Comparative genomics to investigate genome function and adaptations in the newly sequenced *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*

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A Thesis presented for the degree of

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Dedication

I would like to dedicate this dissertation to my family

and my fiancée Ratchaneekorn.

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ABSTRACT

Brachyspira hyodysenteriae and *Brachyspira pilosicoli* are anaerobic intestinal spirochaetes that are the aetiological agents of swine dysentery and intestinal spirochaetosis, respectively. As part of this PhD study the genome sequence of *B. hyodysenteriae* strain WA1 and a near complete sequence of *B. pilosicoli* strain 95/1000 were obtained, and subjected to comparative genomic analysis. The *B. hyodysenteriae* genome consisted of a circular 3.0 Mb chromosome, and a 35,940 bp circular plasmid that has not previously been described. The incomplete genome of *B. pilosicoli* contained 4 scaffolds. There were 2,652 and 2,297 predicted ORFs in the *B. hyodysenteriae* and *B. pilosicoli* strains, respectively. Of the predicted ORFs, more had similarities to proteins of the enteric *Clostridium* species than they did to proteins of other spirochaetes. Many of these genes were associated with transport and metabolism, and they may have been gradually acquired through horizontal gene transfer in the environment of the large intestine.

A construction of central metabolic pathways of the *Brachyspira* species identified a complete set of coding sequences for glycolysis, gluconeogenesis, a non-oxidative pentose phosphate pathway, nucleotide metabolism and a respiratory electron transport chain. A notable finding was the presence of *rfb* genes on the *B. hyodysenteriae* plasmid, and their apparent absence from *B. pilosicoli*. As these genes are involved in rhamnose biosynthesis it is likely that the composition of the *B. hyodysenteriae* lipooligosaccharide O-sugars is different from that of *B. pilosicoli*. O-antigen differences in these related species could be associated with differences in their specific niches, and/or with their disease specificity. Overall, comparison of *B. hyodysenteriae* and *B. pilosicoli* protein content and analysis of their central metabolic pathways showed that they have

diverged markedly from other spirochaetes in the process of adapting to their habitat in the large intestine.

The presence of overlapping genes in the two *Brachyspira* species and in other spirochaete species also was investigated to determine their functional role, if any. The number of overlapping genes in the 12 spirochaete genomes examined ranged from 11-45%. Of these, 80% were unidirectional. Overlapping genes were found non-uniform distributed within the *Brachyspira* genomes such that 70-80% of them occurred on the same strand (unidirectional, →→/←←), with 16-28% occurring on opposite DNA strands (divergent, ←→). The remaining 4-6% of overlapping genes were convergent (→←). The majority of the unidirectional overlap regions were relatively short, with >50% of the total observations overlapping by >4 bp. A small number of overlapping gene pairs was duplicated within each genome and there were some triplet overlapping gene pairs. Unique orthologous overlapping gene pairs were identified within the various spirochaete genera. Over 75% of the overlapping genes in the *Brachyspira* species were in the same or related metabolic pathway. This finding suggests that overlapping genes are not only likely to be the result of functional constraints but also are constrained from a metabolomic context. Of the remaining 25% overlapping genes, 50% contained one hypothetical gene with unknown function. In addition, in one of the orthologous overlapping gene pairs in the *Brachyspira* species, a promoter was shared, indicating the presence of a novel class of overlapping gene operon in these intestinal spirochaetes.

Declaration

The work in this thesis is based on research carried out at the Centre for Comparative Genomics (CCG), Murdoch University, Australia. I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary institution.

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(Phatthanaphong Wanchanthuek)

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List of publications:

1) **Wanchanthuek, P**., Hampson, D.J., and Bellgard, M. 2009. Analysis of overlapping genes in the newly sequenced of *Brachyspira* and other spirochaetes indicates their likely role in streamlining metabolic pathways (*in preparation*).

2) Bellgard, M*., **Wanchanthuek, P***., La, T., Ryan, K., Moolhuijzen1, P., Zlbertyn, A., Shaban, B., Motro, Y., Dunn, D., Schibeci, D., Hunter, A., Barrero, R., Phillips, N., and Hampson, D. 2009. Genome sequence of the pathogenic intestinal spirochete *Brachyspira hyodysenteriae* reveals adaptations to its lifestyle in the porcine large intestine. *PLoS ONE* 4, e4641.

* These authors contributed equally to this work.

3) **Wanchanthuek, P**., Hampson, D.J. and Bellgard, M. 2008. Conservation and metabolic functional significance of overlapping gene in the bacterial genomes. The 20th Annual Meeting and International Conference of the Thai Society for Biotechnology. October 14-17, 2008 Maha Sarakham, Thailand.

4) **Wanchanthuek, P**., Ryan, K., Moolhuijzen, P., Albertyn, Z., Shaban, B., La, T., Hampson, D.J. and Bellgard, M. 2008. Comparison of *Brachyspira* central metabolism pathway using the genomic DNA sequence. The International Conference on Genome Informatics. Gold Coast, Australia, 1-3 December 2008.

5) **Wanchanthuek, P**., Ryan, K., Moolhuijzen, P., Albertyn, Z., Shaban, B., La, T., Hampson, D.J. and Bellgard, M. 2008. A plasmid-borne O-antigen in *Brachyspira hyodysenteriae*. The Seventh Asia Pacific Bioinformatics Conference, Beijing, China, 13-16 January 2009.

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