

## Supplemental data, Section 1:

In the following section, we described the conflicting knowledge of some of the dead ends that are listed in Table S4 and our decision on how to resolve them.

### *Thiamine Biosynthesis:*

It has been observed in *in vivo* studies that thiamine uptake is essential for *H. pylori* growth (10). Therefore, a transport system for thiamine was included in *iIT341* GSM/GPR although no gene has been annotated for thiamine transport (15). On the other hand, three genes of the thiamin biosynthesis pathway have been identified in the genome (15) and the corresponding reactions were included in *iIT341* GSM/GPR. Since the reactions associated with these three genes do not describe the complete thiamine biosynthesis pathway, the network still had to uptake extracellular thiamine from the media.

### *Sulfate Metabolism:*

Cysteine and methionine contain a sulfur group that can be used by the network to synthesize sulfur containing compounds such as biotin. The previous model, *iCS291*, could not utilize methionine and thus the uptake of cysteine or inorganic sulfate was essential. However, in *iIT341* GSM/GPR, methionine can be utilized in place of these two compounds. This difference is a result of the changes in the methionine metabolism introduced in *iIT341* GSM/GPR. These changes included the deletion of METH (methionine synthase) and the inclusion of a sink reaction for S-adenosylhomocysteine in *iIT341* GSM/GPR (see Properties of the *iIT341* GSM/GPR metabolic network and Methionine Metabolism).

### *Phenylalanine Metabolism:*

In *H. pylori*, L-phenylalanine is thought to be an essential amino acid (10, 12). In a large number of prokaryotic and eukaryotic organisms, the PheA protein is bifunctional with a chorismate synthetase domain and a prephenate dehydratase domain (1, 5, 6). In *H. pylori*, the gene locus HP0291 shows sequence homology with the chorismate

synthetase domain of PheA, while the prephenate dehydratase domain is missing(1). Prephenate is an unstable precursor that spontaneously converts to phenylpyruvate(1). Thus, the biosynthesis pathway of L-phenylalanine is complete and *iIT341* GSM/GPR is able to synthesize L- phenylalanine. It has been suggested that the requirement of L-phenylalanine observed *in vitro* might be a consequence of the laboratory growth conditions(1, 3).

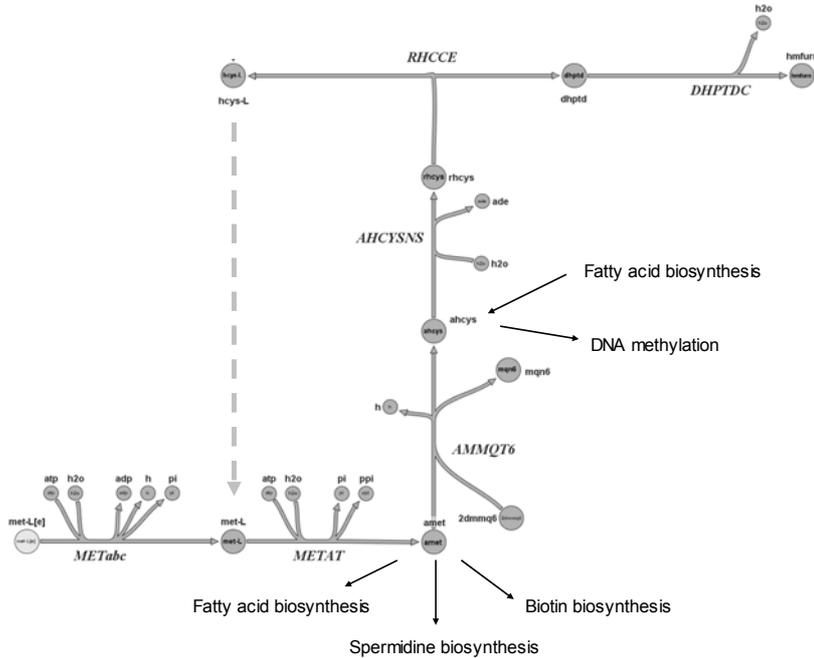
#### *Glutathione metabolism:*

The enzyme  $\gamma$ -glutamyltransferase has been rarely found in bacteria(14), but it has been annotated in the *H. pylori* genome (HP1118) with high sequence similarity to an ortholog in *Escherichia coli* K12(1, 15). This enzyme has an important function in the glutathione metabolism in mammalian cells. However, since the glutathione pathway is entirely absent in *H. pylori* except for this gene, the corresponding reaction is not connected to the rest of the network. It has been suggested that this gene product has a different function in *H. pylori* than that in mammalian cells. The gene has been found to be essential for the colonization of *H. pylori* in the human stomach(2). In addition, it has been hypothesized that this gene plays a role in the transport of amino acids (9).

#### *Biotin Biosynthesis:*

We have not found any references in the literature reporting biotin as an essential compound in minimal media for *H. pylori*. In addition, the genome annotation did not identify a transport system for biotin or for pimelate, a precursor of biotin(1, 15). However, it has been observed that *Bacillus sphaericus*, *Escherichia coli*, and *Pseudomonas dentrificans* are able to take up pimelate by passive diffusion (11). Therefore, it appears that such diffusion of pimelate might also be possible for *H. pylori*, although we were not able to find any report for this ability. Interestingly, four out of five genes of the biotin biosynthetic pathway have been annotated in the *H. pylori* genome(1, 15), but the gene encoding for pimelyl-CoA synthetase (EC 6.2.1.14) is missing. Since this gene has been identified in several other bacteria (4), we decided to include both the reaction catalyzed by pimelyl-CoA synthetase and a transport reaction for pimelate in *iIT341* GSM/GPR.

## Methionine Metabolism:



**Figure 1:** Schematic representation of the cycle of methyl group transfer. Dotted line: missing gene/gene product in *H. pylori*.

Experimental studies have shown that L-methionine is an essential amino acid for *H. pylori* (10, 12). The gene encoding for the enzyme that converts L-homocysteine to L-methionine (the last step in the L-methionine biosynthesis pathway) is not present in the *H. pylori* genome (1, 15). This reaction is also part of a cycle that transfers and recycles an activated methyl group from methionine to S-adenosylmethionine, and then to S-adenosylhomocysteine (Figure 1). S-adenosylmethionine is an important methyl group donor in the *H. pylori* metabolic network, but S-adenosylhomocysteine is not very connected with other reactions because it mostly participates in non-metabolic functions, such as DNA-methylation. Since S-adenosylhomocysteine must be consumed at steady state by the network, its consumption in the network was nominally represented by the reaction abbreviated “sink\_ahcys”.

### *Methionine salvage pathway*

The methionine salvage pathway is used to recycle methionine from methylthioadenosine. Genes participating in this pathway have been recently identified in various bacteria such as *Bacillus subtilis*, *Klebsellia pneumoniae*, *Leptospira interrogans*, *Thermobacter tengcongensis* and *Xylella fastidiosa* by Sekovska *et al.* (13). Although these organisms are not directly related to *H. pylori*, this study showed that methionine salvage pathway can take on slightly different routes in different bacteria. Therefore, it is possible the *H. pylori* contains a “non-tradition” salvage pathway, whose gene products could not be identified by homology. However, in absence of this information, we decided that *iIT341* GSM/GPR would have a methionine salvage pathway similar to that of *Bacillus subtilis*, although only one gene in this pathway (HP0089) has been annotated in the *H. pylori* genome. It was necessary to include this pathway in the model so that 5-methylthioadenosine, a by-product of spermidine biosynthesis, could be consumed. Therefore, indirectly, the addition of the methionine salvage pathway allowed the spermidine biosynthesis pathway to be functional.

### *Quinone Biosynthesis:*

Though ubiquinone has not been identified in *H. pylori* (8), the three genes (HP0396, HP1360, HP1483) normally associated with ubiquinone biosynthesis were annotated with extensive homology to the UPF0096 family (1, 15). On the other hand, menaquinone 6 was observed to be most abundant type of quinone in *H. pylori* (8), but no gene specific for menaquinone biosynthesis has been found (1, 15). The gene product of HP1483 can perform the last step of ubiquinone biosynthesis as well as menaquinone biosynthesis (7). We decided to include a complete biosynthesis pathway for menaquinone in the network, but only included three mentioned genes and reactions in the synthesis pathway of ubiquinone. As a result, these three reactions are not connected with other reactions in the network.

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