

Comparison of *Brachyspira* central metabolism pathways using genomic sequence data

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1 Introduction

The *Brachyspira hyodysenteriae* and *B. pilosicoli* are anaerobic spirochaetes that colonize the large intestine and cause diarrhea. We recently sequenced the 3.0 and 2.6 Mb genomes of *B. hyodysenteriae* and *B. pilosicoli*, respectively. The complete genome sequences were analyzed, and putative biological functions were assigned to 1,829 (69%) of the 1,685 (73%) predicted protein-encoding genes. Annotation of the genome, however, is only the first step toward understanding the function of genes. To understand biological function, individual genes have to be placed into a biological context, including the context of metabolic pathways. Reconstruction of metabolic pathways therefore will assist in our understanding *in silico* predictions of gene function and the metabolic (potential) capabilities of an organism. Many databases and tools that aid metabolic reconstruction can be found on the World Wide Web (1). We have used genomic information to construct the major metabolic pathways of *B. hyodysenteriae* and *B. pilosicoli* and performed a comparative analysis of the metabolic genes and pathways of *B. hyodysenteriae* and *B. pilosicoli*. Overall, the metabolic pathways of *B. hyodysenteriae* were similar to *B. pilosicoli*, but there were notable differences in several pathways including nucleotidesugar metabolism, lipopolysaccharide biosynthesis and the synthesis of cell wall components. Our results provide a useful guide to the post-genomic analysis of *B. hyodysenteriae* and *B. pilosicoli* and lead to a better understanding of the predicted structure and functional of these spirochaetes.

2 Materials and Methods

2.1 Organisms

The complete genomic sequences of *B. hyodysenteriae* WA-1 and a draft of the genome sequence of *B. pilosicoli* 95/1000 were recently obtained and annotated at the Centre for Comparative Genomics (CCG), Murdoch University.

2.2 Prediction and annotation of protein coding sequences

Metabolic and genetic analysis of *B. hyodysenteriae* and *B. pilosicoli* was based on previous published annotation data. Annotation of COGs of putative functional genes was further confirmed by performing a BLAST search against the COG database (e-value >1-5, multiple assignments per protein allowed) (7). Metabolic pathways were subsequently analyzed using the Kyoto encyclopedia of genes and genomes (KEGGs) metabolic database (2, 4). Each gene that was implicated in a metabolic pathway was manually confirmed by a BLAST search of KEGG genes using BLASTP program (e-value >1-5).

3 Results

Analysis of the genome sequences provided a global view of the metabolic potential of *B. hyodysenteriae* and *B. pilosicoli* (Fig. 1). Of the 2,652 (*B. hyodysenteriae*) and 2,297 (*B. pilosicoli*) predicted proteins, 801 (30%) and 781 (34%) predicted proteins were identified as enzymes, of which 375 (14%) and 365 (15%) were assigned Enzyme Commission (EC) numbers. This is considerably fewer than the roughly one-quarter to one-third of the genes in bacterial and archeal genomes that can be mapped to KEGG pathway diagrams (3). This suggests either that *B. hyodysenteriae* and *B. pilosicoli* have a similar proportion of its genome devoted to enzymes, or that enzymes are more difficult to identify in *B.*

hyodysenteriae and *B. pilosicoli* by sequence similarity methods. However, many biochemical pathways could be reconstructed in their entirety, suggesting that this similarity-searching approach was for the most part successful, and that the relative paucity of enzymes in *B. hyodysenteriae* and *B. pilosicoli* may relate to their life-style in large intestine.

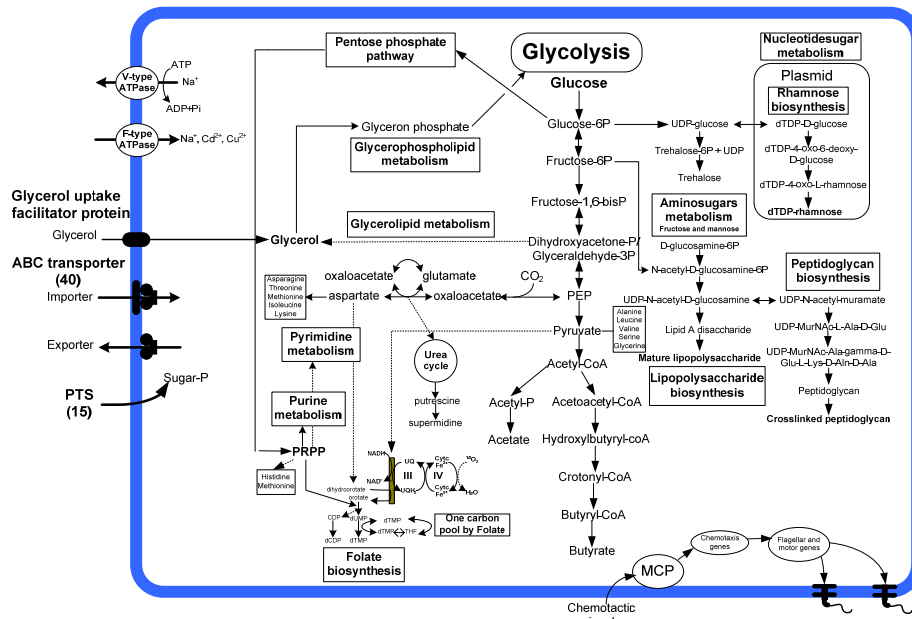


Figure 1. Key metabolic pathways in *B. hyodysenteriae* and *B. pilosicoli*

Genome sequence analysis revealed that the enzymes of these pathways are present in *B. hyodysenteriae* and *B. pilosicoli*. The central metabolic pathways are a glycolytic pathway and non-oxidative pentose phosphate pathway (Fig. 1). Briefly, the central carbon metabolic pathway in *B. hyodysenteriae* and *B. pilosicoli* are most similar to that in *Leptospira*. *B. hyodysenteriae* and *B. pilosicoli* have a complete glycolysis pathway and pentose phosphate pathway, but an incomplete TCA cycle. This incomplete reductive type of cycle probably functions primarily in carbon assimilation and the generation of precursor metabolites for biosynthesis (5). Some of the previously identified metabolic characteristics of *Brachyspira*, such as the absence of the TCA (6), were confirmed by genomic analysis. A complete set of genes for glycolysis, nucleotide metabolism and lipopolysaccharide biosynthesis, and respiratory electron transport chain were identified in *B. hyodysenteriae* and *B. pilosicoli* (Fig. 1). This was consistent with the notion that the organisms generate ATP by oxidative phosphorylation.

References

- [1] Duarte, N. C., Becker, S. A., Jamshidi, N., Thiele, I., Mo, M. L., Vo, T. D., Srivas, R. & Palsson, B. O. (2007). Global reconstruction of the human metabolic network based on genomic and bibliomic data. *Proceedings of the National Academy of Sciences of the United States of America* 104, 1777-1782.
- [2] Kanehisa, M. & Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, 28, 27-30.
- [3] Kanehisa, M. (2002). The KEGG database. Novartis Foundation symposium 247, 91-101; discussion 101-103, 119-128, 244-152.
- [4] Kanehisa, M., Goto, S., Kawashima, S. & Nakaya, A. (2002). The KEGG databases at GenomeNet. *Nucleic Acids Research*, 30, 42-46.
- [5] Romano, A. H. & Conway, T. (1996). Evolution of carbohydrate metabolic pathways. *Research in Microbiology*, 147, 448-455.
- [6] Stanton, T. B. (1989). Glucose metabolism and NADH recycling by *Treponema hyodysenteriae*, the agent of swine dysentery. *Applied and Environmental Microbiology*, 55, 2365-2371.
- [7] Tatusov, R. L., Galperin, M. Y., Natale, D. A. & Koonin, E. V. (2000). The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Research*, 28, 33-36.