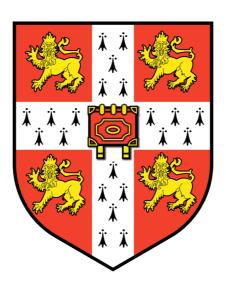
Functional and evolutionary analyses of pneumococcal genome variation

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This dissertation is submitted for the degree of Doctor of Philosophy

Declaration

Declaration

I hereby declare that this dissertation is my own work and contains nothing that is the

outcome of work done in collaboration with others, except where specifically

indicated here or elsewhere in the text.

All sequence data were produced by the sequencing and research and development

teams at the Wellcome Trust Sanger Institute. The annotation of complete genome

sequences was performed in conjunction with Dr. Stephen Bentley (Wellcome Trust

Sanger Institute, Cambridge). Phylogenetic analyses of bacterial genome sequences

were performed in collaboration with Dr. Simon Harris (Wellcome Trust Sanger

Institute, Cambridge). All microarray experiments and statistical analyses were

performed by the BµG@S group (St. George's Hospital, London). All statistical

analyses of Omnilog data were performed by Lars Barquist (Wellcome Trust Sanger

Institute, Cambridge).

None of the work described herein has been previously submitted for the purpose of

obtaining another degree. This dissertation does not exceed 60,000 words in length, as

required by the School of Biological Sciences.

Nicholas Jason Croucher

August 2011

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p.s. Mum, to save you flicking through to the end: no, I still haven't cured anything.

Abstract

Functional and evolutionary analyses of pneumococcal genome variation

Nicholas Jason Croucher

Streptococcus pneumoniae (the pneumococcus) is a human nasopharyngeal commensal and respiratory pathogen responsible for a high burden of morbidity and mortality worldwide. The bacterium's primary virulence factor appears to be its polysaccharide capsule, of which there are more than 90 different serologicallydistinguishable types (serotypes). Although this categorisation was originally used for tracing pneumococcal epidemiology, the bacterium is naturally transformable, and hence is able to switch serotypes through horizontal exchange of capsule biosynthesis (cps) gene clusters. Therefore, following the emergence of multidrug-resistant lineages in the late 1970s, superior, multilocus-based typing schemes were devised for following pneumococcal evolution. Increasing antibiotic resistance also motivated the development of a heptavalent conjugate polysaccharide vaccine, which targeted seven S. pneumoniae serotypes, leading to a decrease in pneumococcal disease. However, this impact has been ameliorated by an increase in disease resulting from replacement by non-vaccine serotypes and switching of cps loci by strains previously expressing vaccine serotypes. This thesis describes the application of second-generation sequencing technologies to investigating the mechanisms by which the pneumococcus evolves, especially in response to such clinical interventions.

The first part concerns the Pneumococcal Molecular Epidemiology Network clone 1 (PMEN1) lineage, one of the first multidrug-resistant pneumococcal genotypes to become a worldwide problem. Complete sequencing of the *S. pneumoniae* ATCC 700669 type strain, combined with draft sequencing of a global collection of 240 isolates, quantified the impact of recombination across the chromosome, as well as revealing the diversity of conjugative elements and prophage in the population. The acquisition of antibiotic resistances and the evasion of the conjugate polysaccharide vaccine were both evident in among the strains. *In vitro* transformation experiments, in the same genetic background, were then used to perform a more detailed

investigation of the types of homologous recombination events seen in the global population.

The second part of this dissertation describes the use of RNA sequencing to investigate the functional consequences of genomic variation. A novel method was developed and validated, and, when applied to *S. pneumoniae* ATCC 700669, revealed a family of expressed putative coding sequences that were formed by extended forms of the BOX interspersed repeat. This technique was also applied to two closely related strains of the PMEN31 lineage, both isolated from a single case of disease. This allowed the functional consequences of a small number of distinguishing polymorphisms on the global transcriptome to be ascertained, providing an insight into the level of pneumococcal evolution that can occur within an individual. Sequencing further members of this lineage showed that, although highly successful, this lineage has a much more static genotype than that of PMEN1.

The different mechanisms of pneumococcal genome variation are associated with evolution over different timescales, and in response to different selection pressures, but clearly interact in a number of ways. Hence the use of whole genome sequencing, surveying all the variation throughout the chromosome, will be crucial for greater understanding, and therefore improved control, of this important pathogen.

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Abbreviations

aa	Amino acid
ABC	ATP-binding cassette
ATCC	American type culture collection
ATP	Adenosine triphosphate
BAC	Bacterial artificial chromosome
BHI	Brain heart infusion
BLAST	Basic local alignment search tool
bp	Base pair
CBP	Choline-binding protein
CC	Clonal complex
cDNA	Complementary DNA
CDS	Coding sequences
CGH	Comparative genome hybridisation
CSF	Cerebrospinal fluid
CSP	Competence stimulating peptide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
ds	Double stranded
DUS	DNA uptake sequence
EDTA	Ethylenediaminetetraacetic acid
EMBL	European Molecular Biology Laboratories
FR	Flanking region
GC	Guanine and cytosine
GMP	Guanosine monophosphate
HIV	Human immunodeficiency virus
HMM	Hidden Markov model
ICE	Integrative and conjugative element
IPD	Invasive pneumococcal disease
IS	Insertion sequence
LB	Luria broth
MCS	Multiple cloning site

MEPS	. Minimum efficiently processed segment
MGE	. Mobile genetic element
MITE	. Miniature inverted repeat transposable element
MLEE	. Multilocus enzyme electrophoresis
MLST	Multilocus sequence typing
MMR	Mismatch repair
MR	Mosaic recombination
mRNA	. Messenger RNA
nt	. Nucleotide
NTP	. Nucleoside triphosphate
ORF	. Open reading frame
PBP	. Penicillin binding protein
PCR	. Polymerase chain reaction
PCV	. Polysaccharide conjugate vaccine
PEP	Phosphoenolpyruvate
PFGE	. Pulsed field gel electrophoresis
PMEN	. Pneumococcal molecular epidemiology network
PPI-1	. Pneumococcal pathogenicity island 1
PTS	. Phosphotransferase system
PUS	patAB upregulatory SNP
rDNA	. Ribosomal DNA
RNA	. Ribonucleic acid
RPKM	. Reads per kilobase per million mapped reads
rRNA	. Ribosomal RNA
RSS	. Recombined sequence segment
RT-PCR	. Reverse transcription PCR
RUP	. Repeat unit of pneumococcus
SGST	. Second generation sequencing technology
SNP	. Single nucleotide polymorphism
SS	. Single stranded
ST	. Sequence type
TCA	. Tricarboxylic acid
TIR	. Terminal inverted repeats
tRNA	. Transfer RNA

TSD	. Target sequence duplication
USS	. Uptake signal sequence
UTR	. Untranslated region
UV	. Ultraviolet
VNTR	. Variable number tandem repeat