[c] + ca2[c] + h[c] + pi[c] | MA4082 |
| CAT | (2) h2o2[c] -> (2) h2o[c] + o2[c] | MA0972 |
| CAt6 | ca2[c] + na1[e] <==> ca2[e] + na1[c] | ( MA2008 or MA2817 or MA3021 ) |
| CBIabc | atp[c] + cbi[e] + h2o[c] --> adp[c] + cbi[c] + h[c] + pi[c] | (MA4604 and MA4605 and MA4606) |
| CBIAT | atp[c] + cbi[c] + h[c] -> adocbi[c] + pppi[c] | MA2084 |
| CBL1abc | atp[c] + cbl1[e] + h2o[c] --> adp[c] + cbl1[c] + h[c] + pi[c] | (MA4604 and MA4605 and MA4606) |
| CBL1HBIabc | atp[c] + cbl1hbi[e] + h2o[c] --> adp[c] + cbl1hbi[c] + h[c] + pi[c] | (MA4604 and MA4605 and MA4606) |
| CBLAT2 | atp[c] + cbl1hbi[c] + h[c] -> adocblhbi[c] + pppi[c] | MA2084 |
| CBLD | cbl1[c] -> cbi[c] + unknown_cbl1deg[c] | |
| CBPS | (2) atp[c] + gln-L[c] + h2o[c] + hco3[c] -> (2) adp[c] + cbp[c] + glu-L[c] + (2) h[c] + pi[c] | (MA2143 and MA2144) |
| CD2abc1 | atp[c] + cd2[c] + h2o[c] --> adp[c] + cd2[e] + h[c] + pi[c] | (MA0549 or MA3366 or MA3632) |
| CD2t4 | cd2[c] + h[e] + k[e] <=> cd2[e] + h[c] + k[c] | MA1117 |
| CDGGGPP3 | cdgggp[c] + glyc1p[c] -> cmp[c] + dggpgp[c] + h[c] | (MA0264 or MA0525) |
| CDGGGPP4 | 3hcdgggp[c] + glyc1p[c] -> 3hdggpgp[c] + cmp[c] + h[c] | (MA0264 or MA0525) |
| CDGGGS | ctp[c] + dgggp[c] + h[c] -> cdgggp[c] + ppi[c] | MA2010 |
| CDGGGS2 | 3hdgggp[c] + ctp[c] + h[c] -> 3hcdgggp[c] + ppi[c] | MA2010 |
| CDGGGSAT | cdgggp[c] + ser-L[c] -> cmp[c] + dgggps[c] + h[c] | MA0116 |
| CDGGGSAT2 | 3hcdgggp[c] + ser-L[c] -> 3hdgggps[c] + cmp[c] + h[c] | MA0116 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| CDGGIPT | cdgggp[c] + inost[c] -> cmp[c] + dggpi[c] + h[c] | MA0525 |
| CDGGIPT2 | 3hcdgggp[c] + inost[c] -> 3hdggpi[c] + cmp[c] + h[c] | MA0525 |
| CF3Ha | f390a[c] + h2o[c] -> amp[c] + f420-2[c] + (2) h[c] | |
| CF3Hg | f390g[c] + h2o[c] -> f420-2[c] + gmp[c] + h[c] | |
| CF3Sa | atp[c] + f420-2[c] + h[c] -> f390a[c] + ppi[c] | MA4074 |
| CF3Sg | f420-2[c] + gtp[c] -> f390g[c] + ppi[c] | MA4074 |
| CH2ACH | h2acon-C[c] + h2o[c] <=> tih2cit[c] | (MA3085 and MA3751) |
| CH3ACH | h2o[c] + h3acon-C[c] <=> tih3cit[c] | (MA3085 and MA3751) |
| CH4St | ch4s[e] <==> ch4s[c] | |
| CH4t | ch4[c] <=> ch4[e] | |
| CHACH | h2o[c] + hacon-C[c] <=> ihcit-T[c] | (MA3085 and MA3751) |
| CHORM | chor[c] <=> pphn[c] | MA1377 |
| CHORS | 3psme[c] -> chor[c] + pi[c] | MA0550 |
| CHRPL | chor[c] -> 4hbz[c] + pyr[c] | (MA2986 and MA2987) |
| Clt | cl[e] <==> cl[c] | MA3609 |
| CM5HBCMT | com[c] + m5hbc[c] --> mcom[c] + 5hbc_red[c] + h[c] | |
| CMLDC | 2c25dho[c] + h[c] -> co2[c] + 5odhf2a[c] | (MA0409 or MA2469) |
| CMPSAS | acnam[c] + ctp[c] <=> cmpacna[c] + ppi[c] | MA3766 |
| CO2t | co2[c] <=> co2[e] | |
| Coabc | atp[c] + cobalt2[e] + h2o[c] --> adp[c] + cobalt2[c] + h[c] + pi[c] | (MA0393 or MA0869 or MA1748 or MA3552) and (MA0394 or MA0870 or MA1747 or MA3551) and MA3553 |
| COBALTt5 | cobalt2[c] <==> cobalt2[e] | MA1721 |
| COBS | 7mht[c] + atp[c] -> adp[c] + cob[c] + h[c] | MA0339 |
| COCHL | atp[c] + cobalt2[c] + h2o[c] + hgbam[c] -> adp[c] + co2dam[c] + (4) h[c] + pi[c] | (MA0346 or MA0384 or MA0385 or MA0872) |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| CODH2 | co[c] + (2) fdox[c] + h2o[c] -> co2[c] + (2) fdred[c] + (2) h[c] | ( (MA1016 or MA3860 or MA4399) and (MA1011 or MA3865) ) or ( MA3283 and (MA1309 or MA3282) ) |
| CODH3 | co[c] + coa[c] + mh4spt[c] -> h4spt[c] + accoa[c] | ( (MA1012 or MA3864) and (MA1014 or MA3862) and (MA1015 or MA3861) ) |
| CODH2_SIDERXN | co[c] + h2o[c] --> for[c] + h[c] | (MA1011 or MA3865) and (MA1016 or MA3860 or MA4399) |
| CODHr | accoa[c] + (2) fdox[c] + h2o[c] + h4spt[c] <=> co2[c] + coa[c] + (2) fdred[c] + (2) h[c] + mh4spt[c] | (MA1016 or MA3860 or MA4399) and (MA1015 or MA3861) and (MA1014 or MA3862) and (MA1013 or MA3863) and (MA1012 or MA3864) and (MA1011 or MA3865) |
| COMS | h2o[c] + sec[c] -> com[c] + nh4[c] + pyr[c] | |
| COt | co[c] <=> co[e] | |
| CPC2MT | amet[c] + copre2[c] -> ahcys[c] + copre3[c] + h[c] | MA4262 |
| CPC3MT | amet[c] + copre3[c] -> ahcys[c] + copre4[c] | MA4259 |
| CPC4MT | amet[c] + copre4[c] -> ahcys[c] + copre5[c] + h[c] | MA4261 |
| CPC5MT | amet[c] + copre5[c] + h2o[c] -> acald[c] + ahcys[c] + copre6[c] + (2) h[c] | MA0521 |
| CPC6MT | (2) amet[c] + codhpre6[c] -> (2) ahcys[c] + co2[c] + copre8[c] + (2) h[c] | MA0520 |
| CPC6R | copre6[c] + nadph[c] + (2) h[c] -> codhpre6[c] + nadp[c] | |
| CPC8MM | copre8[c] + h[c] -> cobya[c] | MA4258 |
| CS | cit[c] + coa[c] + h[c] <=> accoa[c] + h2o[c] + oaa[c] | MA0249 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| CSND | csn[c] + h[c] + h2o[c] --> nh4[c] + ura[c] | MA2341 |
| CTPRIBFLVTX | ctp[c] + ribflv[c] -> cdp[c] + fmn[c] + h[c] | MA0547 |
| CTPS1 | atp[c] + nh4[c] + utp[c] -> adp[c] + ctp[c] + (2) h[c] + pi[c] | MA3279 |
| CTPS2 | atp[c] + gln-L[c] + h2o[c] + utp[c] -> adp[c] + ctp[c] + glu-L[c] + (2) h[c] + pi[c] | MA3279 |
| Cuabc | atp[c] + cu2[e] + h2o[c] --> adp[c] + cu2[c] + h[c] + pi[c] | MA1342 |
| Cut1 | atp[c] + cu2[c] + h2o[c] --> adp[c] + cu2[e] + h[c] + pi[c] | (MA0166 or MA1342) |
| CYRDAAT | atp[c] + co1dam[c] + h[c] -> adcobdam[c] + pppi[c] | MA2084 |
| CYRDAR | (2) co2dam[c] + nadh[c] -> (2) co1dam[c] + h[c] + nad[c] | |
| CYRDAS | (2) atp[c] + cobya[c] + (2) gln-L[c] + h2o[c] -> (2) adp[c] + co2dam[c] + (2) glu-L[c] + h[c] + ppi[c] | (MA0106 or MA1431 or MA3626) |
| CYSDS | cys-L[c] + h2o[c] -> h2s[c] + nh4[c] + pyr[c] | MA2532 |
| CYSSr | acser[c] + h2s[c] -> ac[c] + cys-L[c] + h[c] | MA2720 |
| CYSt2r | cys-L[e] + h[e] <==> cys-L[c] + h[c] | |
| CYSTA | akg[c] + cys-L[c] <==> glu-L[c] + mercppyr[c] | (MA0636 or MA1385 or MA1819) |
| CYSTRS | atp[c] + cys-L[c] + trnacys[c] -> amp[c] + ppi[c] + cystrna[c] | MA0749 |
| CYTDK1 | atp[c] + cytd[c] --> adp[c] + cmp[c] + h[c] | MA1373 |
| CYTK1 | atp[c] + cmp[c] <=> adp[c] + cdp[c] | MA1104 |
| CYTK2 | atp[c] + dcmp[c] -> adp[c] + dcdp[c] | MA1104 |
| CYTK5 | ctp[c] + dcmp[c] <=> cdp[c] + dcdp[c] | MA1104 |
| DADK | atp[c] + damp[c] <=> adp[c] + dadp[c] | MA1096 |
| DAPDC | 26dap-M[c] + h[c] -> co2[c] + lys-L[c] | MA0726 |
| DAPE | 26dap-LL[c] <==> 26dap-M[c] | |
| DAPNH4T | glu-L[c] + thdp[c] + h2o[c] --> akg[c] + 26dap-LL[c] | MA1712 |
| DATPHs | datp[c] + h[c] + h2o[c] -> ditp[c] + nh4[c] | |
| DB4PS | ru5p-D[c] -> db4p[c] + for[c] + h[c] | MA0548 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| DBTSr | atp[c] + co2[c] + dann[c] <=> adp[c] + dtbt[c] + (3) h[c] + pi[c] | |
| DCMPDA | dcmp[c] + h[c] + h2o[c] -> dump[c] + nh4[c] | (MA0136 or MA0137) |
| DCTPD | dctp[c] + h[c] + h2o[c] -> dutp[c] + nh4[c] | MA0440 |
| DGGGPS | ggdp[c] + gggp[c] -> dgggp[c] + ppi[c] | MA0961 |
| DGGGPS2 | 3hggdp[c] + gggp[c] -> 3hdgggp[c] + ppi[c] | MA0961 |
| DGGPGP | dggpgp[c] + h2o[c] -> dggpg[c] + pi[c] | MA1569 |
| DGGPGP2 | 3hdggpgp[c] + h2o[c] -> 3hdggpg[c] + pi[c] | MA1569 |
| DGLY3POX | (2) fdox[c] + g3p[c] + h2o[c] -> 3pg[c] + (2) fdred[c] + (3) h[c] | MA1714 |
| DGLYOX | glyald[c] + h2o[c] + nad[c] --> glyc-R[c] + (2) h[c] + nadh[c] | MA2860 |
| DHAD1 | 23dhmb[c] -> 3mob[c] + h2o[c] | (MA1802 or MA3373) |
| DHAD2 | 23dhmp[c] -> 3mop[c] + h2o[c] | (MA1802 or MA3373) |
| DHDPRx | nad[c] + thdp[c] <=> 23dhdp[c] + h[c] + nadh[c] | MA4474 |
| DHDPRy | nadp[c] + thdp[c] <=> 23dhdp[c] + h[c] + nadph[c] | MA4474 |
| DHDPS | aspsa[c] + pyr[c] -> 23dhdp[c] + (2) h2o[c] | MA4473 |
| DHFR | dhf[c] + h[c] + nadph[c] <=> nadp[c] + thf[c] | |
| DHFS | atp[c] + dhpt[c] + glu-L[c] --> adp[c] + dhf[c] + h[c] + pi[c] | |
| DHNPA2 | dhnpt[c] -> 6hmhpt[c] + gcald[c] + h[c] | |
| DHORD7 | dhor-S[c] + f420-2[c] + h[c] <=> f420-2h2[c] + orot[c] | (MA0583 and MA0584) |
| DHORTS | dhor-S[c] + h2o[c] <=> cbasp[c] + h[c] | MA0892 |
| DHPCPH | dhp23cp[c] + h2o[c] -> dhpmp[c] + h[c] | |
| DHPS2 | 4abz[c] + 6hmhptpp[c] + h[c] -> dhpt[c] + ppi[c] | MA3516 |
| DHPS3 | 6hmhptpp[c] + Brfap[c] + h[c] -> dhrfap[c] + ppi[c] | (MA1946 or MA3419) |
| DHQAT | 3dhq[c] + nh4[c] --> 4adhq[c] + h2o[c] | |
| DHQD | 3dhq[c] <=> 3dhsk[c] + h2o[c] | MA4593 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| DHQS2 | dohdu[c] -> 3dhq[c] | MA4592 |
| DIPS | amet[c] + caphis[c] -> ahcys[c] + cmaphis[c] + h[c] | MA1370 |
| DKFPASPL | aspsa[c] + dkfp[c] + nadh[c] + h[c] -> dohau[c] + g3p[c] + nad[c] | MA4591 |
| DKFPR | dkfp[c] + h[c] + nadh[c] <=> dkmp[c] + nad[c] | |
| DKFPS2 | mthgxl[c] + fdp[c] <=> dkfp[c] + g3p[c] | (MA2666 or MA3889) |
| DKFPS3 | f1p[c] + mthgxl[c] <=> dkfp[c] + gald[c] | (MA2666 or MA3889) |
| DMAMT | dma[c] + h[c] + 5hbc_red[c] -> mma[c] + m5hbc[c] | ( MA0146 and (MA0532 or MA0933 or MA2425) and (MA0527 or MA0934 or MA2424) and (MA0150 or MA0849 or MA4360) ) |
| DMAt | h[e] + dma[e] <==> h[c] + dma[c] | MA0143 |
| DMATT | dmpp[c] + ipdp[c] -> grdp[c] + ppi[c] | MA0606 |
| DMATT2 | ipdp[c] + m3hdp[c] -> 3hgrdp[c] + ppi[c] | MA0606 |
| DMHDRFS | 34hpp[c] + 4r5au[c] + h2o[c] + (2) nadp[c] -> ddhrb[c] + (3) h[c] + (2) nadph[c] + nh4[c] + oxa[c] | ( (MA1490 or MA1489) and MA1491) |
| DMSt | dms[e] <==> dms[c] | |
| dna_met | (0.58) datp[c] + (0.44) dctp[c] + (0.44) dgtp[c] + (0.59) dttp[c] <=> (2.05) ppi[c] + dna_met[c] | |
| DNADDP | dnad[c] + h2o[c] -> amp[c] + (2) h[c] + nicrnt[c] | MA1439 |
| DNMPPA | dhpmp[c] + h2o[c] -> dhnpt[c] + pi[c] | |
| DOHDUS | dohau[c] + h2o[c] + nad[c] -> dohdu[c] + h[c] + nadh[c] + nh4[c] | MA4592 |
| DPCOAK | atp[c] + dpcoa[c] -> adp[c] + coa[c] + h[c] | |
| DPR | h[c] + nadph[c] + 2dhp[c] -> nadp[c] + pant-R[c] | |
| DRBK | atp[c] + drib[c] --> 2dr5p[c] + adp[c] + h[c] | MA1373 |
| DROPPRx | 25dhpp[c] + h[c] + nadh[c] -> 25dthpp[c] + nad[c] | MA4092 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| DROPPRy | 25dhpp[c] + h[c] + nadph[c] -> 25dthpp[c] + nadp[c] | MA4092 |
| DRTPPD | 25dthpp[c] + h[c] + h2o[c] -> 5aprbu[c] + nh4[c] | |
| DTMPK | atp[c] + dtmp[c] <=> adp[c] + dtdp[c] | MA4433 |
| DURIPP | duri[c] + pi[c] -> 2dr1p[c] + ura[c] | MA3242 |
| DUTPDP | dutp[c] + h2o[c] -> dump[c] + h[c] + ppi[c] | MA0440 |
| ENO | 2pg[c] <=> h2o[c] + pep[c] | MA1672 |
| ETHAt6 | etha[e] + h[e] <==> etha[c] + h[c] | MA0143 |
| F430S1 | (2) atp[c] + dscl[c] + (2) gln-L[c] + (2) h2o[c] + ni2[c] -> (2) adp[c] + f430p1[c] + (2) glu-L[c] + (4) h[c] + (2) pi[c] | (MA0877 or MA0882) |
| F430S2 | f430p1[c] + h[c] + nadh[c] -> f430p2[c] + nad[c] | |
| F430S3 | f430p2[c] + h[c] + nadh[c] -> f430p3[c] + nad[c] | |
| F430S4 | f430p3[c] -> sf430a[c] | |
| F430S5 | h[c] + sf430a[c] -> f430[c] + h2o[c] | |
| F4D | f420-2h2[c] + h[c] + mphen[c] -> f420-2[c] + (2) h[e] + mphenh2[c] | (MA1494 and MA1495 and MA1496 and MA1497 and MA1498 and MA1499 and MA1500 and MA1501 and MA1502 and MA1503 and MA1504 and MA1505 and MA1506 and (MA1507 or MA1509) and MA3732) |
| F4H2O | (2) f420-2h2[c] + o2[c] <=> (2) f420-2[c] + (2) h2o[c] + (2) h[c] | MA3381 |
| F4MTSPD | f420-2h2[c] + menylh4spt[c] <=> f420-2[c] + (2) h[c] + mleneh4spt[c] | MA4430 |
| F4MTSPR | f420-2h2[c] + mleneh4spt[c] <=> f420-2[c] + mh4spt[c] + h[c] | MA3733 |
| FAE | fald[c] + h4spt[c] --> mleneh4spt[c] + h2o[c] | MA3006 |
| FAPH | fapy[c] + h2o[c] --> for[c] + 25dhpp[c] + | |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| | h[c] | |
| FBA | fdp[c] <=> dhap[c] + g3p[c] | ( MA0439 or MA4591) |
| FBA2 | f1p[c] <=> dhap[c] + glyald[c] | ( MA0439 or MA4591) |
| FBP | fdp[c] + h2o[c] -> f6p[c] + pi[c] | MA1152 |
| FBP2 | fdp[c] + h2o[c] --> pi[c] + f1p[c] | |
| FCLPA | fc1p[c] -> dhap[c] + lald-L[c] | MA0263 |
| FE2abc | atp[c] + fe2[e] + h2o[c] --> adp[c] + fe2[c] + h[c] + pi[c] | (MA3477 and (MA3478 or MA3479)) |
| FE3abc | atp[c] + fe3[e] + h2o[c] --> adp[c] + fe3[c] + h[c] + pi[c] | (MA1230 or MA1231 or MA1232 or MA3357) and (MA1233 or MA3358) and (MA1234 or MA3359) |
| FEDCabc | atp[c] + (2) cit[e] + fe3[e] + h2o[c] --> adp[c] + (2) cit[c] + fe3[c] + h[c] + pi[c] | ( (MA0950 and MA0951 and MA0952) or (MA2148 and MA2149 and MA2150) ) |
| FMFD_b_ | co2[c] + (2) fdred[c] + h[c] + mfr(b)[c] <=> (2) fdox[c] + formmfr(b)[c] + h2o[c] | ( ( MA0304 and MA0305 and MA0306 and MA0307 and MA0308 and MA0309 ) or ( MA4174 and MA4175 and MA4176 and MA4177 and (MA4178 or MA1241) ) or ( MA0835 and MA0834 and MA0833 and MA0832 ) or ( MA2877 and MA2878 and MA2879 and MA2880 and MA2881 and MA2882 ) or ( MA0671 and MA0381 and MA0382 ) ) |
| FMFTSPFT_b_ | formmfr(b)[c] + h[c] + h4spt[c] <=> formh4spt[c] + mfr(b)[c] | MA0010 |
| FMNAT | atp[c] + fmn[c] + h[c] --> fad[c] + ppi[c] | MA1820 |
| FMNAT_CTP_ | ctp[c] + fmn[c] + h[c] --> fcd[c] + ppi[c] | MA1820 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| FMNAT_GTP_ | gtp[c] + fmn[c] + h[c] --> fgd[c] + ppi[c] | MA1820 |
| FOLR2 | fol[c] + nadph[c] + (2) h[c] -> dhf[c] + nadp[c] | |
| FOLt | fol[e] + h[e] <==> fol[c] + h[c] | |
| FORt | for[c] + h[c] <=> for[e] + h[e] | |
| FRTT | frdp[c] + ipdp[c] -> ggdp[c] + ppi[c] | (MA1831 or MA3723 or MA4150 or MA4402) |
| FRTT2 | 3hfrdp[c] + ipdp[c] -> 3hggdp[c] + ppi[c] | (MA1831 or MA3723 or MA4150 or MA4402) |
| FUM | mal-L[c] <=> fum[c] + h2o[c] | ( MA1001 or (MA2497 and MA2498) ) |
| G1PACT | accoa[c] + gam1p[c] -> acgam1p[c] + coa[c] + h[c] | MA3025 |
| G1PDH | glyc1p[c] + nad[c] -> dhap[c] + h[c] + nadh[c] | MA3686 |
| G1PTT | dttp[c] + g1p[c] + h[c] -> dtdpglu[c] + ppi[c] | (MA2183 or MA3022 or MA3777) |
| G1SATi | glu1sa[c] -> 5aop[c] + h[c] | MA0581 |
| G5SADr | glu5sa[c] <=> 1pyr5c[c] + h[c] + h2o[c] | |
| G5SD2 | glu5p[c] + h[c] + nadh[c] -> glu5sa[c] + nad[c] + pi[c] | MA4100 |
| GALT | gal1p[c] + h[c] + utp[c] <==> ppi[c] + udpgal[c] | MA3680 |
| GALTNS | udpgal[c] -> galactan[c] + h[c] + udp[c] | |
| GALUi | g1p[c] + h[c] + utp[c] <=> ppi[c] + udpg[c] | MA4459 |
| GAPD | g3p[c] + nad[c] + pi[c] <=> 13dpg[c] + h[c] + nadh[c] | (MA1018 or MA3345) |
| GARFT | 10fthf[c] + gar[c] <=> fgam[c] + h[c] + thf[c] | (MA0316 or MA3522) |
| GCALDDr | gcald[c] + h2o[c] + nad[c] <=> glyclt[c] + (2) h[c] + nadh[c] | MA2860 |
| GCALDt | gcald[e] <==> gcald[c] | |
| GCC | dhlpro[c] + nad[c] -> h[c] + lpro[c] + nadh[c] | MA1652 |
| GF4GL_0 | f420-0[c] + glu-L[c] + gtp[c] -> f420-1[c] + gdp[c] + h[c] + pi[c] | (MA0135 or MA3512) |
| GF4GL_1 | f420-1[c] + glu-L[c] + gtp[c] -> f420-2[c] + gdp[c] + h[c] + pi[c] | (MA0135 or MA3512) |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| GF4GL_2 | f420-2[c] + glu-L[c] + gtp[c] -> f420-3[c] + gdp[c] + h[c] + pi[c] | (MA0135 or MA3512) |
| GF4GL_3 | f420-3[c] + glu-L[c] + gtp[c] -> f420-4[c] + gdp[c] + h[c] + pi[c] | (MA0135 or MA3512) |
| GF4GL_4 | f420-4[c] + glu-L[c] + gtp[c] -> f420-5[c] + gdp[c] + h[c] + pi[c] | (MA0135 or MA3512) |
| GF4GL_5 | f420-5[c] + glu-L[c] + gtp[c] -> f420-6[c] + gdp[c] + h[c] + pi[c] | (MA0135 or MA3512) |
| GF4GL_6 | f420-6[c] + glu-L[c] + gtp[c] -> f420-7[c] + gdp[c] + h[c] + pi[c] | (MA0135 or MA3512) |
| GF6PTA | f6p[c] + gln-L[c] -> gam6p[c] + glu-L[c] | MA3023 |
| GGDPRED | ggdp[c] + (3) h[c] + (3) nadph[c] -> (3) nadp[c] + phydp[c] | MA1484 |
| GGGPS | ggdp[c] + glyc1p[c] -> gggp[c] + ppi[c] | MA3969 |
| GHMT2 | gly[c] + h2o[c] + mlthf[c] <=> ser-L[c] + thf[c] | MA3520 |
| GK__adp_ | adp[c] + glc-D[c] -> amp[c] + g6p[c] + h[c] | (MA3562 or MA3563) |
| GK1 | atp[c] + gmp[c] <=> adp[c] + gdp[c] | |
| GLCGSD | h2o[c] + glycogen[c] -> glc-D[c] | (MA1190 or MA4050) |
| GLCNt2r | glcn[e] + h[e] <==> glcn[c] + h[c] | MA0021 |
| GLCP | pi[c] + glycogen[c] -> g1p[c] | (MA0905 or MA1874 or MA2628) |
| GLCS2 | udpg[c] -> h[c] + udp[c] + glycogen[c] | MA3679 |
| GLNS | atp[c] + glu-L[c] + nh4[c] -> adp[c] + gln-L[c] + h[c] + pi[c] | MA4216 |
| GLNTRAT | atp[c] + gln-L[c] + h2o[c] + glutrna(gln)[c] -> adp[c] + glu-L[c] + h[c] + pi[c] + glntrna[c] | (MA1317 and MA2862 and MA4522 and MA4523 and MA4524) |
| GLU5K | atp[c] + glu-L[c] -> adp[c] + glu5p[c] | MA4101 |
| GLUDC | glu-L[c] + h[c] -> 4abut[c] + co2[c] | MA1949 |
| GLUDxi | akg[c] + h[c] + nadh[c] + nh4[c] --> glu-L[c] + h2o[c] + nad[c] | MA3169 |
| GLUDyi | akg[c] + h[c] + nadph[c] + nh4[c] --> glu-L[c] + h2o[c] + nadp[c] | MA3169 |
| GLUPRT | gln-L[c] + h2o[c] + prpp[c] -> glu-L[c] + ppi[c] + pram[c] | MA3193 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| GLUS_F420_ | akg[c] + f420-2h2[c] + gln-L[c] -> f420-2[c] + (2) glu-L[c] + h[c] | (MA3787 or MA4216 or MA4217 or MA4218) |
| GLUt2r | glu-L[e] + h[e] --> glu-L[c] + h[c] | MA2961 |
| GLUt4 | glu-L[e] + na1[e] --> glu-L[c] + na1[c] | MA2961 |
| GLUTRR | glutrna[c] + (2) h[c] + nadph[c] -> glu1sa[c] + nadp[c] + trnaglu[c] | MA0577 |
| GLUTRS | atp[c] + glu-L[c] + trnaglu[c] --> amp[c] + glutrna[c] + ppi[c] | MA0587 |
| GLUTRS_Gln_ | atp[c] + glu-L[c] + trnagln[c] -> amp[c] + ppi[c] + glutrna(gln)[c] | MA0587 |
| GLXO1 | h2o[c] + nad[c] + glx[c] <=> nadh[c] + (2) h[c] + oxa[c] | MA2860 |
| GLYALDOX | (2) fdox[c] + glyald[c] + h2o[c] -> (2) fdred[c] + glyc-R[c] + (3) h[c] | (MA2962 or MA3989) |
| GLYBabc | atp[c] + glyb[e] + h2o[c] --> adp[c] + glyb[c] + h[c] + pi[c] | (MA2145 and MA2146 and MA2147) |
| GLYCDx | glyc[c] + nad[c] --> dha[c] + h[c] + nadh[c] | MA0591 |
| GLYOX | lgt-S[c] + h2o[c] --> gthrd[c] + h[c] + lac-D[c] | MA2320 |
| GLYt4r | gly[e] + na1[e] --> gly[c] + na1[c] | MA2837 |
| GLYTRS | atp[c] + gly[c] + trnagly[c] -> amp[c] + ppi[c] + glytrna[c] | MA0097 |
| GMAND | gdpmann[c] <=> gdpddman[c] + h2o[c] | (MA1173 or MA1174) |
| GMPS | atp[c] + nh4[c] + xmp[c] -> amp[c] + gmp[c] + (2) h[c] + ppi[c] | (MA4511 and MA4590) |
| GMPS2 | atp[c] + gln-L[c] + h2o[c] + xmp[c] -> amp[c] + glu-L[c] + gmp[c] + (2) h[c] + ppi[c] | (MA4511 and MA4590) |
| GRTT | grdp[c] + ipdp[c] -> frdp[c] + ppi[c] | (MA0606 or MA1831 or MA3723 or MA4150 or MA4402) |
| GRTT2 | 3hgrdp[c] + ipdp[c] -> 3hfrdp[c] + ppi[c] | (MA0606 or MA1831 or MA3723 or MA4150 or MA4402) |
| GSNK | atp[c] + gsn[c] --> adp[c] + gmp[c] + h[c] | MA1373 |
| GTHOr | gthox[c] + h[c] + nadph[c] -> (2) gthrd[c] + nadp[c] | (MA1019 or MA4013) |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| GTPCHIII | gtp[c] + (3) h2o[c] --> fapy[c] + (2) pi[c] + (2) h[c] | |
| GTPCHIV | gtp[c] + h2o[c] --> for[c] + ppi[c] + dhp23cp[c] | MA4517 |
| GTPHs | gtp[c] + h[c] + h2o[c] -> nh4[c] + xtp[c] | |
| GUACYC | gtp[c] -> 35cgmp[c] + ppi[c] | MA4044 |
| GUAD | gua[c] + h[c] + h2o[c] -> nh4[c] + xan[c] | MA3407 |
| GUAPRT | gmp[c] + ppi[c] <=> gua[c] + prpp[c] | MA0717 |
| H2MPTR | f420-2h2[c] + h2mpt[c] -> f420-2[c] + h4mpt[c] + h[c] | |
| H2Ot | h2o[e] <=> h2o[c] | |
| H2St | h2s[e] <==> h2s[c] | |
| H2td | h2[c] <=> h2[e] | |
| H4MPTGL_atp_ | atp[c] + glu-L[c] + h4mpt[c] -> adp[c] + h[c] + h4spt[c] + pi[c] | MA3268 |
| H4MPTGL_gtp_ | glu-L[c] + gtp[c] + h4mpt[c] -> gdp[c] + h[c] + h4spt[c] + pi[c] | MA3268 |
| H4MPTS10 | dhadrp[c] + h2o[c] -> dhadr[c] + pi[c] | |
| H4MPTS11 | dhadr[c] + prpp[c] -> dhadrpr[c] + ppi[c] | |
| H4MPTS12 | atp[c] + dhadrpr[c] -> adp[c] + dhadrdpr[c] | |
| H4MPTS13 | atp[c] + dhadrdpr[c] -> adp[c] + dhadrtpr[c] | |
| H4MPTS14 | S2hglut[c] + dhadrtpr[c] -> dmh2mpt[c] + ppi[c] | |
| H4MPTS15 | amet[c] + dmh2mpt[c] -> ahcys[c] + h[c] + mmh2mpt[c] | |
| H4MPTS16 | amet[c] + mmh2mpt[c] -> ahcys[c] + h[c] + h2mpt[c] | |
| H4MPTS9 | dhrfap[c] + f420-2h2[c] -> dhadrp[c] + f420-2[c] + h[c] | |
| HASD | 3hdpgps[c] + h[c] -> 3hdpgpe[c] + co2[c] | MA0115 |
| HATGH | 3hdggpg[c] + (7) h[c] + (7) nadph[c] -> 3hdpgpg[c] + (7) nadp[c] | (MA0691 or MA0692 or MA1484) |
| HATIH | 3hdggpi[c] + (7) h[c] + (7) nadph[c] -> 3hdpgpi[c] + (7) nadp[c] | (MA0691 or MA0692 or MA1484) |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| HATSH | 3hdgggps[c] + (7) h[c] + (7) nadph[c] -> 3hdpgps[c] + (7) nadp[c] | (MA0691 or MA0692 or MA1484) |
| HBZOPT | 4hbz[c] + octdp[c] <=> 3ophb[c] + ppi[c] | MA0961 |
| HCARNHYD | 4abut[c] + his-L[c] + h[c] -> h2o[c] + hcarn[c] | MA2653 |
| HCITS | accoa[c] + akg[c] + h2o[c] -> coa[c] + h[c] + hcit[c] | MA3342 |
| HCO3E | co2[c] + h2o[c] <=> h[c] + hco3[c] | MA2536 |
| HDR | (2) h[c] + hsfd[c] + mphenh2[c] -> cob[c] + com[c] + (2) h[e] + mphen[c] | (MA0526 or MA0688) and MA0687 |
| HDR-2 | (4) fdred[c] + f420-2[c] + hsfd[c] + (5) h[c] -> (4) fdox[c] + com[c] + cob[c] + f420-2h2[c] | (MA2868 or MA3128) and (MA3126 or MA4237) and (MA3127 or MA4236) and MA2867 |
| HETZK | 4mhetz[c] + atp[c] -> 4mpetz[c] + adp[c] + h[c] | MA2723 |
| HEX7 | atp[c] + fru[c] -> adp[c] + f6p[c] + h[c] | MA1840 |
| HIBD | 3hmp[c] + nad[c] <=> 2mop[c] + h[c] + nadh[c] | MA0614 |
| HISTD | h2o[c] + histd[c] + (2) nad[c] -> (3) h[c] + his-L[c] + (2) nadh[c] | MA3201 |
| HISTP | h2o[c] + hisp[c] -> histd[c] + pi[c] | MA0219 |
| HISTRS | atp[c] + his-L[c] + trnahis[c] -> amp[c] + ppi[c] + histrna[c] | MA0943 |
| HKt | atp[c] + h2o[c] + k[e] --> adp[c] + h[e] + k[c] + pi[c] | MA3999 |
| HMBS | h2o[c] + (4) ppbng[c] -> hmbil[c] + (4) nh4[c] | MA0582 |
| HMGCOARi | (2) h[c] + hmgcoa[c] + (2) nadph[c] -> coa[c] + mev-R[c] + (2) nadp[c] | MA3073 |
| HMGCOASi | coa[c] + h[c] + hmgcoa[c] -> aacoa[c] + accoa[c] + h2o[c] | MA4041 |
| HMPK1 | 4ahmmp[c] + atp[c] -> 4ampm[c] + adp[c] + (2) h[c] | MA3197 |
| HPACt2r | 4hphac[e] + h[e] <==> 4hphac[c] + h[c] | |
| HPPK2 | 6hmhpt[c] + atp[c] -> 6hmhptpp[c] + amp[c] + h[c] | |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| HPYRRx | h[c] + hpyr[c] + nadh[c] -> glyc-R[c] + nad[c] | MA1334 |
| HPYRRy | h[c] + hpyr[c] + nadph[c] -> glyc-R[c] + nadp[c] | MA1334 |
| HSDx | hom-L[c] + nad[c] <=> aspsa[c] + h[c] + nadh[c] | MA2572 |
| HSDy | hom-L[c] + nadp[c] <=> aspsa[c] + h[c] + nadph[c] | MA2572 |
| HSERTA | accoa[c] + hom-L[c] -> achms[c] + coa[c] | MA2714 |
| HSK | atp[c] + hom-L[c] -> adp[c] + h[c] + phom[c] | |
| HSPMS | (2) ptrc[c] -> hspmd[c] + nh4[c] | MA1636 |
| HSTPT | akg[c] + hisp[c] <=> glu-L[c] + imacp[c] | (MA0118 or MA0942) |
| HXPRT | hxan[c] + prpp[c] -> imp[c] + ppi[c] | MA1687 |
| ICITRED | icit[c] + nadp[c] <=> h[c] + nadph[c] + osuc[c] | MA4265 |
| IG3PS | gln-L[c] + prlp[c] <=> aicar[c] + eig3p[c] + glu-L[c] + h[c] | (MA0541 and MA0913) |
| IGPDH | eig3p[c] -> h2o[c] + imacp[c] | MA0219 |
| IGPS | 2cpr5p[c] + h[c] <=> 3ig3p[c] + co2[c] + h2o[c] | MA2992 |
| ILEt2r | h[e] + ile-L[e] <==> h[c] + ile-L[c] | (MA3437 and MA3438) |
| ILETA | akg[c] + ile-L[c] <=> 3mop[c] + glu-L[c] | MA4349 |
| ILETRS | atp[c] + ile-L[c] + trnaile[c] -> amp[c] + ppi[c] + iletrna[c] | MA2431 |
| IMPC | h2o[c] + imp[c] <=> fprica[c] | MA4012 |
| IMPD | h2o[c] + imp[c] + nad[c] -> h[c] + nadh[c] + xmp[c] | |
| IND3ACt2r | h[c] + ind3ac[c] <==> h[e] + ind3ac[e] | |
| INDPYRD | h[c] + indpyr[c] -> co2[c] + id3acald[c] | MA0594 |
| INS2D | inost[c] + nad[c] -> h[c] + nadh[c] + 2ins[c] | MA4448 |
| INSK | atp[c] + ins[c] --> adp[c] + h[c] + imp[c] | MA1373 |
| IOR | coa[c] + (2) fdox[c] + indpyr[c] <=> co2[c] + (2) fdred[c] + h[c] + indaccoa[c] | ( (MA1022 or MA1727 or MA1982) and (MA1023 or MA1726) ) |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| IOR2 | coa[c] + (2) fdox[c] + phpyr[c] <=> co2[c] + (2) fdred[c] + h[c] + phaccoa[c] | ( ( (MA1022 or MA1727 or MA1982) and (MA1023 or MA1726) ) or (MA2909 and MA2910 and MA2911) ) |
| IOR3 | 34hpp[c] + coa[c] + (2) fdox[c] <=> co2[c] + (2) fdred[c] + h[c] + hphaccoa[c] | ( (MA1022 or MA1727 or MA1982) and (MA1023 or MA1726) ) |
| IPDDI3x | f420-2[c] + (2) h[c] + ipdp[c] + nadh[c] -> dmpp[c] + f420-2h2[c] + nad[c] | MA0604 |
| IPDDI3y | f420-2[c] + (2) h[c] + ipdp[c] + nadph[c] -> dmpp[c] + f420-2h2[c] + nadp[c] | MA0604 |
| IPDPH | ipdp[c] + h2o[c] <=> m3hdp[c] | |
| IPMD | 3c2hmp[c] + nad[c] <=> 3c4mop[c] + h[c] + nadh[c] | (MA0201 or MA3748) |
| IPPK | ipp[c] + atp[c] --> adp[c] + ipdp[c] | MA0603 |
| IPPMIa | 3c2hmp[c] <=> 2ippm[c] + h2o[c] | (MA0202 or MA1223) and (MA1393 or MA0642 or MA4415) |
| IPPMIb | 2ippm[c] + h2o[c] <=> 3c3hmp[c] | (MA0202 or MA1223) and (MA1393 or MA0642 or MA4415) |
| IPPS | 3mob[c] + accoa[c] + h2o[c] -> 3c3hmp[c] + coa[c] + h[c] | MA4615 |
| KARA1 | 23dhmb[c] + nadp[c] <=> alac-S[c] + h[c] + nadph[c] | MA3790 |
| KARA2 | 2ahbut[c] + h[c] + nadph[c] <=> 23dhmp[c] + nadp[c] | MA3790 |
| KCCt | cl[c] + k[c] --> cl[e] + k[e] | MA4506 |
| Kt2r | h[e] + k[e] <==> h[c] + k[c] | (MA1527 or MA1550 or MA2034 or MA2218 or MA2448 or MA3398) |
| LCAD | h2o[c] + lald-L[c] + nad[c] --> (2) h[c] + lac-L[c] + nadh[c] | (MA2860 or MA4086) |
| LCYSTAT | Lcyst[c] + akg[c] <==> 3spyr[c] + glu-L[c] | (MA0636 or MA1385 or MA1819) |
| LCYSTS | pser-L[c] + so3[c] + h[c] --> Lcyst[c] + pi[c] | MA3297 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| LEUt2r | h[e] + leu-L[e] <==> h[c] + leu-L[c] | (MA3437 and MA3438) |
| LEUTA | akg[c] + leu-L[c] <=> 4mop[c] + glu-L[c] | MA4349 |
| LEUTRS | atp[c] + leu-L[c] + trnaleu[c] -> amp[c] + ppi[c] + leutrna[c] | MA1611 |
| lipid_met | (0.005) dpgpg[c] + (0.214) 3hdpgpg[c] + (0.027) dpgpi[c] + (0.287) 3hdpgpi[c] + (0.005) dpgpe[c] + (0.057) 3hdpgpe[c] + (0.011) dpgps[c] + (0.244) 3hdpgps[c] + (0.148) gdpgpi[c] <=> lipid_met[c] | |
| LPFPLT | ddhrb[c] + lppg[c] -> f420-0[c] + gmp[c] + h[c] | MA0714 |
| LPPGS | 2plac-L[c] + gtp[c] + h[c] -> lppg[c] + ppi[c] | MA1488 |
| LYSAM | lys-L[c] -> 36dahx[c] | MA3979 |
| LYSt3r | h[e] + lys-L[c] <==> h[c] + lys-L[e] | MA2108 |
| LYSTRS | atp[c] + lys-L[c] + trnalys[c] -> amp[c] + ppi[c] + lystrna[c] | (MA0534 or MA0760) |
| MAN1PT | gtp[c] + h[c] + man1p[c] <=> gdpmann[c] + ppi[c] | MA3140 |
| MAN1PT2r | gdp[c] + h[c] + man1p[c] <=> gdpmann[c] + pi[c] | MA3781 |
| MAN6PI | man6p[c] <=> f6p[c] | MA3781 |
| MCMMT | meoh[c] + 5hbc_red[c] + h[c] --> h2o[c] + m5hbc[c] | ( ( MA0455 or MA1616 or MA4392) and (MA0456 or MA1617 or MA4391) and MA4379 and (MA0150 or MA0849 or MA4360) ) |
| MCR | cob[c] + mcom[c] -> ch4[c] + hsfd[c] | (MA4546 and MA4547 and MA4548 and MA4549 and MA4550) |
| MDH | mal-L[c] + nad[c] <=> h[c] + nadh[c] + oaa[c] | MA0819 |
| MDHy | mal-L[c] + nadp[c] <=> h[c] + nadph[c] + oaa[c] | MA0819 |
| ME1_rev | mal-L[c] + nad[c] <=> co2[c] + nadh[c] + pyr[c] | MA1735 |
| ME2 | mal-L[c] + nadp[c] -> co2[c] + nadph[c] + pyr[c] | MA1735 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| MEOHt2 | meoh[e] <==> meoh[c] | |
| METAT | atp[c] + h2o[c] + met-L[c] -> amet[c] + pi[c] + ppi[c] | (MA0216 or MA0962) |
| METGL | h2o[c] + met-L[c] -> 2obut[c] + ch4s[c] + nh4[c] | MA2532 |
| METS | 5mthf[c] + hcys-L[c] -> met-L[c] + thf[c] | (MA0053 or MA0054 or MA3549) |
| METTRS | atp[c] + met-L[c] + trnamet[c] -> amp[c] + ppi[c] + mettrna[c] | MA4046 |
| MEVK1 | atp[c] + mev-R[c] -> 5pmev[c] + adp[c] + h[c] | MA0602 |
| MFRS1 | dhap[c] + pep[c] + h2o[c] -> mfrbi1[c] | |
| MFRS2 | mfrbi1[c] -> mfrbi2[c] + pi[c] | |
| MFRS3 | mfrbi2[c] -> mfrbi3[c] + h2o[c] | |
| MFRS4 | mfrbi3[c] -> mfrbi4[c] | |
| MFRS5 | mfrbi4[c] -> 24sf[c] + h2o[c] | |
| MFRS6 | 24sf[c] + atp[c] + nadh[c] + h[c] -> 2frald[c] + adp[c] + pi[c] + nad[c] | |
| MFRS7 | 2frald[c] + glu-L[c] -> 2mafr[c] + akg[c] | |
| MFRS8 | tym[c] + 2mafr[c] -> aepmamfr[c] + pi[c] | |
| MFRS9 | aepmamfr[c] + glu-L[c] -> 4gaepmamfr[c] + h2o[c] | |
| MFRS10 | 4gaepmamfr[c] + glu-L[c] -> 4ggaepmamfr[c] + h2o[c] | |
| MFRS11 | 4ggaepmamfr[c] + glu-L[c] -> 4gggaepmamfr[c] + h2o[c] | |
| MFRBS | 4gggaepmamfr[c] + glu-L[c] -> mfr(b)[c] + h2o[c] | |
| MG2abc | atp[c] + h2o[c] + mg2[e] -> adp[c] + h[c] + mg2[c] + pi[c] | MA1721 |
| MGSA2 | g3p[c] --> mthgxl[c] + pi[c] | MA4607 |
| MGt5 | mg2[c] <==> mg2[e] | MA1721 |
| MHPGLUT | hcys-L[c] + mhpglu[c] -> hpglu[c] + met-L[c] | (MA0053 or MA0054 or MA3549) |
| MHPGLUT2 | ahcys[c] + h[c] + mhpglu[c] --> amet[c] + hpglu[c] | (MA0053 or MA0054 or MA3549) |
| MI3PP | h2o[c] + mi3p-D[c] -> inost[c] + pi[c] | MA3344 |
| MI3PS | g6p[c] -> mi3p-D[c] | (MA0075 or MA2253) |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| MMAMT | h[c] + mma[c] + 5hbc_red[c] -> nh4[c] + m5hbc[c] | ( (MA0144 or MA2972) and (MA0145 or MA2971) and MA0146 and (MA0150 or MA0849 or MA4360 ) ) |
| MMAt | h[e] + mma[e] <==> h[c] + mma[c] | MA0143 |
| MNabc | atp[c] + h2o[c] + mn2[e] --> adp[c] + h[c] + mn2[c] + pi[c] | (MA0023 and MA0024 and MA0025) |
| MOBDabc | atp[c] + h2o[c] + mobd[e] --> adp[c] + h[c] + mobd[c] + pi[c] | (MA0280 and MA0281 and MA0282) or (MA0323 and MA0324 and MA0325) or (MA1235 and MA1236 and MA1237) or (MA2280 and MA2281 and MA2282) |
| MOHMT | 3mob[c] + h2o[c] + mlthf[c] -> thf[c] + 2dhp[c] | |
| MSS | mh4spt[c] + h2s[c] + (2) na1[c] -> ch4s[c] + h4spt[c] + (2) na1[e] | |
| MTAP | 5mta[c] + pi[c] -> 5mdr1p[c] + ade[c] | MA1409 |
| MTCMMT | dms[c] + h[c] + 5hbc_red[c] -> ch4s[c] + m5hbc[c] | (MA0859 or MA4384 or MA4558) and (MA0150 or MA0849 or MA4360) |
| MTHFC | h2o[c] + methf[c] <=> 10fthf[c] | MA3519 |
| MTHFD2i | mlthf[c] + nad[c] -> methf[c] + nadh[c] + h[c] | MA3519 |
| MTHFR2 | h[c] + mlthf[c] + nadh[c] --> 5mthf[c] + nad[c] | MA3514 |
| MTHFR3 | h[c] + mlthf[c] + nadph[c] -> 5mthf[c] + nadp[c] | MA3514 |
| MTR_BYPASS | h[c] + mh4spt[c] + 5hbc_red[c] --> h4spt[c] + m5hbc[c] | |
| MTRI | 5mdr1p[c] <==> 5mdru1p[c] | MA0076 |
| MTSPC | formh4spt[c] + h[c] <=> h2o[c] + menylh4spt[c] | MA1710 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| MTSPCMMT | h[c] + mh4spt[c] + (2) na1[c] + 5hbc_red[c] <=> h4spt[c] + (2) na1[e] + m5hbc[c] | ( ( MA0150 or MA0849 or MA4360 ) and MA0269 and MA0270 and MA0271 and MA0272 and MA0273 and MA0274 and MA0275 and MA0276 ) |
| N2tr | n2[e] <==> n2[c] | |
| Naabc | atp[c] + h2o[c] + na1[c] --> adp[c] + h[c] + pi[c] + na1[e] | ( MA2433 and MA2434 and MA2435 and MA2436 and MA2437 and MA2438 and MA2439 and MA2440 and MA2441 ) |
| NACUP | nac[e] --> nac[c] | |
| NADDP | h2o[c] + nad[c] -> amp[c] + (2) h[c] + nmn[c] | MA1439 |
| NADK | atp[c] + nad[c] -> adp[c] + h[c] + nadp[c] | MA3343 |
| NADS1 | atp[c] + dnad[c] + nh4[c] -> amp[c] + h[c] + nad[c] + ppi[c] | ( MA1030 or MA3526 or MA3715 ) |
| NADS2 | atp[c] + dnad[c] + gln-L[c] + h2o[c] -> amp[c] + glu-L[c] + h[c] + nad[c] + ppi[c] | ( MA1030 or MA3526 or MA3715 ) |
| NaKt_1 | atp[c] + h2o[c] + k[e] + na1[c] --> adp[c] + h[c] + k[c] + na1[e] + pi[c] | MA4378 |
| NAPRTr | nicrnt[c] + ppi[c] <=> h[c] + nac[c] + prpp[c] | MA2533 |
| NAt3_1 | h[e] + na1[c] <==> h[c] + na1[e] | ( ( MA4566 and MA4567 and MA4568 and MA4569 and MA4570 and MA4572 and MA4665 ) or MA2633 ) |
| NBAHH | carn[c] + h2o[c] -> ala-B[c] + his-L[c] + h[c] | MA2653 |
| NCCt | na1[e] + cl[c] --> na1[c] + cl[e] | MA4506 |
| NDPK1 | atp[c] + gdp[c] <=> adp[c] + gtp[c] | MA1524 |
| NDPK10 | atp[c] + didp[c] <=> adp[c] + ditp[c] | MA1524 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| NDPK2 | atp[c] + udp[c] <=> adp[c] + utp[c] | MA1524 |
| NDPK3 | atp[c] + cdp[c] <=> adp[c] + ctp[c] | MA1524 |
| NDPK4 | atp[c] + dtdp[c] <=> adp[c] + dttp[c] | MA1524 |
| NDPK5 | atp[c] + dgdp[c] <=> adp[c] + dgtp[c] | MA1524 |
| NDPK6 | atp[c] + dudp[c] <=> adp[c] + dutp[c] | MA1524 |
| NDPK7 | atp[c] + dcdp[c] <=> adp[c] + dctp[c] | MA1524 |
| NDPK8 | atp[c] + dadp[c] <=> adp[c] + datp[c] | MA1524 |
| NDPK9 | atp[c] + idp[c] <=> adp[c] + itp[c] | MA1524 |
| NH4t | nh4[e] <=> nh4[c] | ( ( MA3917 or MA3918) and MA4207 ) |
| NIabc | atp[c] + h2o[c] + ni2[e] --> adp[c] + h[c] + ni2[c] + pi[c] | ( MA3455 and (MA3456 or MA3457) and (MA3458 or MA4673) ) |
| NIT_n1p4 | (16) atp[c] + (8) fdred[c] + (16) h2o[c] + n2[c] -> (16) adp[c] + (8) fdox[c] + (6) h[c] + h2[c] + (2) nh4[c] + (16) pi[c] | ( (MA1208 and MA1209 and MA1210) or ( (MA1205 or MA1213 or MA1633 or MA2032 or MA3627 or MA3895) and (MA1212 or MA1214 or MA3896) and (MA1211 or MA1215 or MA3897) and MA3898 and MA3899 and MA4195 and MA3900 and MA3901 and (MA2717 or MA3265) ) or (MA1217 and MA1216 and MA1218 and (MA1219 or MA1631) and (MA1220 or MA1632) ) ) |
| NIt5 | ni2[c] <==> ni2[e] | MA1721 |
| NMNAT | atp[c] + h[c] + nmn[c] <=> nad[c] + ppi[c] | MA3731 |
| NMNHYD | h2o[c] + nmn[c] -> pi[c] + rnam[c] | MA0104 |
| NN5HBPRT | nicrnt[c] + 5hbzid[c] -> h[c] + nac[c] + 5pr5hbz[c] | |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| NNAT | atp[c] + h[c] + nicrnt[c] <=> dnad[c] + ppi[c] | MA3731 |
| NNDPR | (2) h[c] + prpp[c] + quln[c] -> co2[c] + nicrnt[c] + ppi[c] | (MA0322 or MA0955) |
| NT5C | h2o[c] + nicrnt[c] <=> nicrns[c] + pi[c] | MA0104 |
| NTD1 | dump[c] + h2o[c] --> duri[c] + pi[c] | MA0104 |
| NTD10 | h2o[c] + xmp[c] -> pi[c] + xtsn[c] | MA0104 |
| NTD11 | h2o[c] + imp[c] -> ins[c] + pi[c] | MA0104 |
| NTD2 | h2o[c] + ump[c] -> pi[c] + uri[c] | MA0104 |
| NTD3 | dcmp[c] + h2o[c] -> dcyt[c] + pi[c] | MA0104 |
| NTD4 | cmp[c] + h2o[c] -> cytd[c] + pi[c] | MA0104 |
| NTD5 | dtmp[c] + h2o[c] -> pi[c] + thymd[c] | MA0104 |
| NTD6 | damp[c] + h2o[c] <=> dad-2[c] + pi[c] | MA0104 |
| NTD7 | amp[c] + h2o[c] -> adn[c] + pi[c] | MA0104 |
| NTD8 | dgmp[c] + h2o[c] <=> dgsn[c] + pi[c] | MA0104 |
| NTD9 | gmp[c] + h2o[c] -> gsn[c] + pi[c] | MA0104 |
| NTP1 | atp[c] + h2o[c] -> adp[c] + h[c] + pi[c] | MA3706 |
| NTP2 | datp[c] + h2o[c] -> dadp[c] + h[c] + pi[c] | MA3706 |
| NTP3 | gtp[c] + h2o[c] -> gdp[c] + h[c] + pi[c] | MA3706 |
| NTP4 | dgtp[c] + h2o[c] -> dgdp[c] + h[c] + pi[c] | MA3706 |
| NTP5 | ctp[c] + h2o[c] -> cdp[c] + h[c] + pi[c] | MA3706 |
| NTP6 | dctp[c] + h2o[c] -> dcdp[c] + h[c] + pi[c] | MA3706 |
| NTP7 | h2o[c] + utp[c] -> h[c] + pi[c] + udp[c] | MA3706 |
| NTP8 | dutp[c] + h2o[c] -> dudp[c] + h[c] + pi[c] | MA3706 |
| NTP9 | dttp[c] + h2o[c] -> dtdp[c] + h[c] + pi[c] | MA3706 |
| NTPP11 | h2o[c] + xtp[c] -> h[c] + ppi[c] + xmp[c] | MA3706 |
| NTPTP1 | dgtp[c] + h2o[c] --> dgsn[c] + pppi[c] | MA0713 |
| NTRIR2x | (5) h[c] + (3) nadh[c] + no2[c] -> (2) h2o[c] + (3) nad[c] + nh4[c] | (MA0685 or MA3167) |
| NTRIR2y | (5) h[c] + (3) nadh[c] + no2[c] -> (2) h2o[c] + (3) nad[c] + nh4[c] | (MA0685 or MA3167) |
| OCBT | cbp[c] + orn[c] -> citr-L[c] + h[c] + pi[c] | MA3310 |
| OCTDPSYN | hepdp[c] + ipdp[c] -> octdp[c] + ppi[c] | MA4150 |
| OMCDC | 3c4mop[c] + h[c] -> 4mop[c] + co2[c] | |
| OMPDC | h[c] + orot5p[c] -> co2[c] + ump[c] | MA0969 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| OORr | akg[c] + coa[c] + (2) fdox[c] -> co2[c] + (2) fdred[c] + succoa[c] + h[c] | (MA3075 and MA3076) |
| OPAH | 5oxpro[c] + atp[c] + (2) h2o[c] -> adp[c] + glu-L[c] + h[c] + pi[c] | MA2300 |
| ORNCD | orn[c] <=> nh4[c] + pro-L[c] | MA3252 |
| ORNDC | h[c] + orn[c] -> co2[c] + ptrc[c] | MA2728 |
| ORNTAC | acorn[c] + glu-L[c] <=> acglu[c] + orn[c] | MA3564 |
| ORPT | orot5p[c] + ppi[c] <=> orot[c] + prpp[c] | (MA0919 or MA2520 or MA3307) |
| OSUCCL | h[c] + osuc[c] -> akg[c] + co2[c] | MA4265 |
| overall | (0.63) protein_met[c] + (0.24) rna_met[c] + (0.04) dna_met[c] + (0.05) lipid_met[c] + (0.04) trace_met[c] + (0.01) carb_met[c] + (65) atp[c] + (65) h2o[c] <=> biomass_met[c] + (65) h[c] + (65) adp[c] + (65) pi[c] | |
| OXADC | h[c] + oxa[c] -> co2[c] + for[c] | MA2076 |
| P5CRx | nad[c] + pro-L[c] <=> 1pyr5c[c] + (2) h[c] + nadh[c] | (MA0152 or MA4102) |
| P5CRy | nadp[c] + pro-L[c] <=> 1pyr5c[c] + (2) h[c] + nadph[c] | (MA0152 or MA4102) |
| PACCOAL | atp[c] + coa[c] + pac[c] -> amp[c] + phaccoa[c] + ppi[c] | (MA2244 or MA3853) |
| PACCOAL2 | 4hphac[c] + atp[c] + coa[c] -> amp[c] + ppi[c] + hphaccoa[c] | (MA2244 or MA3853) |
| PACCOAL3 | atp[c] + coa[c] + ind3ac[c] -> amp[c] + indaccoa[c] + ppi[c] | (MA2244 or MA3853) |
| PACt2r | h[e] + pac[e] <==> h[c] + pac[c] | |
| PAPSR | paps[c] + trdrd[c] -> (2) h[c] + pap[c] + so3[c] + trdox[c] | MA2894 |
| PC | atp[c] + hco3[c] + pyr[c] -> adp[c] + h[c] + oaa[c] + pi[c] | (MA0674 and MA0675) |
| PFK__adp_ | adp[c] + f6p[c] -> amp[c] + fdp[c] + h[c] | MA3563 |
| PGAMS | uacgam[c] ->polyacgal[c] + h[c] + udp[c] | |
| PGAMT | gam6p[c] -> gam1p[c] | MA3024 |
| PGCD | 3pg[c] + nad[c] <=> 3php[c] + h[c] + nadh[c] | MA0592 |
| PGLCURS | udpglcur[c] -> polyglcur[c] + h[c] + udp[c] | |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| PGI | g6p[c] <=> f6p[c] | MA0821 |
| PGK | 3pg[c] + atp[c] <=> 13dpg[c] + adp[c] | (MA2669 or MA3592) |
| PGLYCP | 2pglyc[c] + h2o[c] -> glyclt[c] + pi[c] | MA3544 |
| PGM | 2pg[c] <=> 3pg[c] | (MA0132 or MA2400 or MA2671 or MA4007) |
| PGMT | g1p[c] <=> g6p[c] | (MA0451 or MA2665) |
| PHEMES | ddcdscl[c] + fe2[c] + (3) nad[c] --> (2) ac[c] + (2) co2[c] + (3) nadh[c] + (4) h[c] + pheme[c] | |
| PHETA1 | akg[c] + phe-L[c] <=> glu-L[c] + phpyr[c] | (MA0636 or MA1385 or MA1819) |
| PHETRS | atp[c] + phe-L[c] + trnaphe[c] -> amp[c] + ppi[c] + phetrna[c] | (MA0171 and MA1956) |
| PIabc | atp[c] + h2o[c] + pi[e] --> adp[c] + h[c] + (2) pi[c] | (MA0887 and MA0888 and MA0889 and MA0890) or (MA3093 and MA3094 and MA3095) |
| PIt | pi[e] + h[e] --> pi[c] + h[c] | ( MA2935 or MA3014 ) |
| PMANM | man6p[c] <=> man1p[c] | (MA0241 or MA2665) |
| PMDPHT | 5aprbu[c] + h2o[c] -> 4r5au[c] + pi[c] | |
| PMPK | 4ampm[c] + atp[c] -> 2mahmp[c] + adp[c] | MA3197 |
| PMVD | 5pmev[c] + h[c] --> co2[c] + h2o[c] + ipp[c] | MA0601 |
| PNTOt2 | h[e] + pnto-R[e] <==> h[c] + pnto-R[c] | |
| POK | atp[c] + pant-R[c] -> adp[c] + ppant-R[c] + h[c] | MA1278 |
| POR2 | accoa[c] + co2[c] + (2) fdred[c] + h[c] <=> coa[c] + (2) fdox[c] + pyr[c] | ( MA0032 and (MA0031 or MA2407) and MA0033 and MA0034 ) |
| POR3 | co2[c] + (2) fdred[c] + h[c] + ppcoa[c] -> 2obut[c] + coa[c] + (2) fdox[c] | ( ( MA0032 and (MA0031 or MA2407) and MA0033 and MA0034 ) or (MA2909 and MA2910 and MA2911) ) |
| PPA | h2o[c] + ppi[c] -> h[c] + (2) pi[c] | MA2676 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| PPA2 | h2o[c] + pppi[c] --> h[c] + pi[c] + ppi[c] | (MA0083 or MA2351) |
| PPA_1 | h2o[c] + ppi[c] --> h[e] + (2) pi[c] | MA3880 |
| PPA_3 | h2o[c] + ppi[c] + na1[c] --> na1[e] + (2) pi[c] + h[c] | MA3879 |
| PPAKr | adp[c] + ppap[c] <=> atp[c] + ppa[c] | MA3606 |
| PPBNGS | (2) 5aop[c] -> h[c] + (2) h2o[c] + ppbng[c] | MA0578 |
| PPC | co2[c] + h2o[c] + pep[c] --> h[c] + oaa[c] + pi[c] | MA2690 |
| PPCDC | 4ppcys[c] + h[c] -> co2[c] + pan4p[c] | MA1280 |
| PPDK | atp[c] + pi[c] + pyr[c] -> amp[c] + h[c] + pep[c] + ppi[c] | MA0608 |
| PPK2 | atp[c] + ppi[c] -> adp[c] + pppi[c] | MA0081 |
| PPKr | atp[c] + pi[c] <=> ppi[c] + adp[c] | MA0081 |
| PPNCL2 | 4ppan[c] + ctp[c] + cys-L[c] -> 4ppcys[c] + cmp[c] + h[c] + ppi[c] | MA1280 |
| PPND | nad[c] + pphn[c] <=> 34hpp[c] + co2[c] + nadh[c] | MA4595 |
| PPNDH | h[c] + pphn[c] <=> co2[c] + h2o[c] + phpyr[c] | MA3150 |
| PPS | atp[c] + h2o[c] + pyr[c] -> amp[c] + (2) h[c] + pep[c] + pi[c] | (MA2458 or MA2667 or MA3408) |
| PPTS | ppant-R[c] + ala-B[c] + atp[c] --> amp[c] + ppi[c] + h[c] + 4ppan[c] | MA1278 |
| PRAGSr | atp[c] + gly[c] + pram[c] -> adp[c] + gar[c] + h[c] + pi[c] | MA3309 |
| PRAIi | pran[c] <=> 2cpr5p[c] | MA2988 |
| PRAIS | atp[c] + fpram[c] -> adp[c] + air[c] + (2) h[c] + pi[c] | MA0130 |
| PRAMPC | h2o[c] + prbamp[c] -> prfp[c] | MA0908 |
| PRASCS | 5aizc[c] + asp-L[c] + atp[c] <=> 25aics[c] + adp[c] + h[c] + pi[c] | MA4063 |
| PRATPP | h2o[c] + prbatp[c] -> h[c] + ppi[c] + prbamp[c] | MA1392 |
| PRE2DC | dscl[c] + (2) h[c] --> (2) co2[c] + ddcdscl[c] | |
| PRFGS | atp[c] + fgam[c] + gln-L[c] + h2o[c] -> adp[c] + fpram[c] + glu-L[c] + h[c] + pi[c] | (MA1963 or MA1964) and MA4055 |
| PRMICIi | prfp[c] -> prlp[c] | (MA0218 or MA4435) |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| PROabc | atp[c] + h2o[c] + pro-L[e] --> adp[c] + h[c] + pi[c] + pro-L[c] | (MA2145 and MA2146 and MA2147) |
| PROt4 | na1[e] + pro-L[e] --> na1[c] + pro-L[c] | (MA0279 or MA1316) |
| protein_met | (0.4) arg-L[c] + (0.85) leu-L[c] + (0.62) ser-L[c] + (0.62) ala-L[c] + (0.65) gly[c] + (0.36) pro-L[c] + (0.49) thr-L[c] + (0.62) val-L[c] + (0.66) ile-L[c] + (0.4) asn-L[c] + (0.48) asp-L[c] + (0.11) cys-L[c] + (0.23) gln-L[c] + (0.71) glu-L[c] + (0.15) his-L[c] + (0.59) lys-L[c] + (0.4) phe-L[c] + (0.33) tyr-L[c] + (0.21) met-L[c] + (0.09) trp-L[c] <=> protein_met[c] | |
| PROTRS | atp[c] + pro-L[c] + trnapro[c] -> amp[c] + ppi[c] + protrna[c] | MA3886 |
| PRPPP | h2o[c] + prpp[c] -> h[c] + pi[c] + r15bp[c] | MA2851 |
| PRPPS | atp[c] + r5p[c] <=> amp[c] + h[c] + prpp[c] | MA1167 |
| PSCVT | pep[c] + skm5p[c] <=> 3psme[c] + pi[c] | MA4544 |
| PSERT | akg[c] + pser-L[c] <=> 3php[c] + glu-L[c] | MA2304 |
| PSP_L | h2o[c] + pser-L[c] -> pi[c] + ser-L[c] | (MA0678 or MA4428 or MA4429) |
| PTAr | accoa[c] + pi[c] <=> actp[c] + coa[c] | MA3607 |
| PTHPS | ahdt[c] -> 6pthp[c] + pppi[c] | (MA0956 or MA4196) |
| PTPATi | atp[c] + h[c] + pan4p[c] <=> dpcoa[c] + ppi[c] | MA3546 |
| PYDXS | gln-L[c] + g3p[c] + r5p[c] --> (3) h2o[c] + pi[c] + pydx5p[c] + glu-L[c] + h[c] | (MA1566 and MA1567) |
| PYK | atp[c] + pyr[c] -> adp[c] + h[c] + pep[c] | MA3890 |
| PYRDC | h[c] + pyr[c] -> acald[c] + co2[c] | MA0594 |
| PYRFALDC | pyr[c] + fald[c] + h[c] --> acetol[c] + co2[c] | (MA3791 and MA3792) |
| PYRS-1 | amet[c] + 1pyr5c[c] --> 1pyr4m5c[c] + ahcys[c] + h[c] | MA0154 |
| PYRS-2 | 1pyr4m5c[c] + lys-L[c] + atp[c] --> pyr-L[c] + adp[c] + pi[c] + h[c] | MA0153 |
| PYRt2 | h[e] + pyr[e] <==> h[c] + pyr[c] | |
| PYRTHMPPTRX | pyr[c] + thmpp[c] + h[c] <=> he2thpp[c] + co2[c] | (MA1354 or MA1958 or MA3792) and MA3791 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| PYRTRS | pyr-L[c] + atp[c] + trnaala[c] -> amp[c] + ppi[c] + pyrtrna[c] | MA0155 |
| QULNS | dhap[c] + iasp[c] -> (2) h2o[c] + pi[c] + quln[c] + h[c] | MA0959 |
| R00430 | gtp[c] + pyr[c] -> gdp[c] + pep[c] | MA3890 |
| R01411 | 5mcyt[c] + h2o[c] <=> nh4[c] + thym[c] | MA2559 |
| R01986 | 4abutn[c] + h2o[c] + nadp[c] -> 4abut[c] + (2) h[c] + nadph[c] | MA2860 |
| R02549 | 4abutn[c] + h2o[c] + nad[c] -> 4abut[c] + (2) h[c] + nadh[c] | MA2860 |
| R03896 | r2mmal[c] <=> citrac[c] + h2o[c] | (MA0202 or MA1223) and MA1393 |
| R04215 | ctp[c] + glnam[c] <=> glnamcmp[c] + ppi[c] | MA3766 |
| R05680 | glyc1p[c] + nadp[c] -> dhap[c] + h[c] + nadph[c] | MA3686 |
| R07405 | 5c5pdriaz[c] <=> 5aizc[c] | MA1376 |
| R08576 | pser-L[c] + trnacys[c] + atp[c] --> cystrna(pser)[c] + amp[c] + ppi[c] | MA0090 |
| R2MMALSYN | accoa[c] + h2o[c] + pyr[c] -> coa[c] + r2mmal[c] + h[c] | MA3793 |
| RBFSa | 4r5au[c] + db4p[c] <=> dmlz[c] + (2) h2o[c] + pi[c] | (MA1817 and MA1818) |
| RBFSb | (2) dmlz[c] -> 4r5au[c] + ribflv[c] | (MA1817 and MA1818) |
| RBK | atp[c] + rib-D[c] <=> adp[c] + h[c] + r5p[c] | MA1373 |
| RBPC | co2[c] + h2o[c] + rb15bp[c] <=> (2) 3pg[c] + (2) h[c] | MA4555 |
| RBPI | r15bp[c] -> rb15bp[c] | MA2851 |
| RH2CD | Rh2cit[c] <=> h2acon-C[c] + h2o[c] | (MA3085 and MA3751) |
| RH3CD | Rh3cit[c] <=> h2o[c] + h3acon-C[c] | (MA3085 and MA3751) |
| RIBFLVt2 | h[e] + ribflv[e] --> h[c] + ribflv[c] | |
| rna_met | (0.48) atp[c] + (0.42) ctp[c] + (0.5) gtp[c] + (0.6) utp[c] <=> (2) ppi[c] + rna_met[c] | |
| RNDR1 | adp[c] + trdrd[c] -> dadp[c] + h2o[c] + trdox[c] | MA1665 |
| RNDR2 | gdp[c] + trdrd[c] -> dgdp[c] + h2o[c] + trdox[c] | MA1665 |
| RNDR3 | cdp[c] + trdrd[c] -> dcdp[c] + h2o[c] + | MA1665 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| | trdox[c] | |
| RNDR4 | trdrd[c] + udp[c] -> dudp[c] + h2o[c] + trdox[c] | MA1665 |
| RNF | (2) h[c] + (3) na1[c] + (2) fdred[c] + mphen[c] --> (3) na1[e] + (2) fdox[c] + mphenh2[c] | (MA0658 and MA0659 and MA0660 and MA0661 and MA0662 and MA0663 and MA0664 and MA0665) |
| RNTR1 | atp[c] + trdrd[c] -> datp[c] + h2o[c] + trdox[c] | MA0072 |
| RNTR2 | gtp[c] + trdrd[c] -> dgtp[c] + h2o[c] + trdox[c] | MA0072 |
| RNTR3 | ctp[c] + trdrd[c] -> dctp[c] + h2o[c] + trdox[c] | MA0072 |
| RNTR4 | trdrd[c] + utp[c] -> dutp[c] + h2o[c] + trdox[c] | MA0072 |
| RPI | r5p[c] <=> ru5p-D[c] | MA1683 |
| RU5PS | fald[c] + ru5p-D[c] <=> ah6p-D[c] | MA4608 |
| **Rxn name** | #N/A | **GPR** |
| RZ5PP2 | h2o[c] + 5pr5hbz[c] -> pi[c] + r5hbzi[c] | MA0940 |
| SADT2 | atp[c] + gtp[c] + h2o[c] + so4[c] -> aps[c] + gdp[c] + pi[c] + ppi[c] | MA3680 |
| SDPTA | akg[c] + sl26da[c] <=> glu-L[c] + sl2a6o[c] | MA0119 |
| SECS | 2tcc[c] + h[c] + nadh[c] -> nad[c] + sec[c] | |
| SERAT | accoa[c] + ser-L[c] <=> acser[c] + coa[c] | (MA2721 or MA3442) |
| SERTRS | atp[c] + ser-L[c] + trnaser[c] -> amp[c] + ppi[c] + sertrna[c] | MA4048 |
| SHCD | Shcit[c] <=> h2o[c] + hacon-C[c] | (MA3085 and MA3751) |
| SHCHCC | cobalt2[c] + dscl[c] -> copre2[c] + (3) h[c] | MA3631 |
| SHCHD2 | dscl[c] + nad[c] -> nadh[c] + scl[c] | MA0576 |
| SHCHF | fe2[c] + scl[c] -> (4) h[c] + sheme[c] | MA0576 |
| SHK3Dr | nadp[c] + skm[c] <=> 3dhsk[c] + h[c] + nadph[c] | MA4594 |
| SHKK | atp[c] + skm[c] -> adp[c] + h[c] + skm5p[c] | (MA1378 or MA3237) |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| SHSL2r | h2s[c] + suchms[c] <==> h[c] + hcys-L[c] + succ[c] | MA2715 |
| SMTZS | 2saa[c] + cys-L[c] -> 2tcc[c] + h2o[c] | |
| SO4t2 | so4[e] + h[e] --> so4[c] + h[c] | MA1999 |
| SPDC | 3spyr[c] + h[c] -> 2saa[c] + co2[c] | MA3298 |
| SPODM | (2) h[c] + (2) o2-[c] -> h2o2[c] + o2[c] | MA2422 |
| SSALx | h2o[c] + nad[c] + sucsal[c] <=> (2) h[c] + nadh[c] + succ[c] | (MA0705 or MA1355) |
| SSALy | h2o[c] + nadp[c] + sucsal[c] <=> (2) h[c] + nadph[c] + succ[c] | (MA0705 or MA1355) |
| SULabc | atp[c] + h2o[c] + so4[e] -> adp[c] + h[c] + pi[c] + so4[c] | (MA0280 and MA0281 and MA0282) or (MA0323 and MA0324 and MA0325) or (MA1235 and MA1236 and MA1237) or (MA2280 and MA2281 and MA2282) |
| SULR2 | h2s[c] + (3) f420-2[c] + (3) h2o[c] + h[c] <=> (3) f420-2h2[c] + so3[c] | (MA3439 or MA4520) |
| TDP | h2o[c] + thmpp[c] -> h[c] + pi[c] + thmmp[c] | MA3706 |
| TDPDRE | dtdp4d6dg[c] -> dtdp4d6dm[c] | (MA1909 or MA3780) |
| TDPDRR | dtdp4d6dm[c] + h[c] + nadph[c] -> dtdprmn[c] + nadp[c] | MA3778 |
| TDPGDH | dtdpglu[c] -> dtdp4d6dg[c] + h2o[c] | (MA2186 or MA3779) |
| THACH | h2o[c] + hacon-T[c] <=> Shcit[c] | |
| THMabc | atp[c] + h2o[c] + thm[e] --> adp[c] + h[c] + pi[c] + thm[c] | |
| THRA2i | athr-L[c] -> acald[c] + gly[c] | MA3520 |
| THRAr | thr-L[c] <=> acald[c] + gly[c] | MA3520 |
| THRPD | h[c] + thrp[c] -> applp[c] + co2[c] | MA0941 |
| THRPS | atp[c] + thr-L[c] -> adp[c] + h[c] + thrp[c] | |
| THRS | h2o[c] + phom[c] -> pi[c] + thr-L[c] | MA1610 |
| THRTRS | atp[c] + thr-L[c] + trnathr[c] -> amp[c] + ppi[c] + thrtrna[c] | MA2896 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| THZPSN | atp[c] + cys-L[c] + tyr-L[c] + dxyl5p[c] -> 4mpetz[c] + ala-L[c] + amp[c] + co2[c] + h[c] + h2o[c] + ppi[c] + 4hba[c] | (MA0236 or MA2718 or MA3264) and MA0255 and MA1466 |
| TIH2CD | nad[c] + tih2cit[c] -> co2[c] + nadh[c] + ohepa[c] | MA3748 |
| TIH3CD | nad[c] + tih3cit[c] -> 2ood[c] + co2[c] + nadh[c] | MA3748 |
| TIHCD | ihcit-T[c] + nad[c] -> co2[c] + nadh[c] + ohexa[c] | MA3748 |
| TMAMT | tma[c] + 5hbc_red[c] + h[c] -> dma[c] + m5hbc[c] | (MA0150 or MA0849 or MA4360) and (MA0528 or MA0932) and (MA0529 or MA0931) and (MA4379 or MA0146) |
| TMAt2 | h[e] + tma[e] <==> h[c] + tma[c] | MA0530 |
| TMDK1 | atp[c] + thymd[c] -> adp[c] + dtmp[c] + h[c] | MA4433 |
| TMDPP | pi[c] + thymd[c] -> 2dr1p[c] + thym[c] | MA3242 |
| TMDS | dump[c] + mlthf[c] <=> dhf[c] + dtmp[c] | MA4543 |
| TMKr | atp[c] + thm[c] <=> adp[c] + h[c] + thmmp[c] | |
| TMPKr | atp[c] + thmmp[c] -> adp[c] + thmpp[c] | MA0069 |
| TMPPP | 2mahmp[c] + 4mpetz[c] + h[c] -> ppi[c] + thmmp[c] | MA2722 |
| TPI | g3p[c] <=> dhap[c] | MA4607 |
| trace_met | (0.243) ptrc[c] + (0.044) hspmd[c] + (0.0009) accoa[c] + (0.00006) coa[c] + (0.0204) nad[c] + (0.00093) nadh[c] + (0.00093) nadp[c] + (0.00371) nadph[c] + (0.00003) succoa[c] + (0.00929) amp[c] + (0.191) com[c] + (0.00037) f420-2[c] + (0.00028) f420-3[c] + (0.00371) f420-4[c] + (0.00279) f420-5[c] + (0.00009) f420-6[c] + (0.00001) f420-7[c] + (0.219) h4spt[c] + (0.044) adocblhbi[c] + (0.019) f430[c] + (0.00464) cob[c] + (0.00093) thf[c] + (0.00005) f390a[c] + (0.00005) f390g[c] + (0.191) mfr(b)[c] <=> | |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| | trace_met[c] | |
| TRDR | h[c] + nadph[c] + trdox[c] -> nadp[c] + trdrd[c] | MA1368 |
| TRPS1 | 3ig3p[c] + ser-L[c] <=> g3p[c] + h2o[c] + trp-L[c] | MA2990 and (MA2991 or MA3198) |
| TRPS2 | indole[c] + ser-L[c] -> h2o[c] + trp-L[c] | (MA2991 or MA3198) |
| TRPS3 | 3ig3p[c] <=> g3p[c] + indole[c] | MA2990 |
| TRPt2r | trp-L[c] + h[c] <=> trp-L[e] + h[e] | MA1417 |
| TRPTA | akg[c] + trp-L[c] <=> glu-L[c] + indpyr[c] | MA0925 |
| TRPTRS | atp[c] + trp-L[c] + trnatrp[c] -> amp[c] + ppi[c] + trptrna[c] | MA0172 |
| TYRCBOX | h[c] + tyr-L[c] -> co2[c] + tym[c] | MA0006 |
| TYRTA | akg[c] + tyr-L[c] <=> 34hpp[c] + glu-L[c] | (MA0636 or MA1385 or MA1819) |
| TYRTRS | atp[c] + tyr-L[c] + trnatyr[c] -> amp[c] + ppi[c] + tyrtrna[c] | MA0815 |
| UAG2EMA | h2o[c] + uacgam[c] <=> h[c] + udp[c] + acmana[c] | |
| UAG4E | uacgam[c] <==> udpacgal[c] | (MA1185 or MA4460 or MA4464) |
| UAGDP | acgam1p[c] + h[c] + utp[c] -> ppi[c] + uacgam[c] | MA3025 |
| UDPG4E | udpg[c] <=> udpgal[c] | (MA1185 or MA4460 or MA4464) |
| UDPGDr | h2o[c] + (2) nad[c] + udpg[c] <=> (3) h[c] + (2) nadh[c] + udpglcur[c] | MA4457 |
| UMPK | atp[c] + ump[c] <=> adp[c] + udp[c] | MA0372 |

Table B.2. (continued)

| Reaction ID | Reaction | GPR |
|---|---|---|
| UNK_CBL1DEGt | unknown_cbl1deg[e] <==> unknown_cbl1deg[c] | |
| UNK_RBFDEGt | unknown_rbfdeg[e] <==> unknown_rbfdeg[c] | |
| UPP3MT | (2) amet[c] + uppg3[c] -> (2) ahcys[c] + dscl[c] + h[c] | MA3033 |
| UPP3S | hmbil[c] -> h2o[c] + uppg3[c] | MA3034 |
| URIDK2r | atp[c] + dump[c] <=> adp[c] + dudp[c] | MA4433 |
| VALt2r | h[e] + val-L[e] <==> h[c] + val-L[c] | (MA3437 and MA3438) |
| VALTA | akg[c] + val-L[c] <=> 3mob[c] + glu-L[c] | MA4349 |
| VALTRS | atp[c] + val-L[c] + trnaval[c] -> amp[c] + ppi[c] + valtrna[c] | MA3052 |
| VOR | 3mob[c] + coa[c] + (2) fdox[c] -> co2[c] + (2) fdred[c] + ibcoa[c] + h[c] | (MA2909 and MA2910 and MA2911) |
| WO4abc | wo4[e] + atp[c] + h2o[c] --> adp[c] + h[c] + pi[c] + wo4[c] | (MA0280 and MA0281 and MA0282) |
| XPPT | prpp[c] + xan[c] -> ppi[c] + xmp[c] | MA4581 |
| YUMPS | r5p[c] + ura[c] <=> h2o[c] + psd5p[c] | (MA0208 and MA3271) |
| ZN2t4 | zn2[c] + h[e] + k[e] <=> zn2[e] + h[c] + k[c] | MA1117 |
| ZNabc | atp[c] + h2o[c] + zn2[e] --> adp[c] + h[c] + pi[c] + zn2[c] | (MA0023 and MA0024 and MA0025) |
| Znabc2 | atp[c] + zn2[c] + h2o[c] --> adp[c] + h[c] + pi[c] + zn2[e] | (MA0549 or MA3366 or MA3632) |

Table B.3. Minimal (High Salt) media used for FBA simulations of the M. acetivorans model. Required and optional refer to whether the component is predicted to be essential based on FBA simulations.

| Model ID | Name | Turned on in simulations |
|---|---|---|
| ni2 | Nickel | Yes (required) |
| nh4 | Ammonia | Yes (required) |
| pi | Phosphate | Yes (required) |
| h2s | Hydrogen sulfide | Yes (required) |
| h2o | Water | Yes (required) |
| h | Proton (H+) | Yes (required) |
| cobalt2 | Cobalt | Yes (required) |
| cu2 | Copper | Yes (optional) |
| fe2 | Iron(II) | Yes (optional) |
| hco3 | Bicarbonate | Yes (optional) |
| na1 | Sodium | Yes (optional) |
| mg2 | Magnesium | Yes (optional) |
| cl | Chlorine | Yes (optional) |
| k | Potassium | Yes (optional) |
| mn2 | Manganese | Yes (optional) |
| mobd | molybdate | Yes (optional) |
| ac | Acetate | User's choice (Carbon source) |
| co | Carbon monoxide | User's choice (Carbon source) |
| dma | Dimethylamine | User's choice (Carbon source) |
| dms | Dimethylsulfide | User's choice (Carbon source) |
| meoh | Methanol | User's choice (Carbon source) |
| mma | Monomethylamine | User's choice (Carbon source) |
| tma | Trimethylamine | User's choice (Carbon source) |
| bo3 | Borate | No |
| cys-L | cysteine | No |
| seo3 | Selenium | No |
| so4 | Sulfate | No |
| wo4 | tungestenate | No |
| 4abz | 4-aminobenzoic acid | No |
| btn | Biotin | No |
| cbl1 | Vitamin B12 | No |
| fol | Folic acid | No |
| lipoate | alpha-Lipoic acid | No |
| nac | Nicotinic acid | No |

Table B.3. (continued)

| Model ID | Name | Turned on in simulations |
|---|---|---|
| pnto-R | (Calcium) pantothenate | No |
| pydxn | Pyrodoxine (HCl) | No |
| ribflv | Riboflavin | No |
| thm | Thiamine (HCl) | No |

Table B.4. Biomass objective function used in the *M. acetivorans* model. The "Overall" component was the actual function that was maximized – the individual components of the overall biomass reaction were further broken down into synthesized subunits shown in the other rows of the table.

| Component | Reaction |
|---|---|
| **Overall** | (0.63) protein_met + (0.24) rna_met + (0.04) dna_met + (0.05) lipid_met + (0.01) carbo_met + (0.04) trace_met --> biomass_met |
| **DNA** | (0.58) datp + (0.44) dctp + (0.44) dgtp + (0.59) dttp --> (2.05) ppi + dna_met |
| **RNA** | (0.48) atp + (0.42) ctp + (0.5) gtp + (0.6) utp --> (2) ppi + rna_met |
| **PROTEIN** | (0.4) arg-L + (0.85) leu-L + (0.62) ser-L + (0.62) ala-L + (0.65) gly + (0.36) pro-L + (0.49) thr-L + (0.62) val-L + (0.66) ile-L + (0.4) asn-L + (0.48) asp-L + (0.11) cys-L + (0.23) gln-L + (0.71) glu-L + (0.15) his-L + (0.59) lys-L + (0.4) phe-L + (0.33) tyr-L + (0.21) met-L + (0.09) trp-L --> protein_met |
| **LIPID** | (0.005) dpgpg + (0.214) 3hdpgpg + (0.027) dpgpi + (0.287) 3hdpgpi + (0.005) dpgpe + (0.057) 3hdpgpe + (0.011) dpgps + (0.244) 3hdpgps + (0.148) gdpgpi --> lipid_met |
| **CARBOHYDRATE** | (1.27) glycogen + (0.06) galactan + (2.49) polyacgal + (1.6) polyglcur --> carb_met |
| **SOLUBLE POOL** | (0.243) ptrc + (0.044) hspmd + (0.0009) accoa + (0.00006) coa + (0.0204) nad + (0.00093) nadh + (0.00093) nadp + (0.00371) nadph + (0.00003) succoa + (0.00929) amp + (0.191) com + (0.00037) f420-2 + (0.00028) f420-3 + (0.00371) f420-4 + (0.00279) f420-5 + (0.00009) f420-6 + (0.00001) f420-7 + (0.219) h4spt + (0.044) adocblhbi + (0.019) f430 + (0.00464) cob + (0.00093) thf + (0.00005) f390a + (0.00005) f390g + (0.191) mfr(b) --> trace_met |

# Appendix C. Experimental Validation Data

This Appendix lists the experimental data cited in the Main Text that was used to validate the model, along with the references for such data.

Table C.1. Growth and secretion rate data and references.

| **Quantity** | **Carbon source** | **Value** | Units | **Source** |
|---|---|---|---|---|
| Specific growth rate | CO | 0.029 | hr^-1 | (60) |
| Specific growth rate | Acetate | 0.023 | hr^-1 | (27, 66) |
| Specific growth rate | Acetate | 0.012-0.015 | hr^-1 | (7) |
| Specific growth rate | Acetate | 0.033 | hr^-1 | (68) |
| Specific growth rate | Methanol | 0.098 | hr^-1 | (27) |
| Specific growth rate | Methanol | 0.1-0.12 | hr^-1 | (7) |
| Specific growth rate | Methanol | 0.058-0.064 | hr^-1 | (51) |
| Specific growth rate | TMA | 0.077 | hr^-1 | (7) |
| Growth yield | Acetate | 2.400 | gDW/mol acetate | (66) |
| Growth yield | Acetate | 2.400 | g DW/mol acetate | (68) |
| Growth yield | Methanol | 4.8-5.6 | gDW/mol meoH | (73) |
| Growth yield | CO | 2.500 | gDW/mol CO | (60) |
| Acetate uptake rate | Acetate | 9.630 | mmol/gDW/hr | (27, 66) |
| Acetate uptake rate | Acetate | 3-10 | mmol/gDW/hr | (68) |
| Methanol uptake rate | Methanol | 17.5-20.4 | mmol/gDW/hr | (73) |
| CO uptake rate | CO | 4.5-9.4 | mmol/gDW/hr | (60) |
| CO uptake rate | CO | 11.600 | mmol/gDW/hr | (60) |
| Methane secretion | Acetate | 3.06-6.8 | mmol CH4/gDW/hr | (7) |
| Methane secretion | Acetate | 2.64-3.12 | mmol CH4/gDW/hr | (34) |
| Methane secretion | Methanol | 18.1-26.5 | mmol CH4/gDW/hr | (73) |
| Methane secretion | CO | 0.36-0.48 | mmol CH4/gDW/hr | (50) |
| Acetate secretion | CO | 0.38-0.42 | mmol ac/gDW/hr | (50) |
| Formate secretion | CO | 1.7-1.9 | mmol for/gDW/hr | (50) |
| DMS secretion | CO | 0.0027-0.0034 | mmol dms/gDW/hr | (50) |

Table C.2. Gene knockout lethality data used to validate the model, with references. "Genes in model": YES means the genes are included with at least one reaction in the model (the reactions associated with the appropriate gene(s) are shown in parenthesis).

| Genotype | Genes in model | Carbon source | Result | Reference |
|---|---|---|---|---|
| ΔackΔpta | YES (ACKr, PTAr) | Acetate | Lethal | (60) |
| ΔackΔpta | YES (ACKr, PTAr) | CO | Lethal | (60) |
| ΔackΔpta | YES (ACKr, PTAr) | Methanol | Viable | (60) |
| *ΔatpDCIXBEFAG* | YES (Naabc) | Methanol | Viable | (64) |
| *ΔatpDCIXBEFAG* | YES (Naabc) | TMA | Viable | (64) |
| *ΔatpDCIXBEFAG* | YES (Naabc) | Acetate | Viable | (64) |
| *ΔcooS1F* | YES (CODH2) | CO | Viable | (61) |
| *ΔcooS2* | YES (CODH2) | CO | Viable | (61) |
| ΔhdrABC | YES (HDR-2) | Methanol | Viable | (7) |
| ΔhdrABC | YES (HDR-2) | TMA | Viable | (7) |
| ΔhdrABC | YES (HDR-2) | Acetate | Viable | (7) |
| ΔhdrED | YES (HDR) | Acetate | Lethal | (7) |
| ΔhdrED | YES (HDR) | Methanol | Lethal | (7) |
| ΔhdrED | YES (HDR) | TMA | Lethal | (7) |
| Δmch | YES (MTSPC) | Acetate | Lethal | (27) |
| Δmch | YES (MTSPC) | Methanol | Lethal | (27) |
| Δmch::ech+ | NO (ECH not in WT) | MeOH | Lethal | (27) |
| Δmch::ech+ | NO (ECH not in WT) | MeOH/H2/CO2 | Viable | (27) |
| *ΔmtaA1* | YES (TMAMT, MCMMT) | Methanol | Lethal | (6) |
| *ΔmtaA1* | YES (TMAMT, MCMMT) | Acetate | Viable | (6) |
| *ΔmtaA1* | YES (TMAMT, MCMMT) | MMA | Viable | (6) |
| *ΔmtaA1* | YES (TMAMT, MCMMT) | DMA | Viable | (6) |
| *ΔmtaA1* | YES (TMAMT, MCMMT) | TMA | Viable | (6) |
| *ΔmtaA2* | NO | Methanol | Viable | (6) |
| *ΔmtaA2* | NO | Acetate | Viable | (6) |
| *ΔmtaA2* | NO | MMA | Viable | (6) |
| *ΔmtaA2* | NO | DMA | Viable | (6) |

Table C.2. (continued).

| Genotype | Genes in model | Carbon source | Result | Reference |
|---|---|---|---|---|
| ΔmtaA2 | NO | TMA | Viable | (6) |
| *ΔmtaB1C1ΔmtaB2C2ΔmtaB3C3* | YES (MCMMC) | Methanol | Lethal | (6) |
| *ΔmtaB1C1ΔmtaB2C2ΔmtaB3C3* | YES (MCMMC) | Acetate | Viable | (6) |
| *ΔmtaB1C1ΔmtaB2C2ΔmtaB3C3* | YES (MCMMC) | MMA | Viable | (6) |
| *ΔmtaB1C1ΔmtaB2C2ΔmtaB3C3* | YES (MCMMC) | DMA | Viable | (6) |
| *ΔmtaB1C1ΔmtaB2C2ΔmtaB3C3* | YES (MCMMC) | TMA | Viable | (6) |
| *ΔmtaA1ΔmtaB1C1ΔmtaB2C2ΔmtaB3C3* | YES (MCMMC) | Methanol | Lethal | (6) |
| *ΔmtaA1ΔmtaB1C1ΔmtaB2C2ΔmtaB3C3* | YES (MCMMC) | MMA | Viable | (6) |
| *ΔmtaA1ΔmtaB1C1ΔmtaB2C2ΔmtaB3C3* | YES (MCMMC) | DMA | Viable | (6) |
| *ΔmtaA1ΔmtaB1C1ΔmtaB2C2ΔmtaB3C3* | YES (MCMMC) | TMA | Viable | (6) |
| *ΔmtaA1ΔmtaB1C1ΔmtaB2C2ΔmtaB3C3* | YES (MCMMC) | Acetate | Lethal | (6) |
| *ΔmtbA* | YES (MMAMT, DMAMT, TMAMT) | Acetate | Viable | (6) |
| *ΔmtbA* | YES (MMAMT, DMAMT, TMAMT) | TMA | Viable | (6) |
| *ΔmtbA* | YES (MMAMT, DMAMT, TMAMT) | Methanol | Viable | (6) |
| *ΔmtbA* | YES (MMAMT, DMAMT, TMAMT) | DMA | Lethal | (6) |
| *ΔmtbA* | YES (MMAMT, DMAMT, TMAMT) | MMA | Lethal | (6) |
| *ΔmtsDΔmtsFΔmtsH* | YES (MTCMMT) | TMA | Viable | (49) |
| *ΔmtsDΔmtsFΔmtsH* | YES (MTCMMT) | Methanol | Viable | (49) |
| *ΔmtsDΔmtsFΔmtsH* | YES (MTCMMT) | Acetate | Viable | (49) |
| *ΔmtsDΔmtsFΔmtsH* | YES (MTCMMT) | CO | Viable | (49) |
| *ΔmtsXΔmtsY, X and Y any two mts genes* | YES (MTCMMT) | DMS | Viable | (49) |
| *ΔmtsDΔmtsFΔmtsH* | YES (MTCMMT) | DMS | Lethal | (49) |
| *ΔrnfXCDGEABY* | YES (RNF) | Acetate | Lethal | (18) |
| *ΔrnfXCDGEABY* | YES (RNF) | Methanol | Viable | (7) |
| *ΔrnfXCDGEABY* | YES (RNF) | DMS | Viable | (7) |
| *ΔrnfXCDGEABY* | YES (RNF) | MMA | Viable | (7) |
| *ΔrnfXCDGEABY* | YES (RNF) | DMA | Viable | (7) |
| *ΔrnfXCDGEABY* | YES (RNF) | TMA | Viable | (7) |
| *ΔlysK* | YES (LYSTRS) | Methanol | Viable | (40) |
| *ΔlysK* | YES (LYSTRS) | TMA | Viable | (40) |
| *ΔlysK* | YES (LYSTRS) | DMA | Viable | (40) |

Table C.2. (continued).

| Genotype | Genes in model | Carbon source | Result | Reference |
|---|---|---|---|---|
| *ΔlysK* | YES (LYSTRS) | MMA | Viable | (40) |
| *ΔlysS* | YES (LYSTRS) | Methanol | Viable | (40) |
| *ΔlysS* | YES (LYSTRS) | TMA | Viable | (40) |
| *ΔlysS* | YES (LYSTRS) | DMA | Viable | (40) |
| *ΔlysS* | YES (LYSTRS) | MMA | Viable | (40) |
| *Δmtr* | YES (MTSPCMMT) | Acetate | Lethal | (59) |
| *Δmtr* | YES (MTSPCMMT) | Methanol | Lethal | (59) |
| *Δmtr* | YES (MTSPCMMT) | TMA | Lethal | (59) |