Integrons in Pseudomonads are Associated with hotspots of genomic diversity.

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Synopsis

Integrons associated with mobile genetic elements have played a central role in the emergence and spread of multiple antibiotic resistance in many pathogenic bacteria. However, the discovery of integrons in the chromosomes of diverse, non-pathogenic bacteria suggests that integrons have a broader role in bacterial evolution. The Pseudomonas stutzeri species complex is a well studied model for bacterial diversity. Members of the complex are genetically closely related, but sub-taxa are not able to be defined by exclusively shared sets of phenotypic characters. Rather, on the basis of total DNA:DNA similarity, Ps. stutzeri strains have been divided into 17 different groups (termed genomovars). Two Ps. stutzeri strains have been found to contain Chromosomal Integrons (CIs). This thesis involved exploration of the hypothesis that a CI was present in the common ancestor of the Ps. stutzeri species complex and assessed the impact of integrons on diversity across all Pseudomonads. The history and significance of integrons is discussed in Chapter 1 as part of a literature review, and general materials and methods are provided in Chapter 2. Chapters 3 – 6 comprise the sections in which data generated during my PhD project are presented. A comprehensive analysis of the relationships between the strains being analysed is presented in Chapter 3. In Chapter 4, results of PCR and hybridisation screening for integrons across the strain collection are presented. In Chapter 5 the recovery of additional integrons and in depth sequence analysis of the recovered integrons are described. Finally, Chapter 6 contains statistical analyses of integron-associated genes and Chapter 7 contains a final discussion the most significant findings. Twenty-three Pseudomonas spp. strains were screened for the presence of integrons. All but three were found to contain integron-like sequences; however, most integron sequences recovered contained inactivated core integrons.
Despite having a chromosomal locus, integrons in *Pseudomonas* were found to have properties indicative of frequent horizontal transfer. Evidence was also obtained which suggests that integrons have been acquired at the same locus on multiple independent occasions. This has not been observed in other families of chromosomal integrons and suggests that the loci at which integrons in *Pseudomonas* are found are hotspots for recombination.
STATEMENT OF CANDIDATE

This work is original and has not been submitted for a higher degree to any other university or institution. The work of others, when drawn upon, is referenced fully.

Approximately 50% of the DNA sequencing performed in Chapter 5 was performed by another researcher. All analyses of DNA sequence data presented in this thesis were performed by the author.

Signed

Neil Wilson

Date
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Abbreviations

59-be – 59-base element
bp – Base pairs
CA – correspondence analysis
CI – chromosomal integron
CTAB – Hexadecyltrimethylammonium bromide
DIG-6-dUTP – digoxygenin-6-dUTP
DNA – Deoxyribonucleic acid
EDTA – Ethylenediaminetetraacetic acid
Gv. – Genomovar
HGT – horizontal gene transfer
IGS1 – 16S-23S rDNA intergenic spacer
IntI – integron integrase
LB broth – Luria-Bertani broth
MI – mobile integron
PCA – principal components analysis
PCR – polymerase chain reaction
RFLP – Restriction fragment length polymorphism
RSCU – relative synonymous codon usage
SSC – Standard Saline Citrate
SDS – Sodium dodecyl sulfate
TBE buffer – Tris borate EDTA buffer
TE buffer – Tris EDTA buffer