Metabolic capabilities *in silico* of the human pathogen *Helicobacter pylori*

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Abstract

The human pathogen *Helicobacter pylori* colonizes the stomach of roughly half of the world's population and is thought to cause gastritis and gastric ulcer. A second version of the metabolic network of this organism, *i*IT341, was reconstructed based on the revised genome annotation and new experimental data. This genome-scale reconstruction represents a detailed review of the current knowledge about *H. pylori*'s metabolism by integrating biochemical and genomic data in a comprehensive framework.

Phenotypic properties of wild type and mutants were investigated *in silico* using constraint-based modeling approaches. Single gene deletion studies predicted 60 percent of *H. pylori*'s metabolic genes (conditional) essential genes in minimal medium. Assessment of its growth capabilities showed that the sensitivity of this micro-aerophilic pathogen to high oxygen concentrations was not attributable to the stoichiometric structure of its metabolic network but that a very small amount of oxygen provided a large percentage of the maximal growth potential of the network. The superoxide dismutase deficient mutant was found to be hyper oxygen-sensitive *in vivo* and *in silico* but this sensitivity could be reversed *in silico* by addition of nitric oxide in medium. Furthermore, L-threonine, L-alanine, D-alanine, L-aspartate and L-serine were found to be the preferred carbon and nitrogen sources for the network based on relative growth rate per carbon and nitrogen. These results illustrate that genome-scale metabolic reconstructions can be used to obtain network-level understanding of cellular functions and to create novel hypotheses on open biological questions.

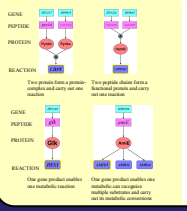
*i*IT341 GSM/GPR

✓Based on the previously published genome-scale metabolic network [1].
✓All network reactions were charge- and mass balanced.
✓Gene-Protein-Reactions (GPR) association were included if known.
✓Provides the first comprehensive map for *H. pylori*'s metabolism.
✓Confidence level were assigned to each network reaction.

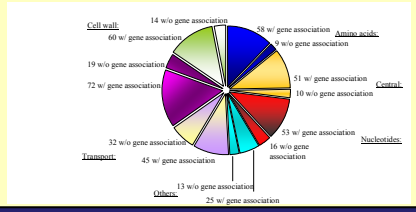
Network content:

	*i*IT341 GSM/GPR
No. of genes included	341
No. of gene associated reactions	355
No. of other reactions	123
No. of total reactions	478
No. of metabolites (internal/ external)	411/76
No. of exchange fluxes	76
Dimension of S-matrix (metabolites x reactions)	485*554

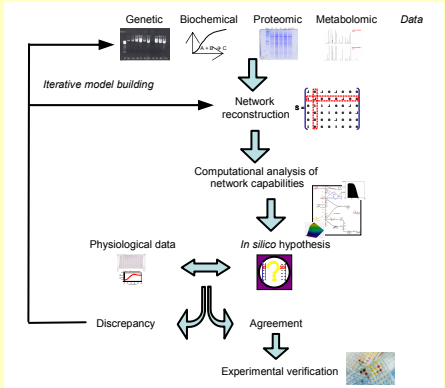
GPR association:



Network reactions per metabolic subsystem:



In silico hypotheses

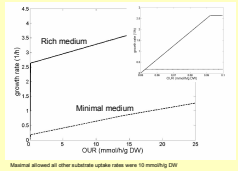


Available 'omics' data are integrated in a comprehensive framework (metabolic, regulatory or signaling reconstruction), and were represented as a stoichiometric matrix. Mathematical methods can be applied to investigate network capabilities under different simulation conditions. Results represent new hypotheses that can be addressed experimentally.

Oxygen sensitivity

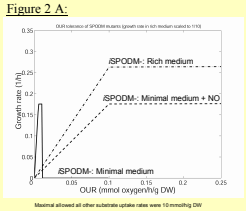
✓Sensitivity of *H. pylori* to high oxygen concentration was not a result of the structure of metabolic network.
✓A very small amount of oxygen provided a large percentage of the maximum possible growth potential of the network (Figure 1).

Figure 1: Growth capacities under different oxygen uptake rates (OUR).



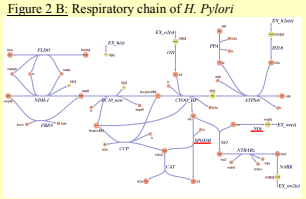
Maximal allowed all other substrate uptake rates were 10 mmol/h/g DW

Figure 2 A:



Maximal allowed all other substrate uptake rates were 10 mmol/h/g DW

Figure 2 B: Respiratory chain of *H. Pylori*



✓Hyper-oxygen-sensitive of superoxide dismutase deficient mutant (*i*SPODM-) could be reversed *in silico* only by addition of Nitric Oxid (NO) in medium (Figure 2 A).
✓Oxygen radicals were removed by nitrite reductive pathway (Figure 2B).

In silico knock-out mutants

Single knockout mutants:

✓60 % and 37.5 % were predicted (conditional) essential metabolic genes in minimal medium and rich medium, respectively.
✓75 % of phenotypes of *in silico* deletion mutants were predicted correctly by model (72 model genes were compared with experimental data).

Double knockout mutants:

✓More than 22,000 possible knock-out mutants were screened .
✓47 conditionally lethal double mutants were identified involving 64 different metabolic genes.

Figure 1: Conditional Essential Genes/ Reactions per subsystem in Minimal Medium

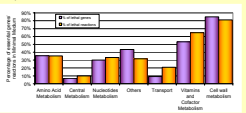
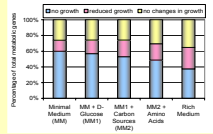


Figure 2: (Conditional) Essential genes in various media

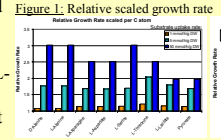


✓A high fraction of essential genes implies that *H. pylori* is especially adapted to its environment and has only limited ability to tolerate environmental disturbances (i.e. substrate supply, acidity or mutagenic agents).

Growth capabilities

✓L-Threonine was the preferred carbon- and nitrogen source (Figure 1A).
✓ L-Threonine, L-,D-Alanine, L-Aspartate, and L-Serine were equally good nitrogen sources at higher uptake rates (Figure 1B).

Figure 1: Relative scaled growth rate



Both experiments were carried out in Minimal Medium + 1 medium compound supplemented.

Table 1: Comparison of growth on amino acids

Amino Acid	Clinical isolate AM1/97	Strain NCTC 11637	Clinical isolate Roberts	Clinical isolate 26695	*i*IT341 GSM/GPR	Amino Acid	Clinical isolate AM1/97	Strain NCTC 11637	Clinical isolate Roberts	Clinical isolate 26695	*i*IT341 GSM/GPR
L-Alanine	+	+	+	+	+	L-Lysine	+	+		(+)	+
L-Arginine	+	+	+	+	+	L-Methionine	+	+		+	+
L-Asparagine	+	+	-	+	+	L-Phenylalanine					(+)
L-Aspartate	+	+		+	+	L-Proline		+	(+)	+	+
L-Cysteine	+	+		+	+	L-Serine				+	+
L-Glutamate	+	+		+	+	L-Tryptophan				(+)	+
L-Glutamine	+	+	-	+	+	L-Threonine	(+)			+	+
L-Histidine	+	+		+	+	L-Tyrosine					(+)
L-Isoleucine		(+)		+		L-Valine	(+)	+		-	
L-Leucine		+		+							

✓ Comparison with clinical strains showed diverse preference of growth enhancing amino acids (Table 1).

Conclusion

Our results demonstrate that genome-scale metabolic reconstructions can be used to i) obtain network-level understanding of cellular functions, and ii) create novel hypotheses on open biological questions. These results can then be addressed with experimental studies. Thus, combined efforts from *in silico* and *in vivo* studies will be particularly useful for organisms which are difficult to cultivate in the laboratory.

References and Acknowledgments

[1] Schilling, C. H., *et al.* 2002. Genome-scale metabolic model of *Helicobacter pylori* 26695. Journal of Bacteriology 184:4582-4593.
[2] Thiele, I., *et al.* 2005. "An Expanded Metabolic Reconstruction of *Helicobacter pylori* (iIT341 GSM/GPR): An in silico genome-scale characterization of single and double deletion mutants", Journal of Bacteriology,187(16): 5818-5830
[3] Thiele, I., *et al. Submitted*. "Metabolic capabilities of *Helicobacter pylori* 26695 in silico: Oxygen tolerance, Reactive oxygen species, growth enhancers and secretion products".
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