Metabolic capabilities \textit{in silico} of the human pathogen \textit{Helicobacter pylori}

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Abstract

The human pathogen \textit{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, \textit{iJT341}, 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 \textit{H. pylori}'s metabolism by integrating biochemical and genomic data in a comprehensive framework.

Phenotypic properties of wild type and mutants were investigated \textit{in silico} using constraint-based modeling approaches. Single gene deletion studies predicted 60 percent of \textit{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 \textit{in vivo} and \textit{in silico} but this sensitivity could be reversed \textit{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.

\textit{iJT341 GSM/GPR}

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

\textbf{GPR association:}

\begin{tabular}{|c|c|}
  \hline
  \textbf{Gene} & \textbf{Protein} \\
  \hline
  \hline
  \end{tabular}

\textbf{Network reactions per metabolic subsystem:}

\begin{tabular}{|c|c|}
  \hline
  \textbf{Subsystem} & \textbf{Reactions} \\
  \hline
  \hline
  \end{tabular}

\textbf{Sensitivity of \textit{H. pylori} to high oxygen concentration was not a result of the structure of metabolic network.}

\textbf{A very small amount of oxygen provided a large percentage of the maximum possible growth potential of the network (Figure 1).}

\textbf{Hyper-oxygen-sensitive of superoxide dismutase deficient mutant (iSPOMD-) could be reversed \textit{in silico} only by addition of Nitric Oxid (NO) in medium (Figure 2 A).}

\textbf{Oxygen radicals were removed by nitrite reductive pathway (Figure 2B).}

\textbf{In silico hypotheses}

\textbf{In silico knock-out mutants}

\begin{itemize}
  \item Single knockout mutants: \textit{60} % and \textit{37.5} % were predicted (conditional) essential metabolic genes in minimal medium and rich medium, respectively.
  \item \textit{75} % of phenotypes of \textit{in silico} deletion mutants were predicted correctly by model (72 model genes were compared with experimental data).
\end{itemize}

\textbf{Double knockout mutants:}

\begin{itemize}
  \item More than \textit{22,000} possible knock-out mutants were screened.
  \item \textit{47} conditionally lethal double mutants were identified involving \textit{64} different metabolic genes.
\end{itemize}

\textbf{Oxygen sensitivity}

\textbf{Growth capabilities}

\begin{itemize}
  \item \textit{L}-Thrreonine was the preferred carbon- and nitrogen source (Figure 1A).
  \item \textit{L}-Thrreonine, \textit{L}-D-Alanine, \textit{L}-Aspartate, and \textit{L}-Serine were equally good nitrogen sources at higher uptake rates (Figure 1B).
\end{itemize}

\textbf{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 \textit{in silico} and \textit{in vivo} studies will be particularly useful for organisms which are difficult to cultivate in the laboratory.

\textbf{References and Acknowledgments}


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