The Analysis of Plasmid Rearrangements Observed in the Soil Bacterium OR168 After the Introduction of Transposon Tn*5*

A thesis presented in fulfilment of the requirements
for the degree of
Master of Science
in Genetics at Massey University, Palmerston North.

David John Gerard Harte

1994

ABSTRACT

Transposon Tn*5* mutagenesis has been used extensively in *Escherichia coli* and various other Gram-negative bacteria to produce both random and site directed mutants. The popularity of Tn*5* as a mutagen stems from its apparent random insertion into the genome, leading to non-leaky polar mutations. It also confers on many bacteria resistance to aminoglycosides, providing a strong selectable marker. The site of insertion can be mapped by Southern DNA hybridisation against a specific Tn*5* probe.

Tn*5*-containing derivatives of the Rhizobia-like soil isolate, OR168, were produced using the broad host-range suicide plasmid vector pSUP1011. After the transfer of pSUP1011 to OR168 via heterogeneric bacterial conjugation, stable OR168::Tn*5* exconjugants were selectively isolated at frequencies of approximately 10^{-4} per recipient. None of the 53 OR168::Tn*5* exconjugants screened showed the parental plasmid profile. Visible alterations to the plasmid profile were common with respect to the native plasmid profile. These events generally showed large deletions from, or additions to, the native replicons of OR168. The alterations also included a low incidence of a decrease in plasmid number. Analysis of the exconjugant population shows that the insertion of Tn*5* into the genome of OR168 may not be strictly random. It was shown that 66% of OR168::Tn*5* exconjugants screened contain a plasmid-borne Tn*5* element, with 90% of those involving Tn*5* insertion in the same episome. There is evidence that events other than classical conservative transposition have occurred after the introduction of pSUP1011 into the OR168 genome.

Screening of the isolated OR168::Tn*5* population for pSUP1011 vector sequences revealed the presence of the pSUP1011-derived RP4-*mob* fragment in 33 of 35 OR168::Tn*5* exconjugants containing a plasmid-borne Tn*5* element. Analysis also revealed the acquisition of Tn*5* alone, presumably by conservative transposition, occurred only twice in the 35 events involving a plasmid target. This suggests that another site within the RP4 fragment can act as a surrogate transposase recognition site. Alternatively, the insertion of the RP4-*mob*::Tn*5* sequence into a plasmid target may involve a site specific recombination process peculiar to the OR168 isolate.

No mechanism was elucidated for the formation of many of the alterations in plasmid mobility. Restriction fragment lengths in the immediate vicinity of the anomalous RP4-*mob*::Tn*5* insertion are identical in different plasmids. This may indicate sequence duplication among the OR168 plasmids. Such duplication may precipitate, through homologous recombination processes, the plasmid instability observed.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AMPPD	3-(2′-Spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy) -phenyl-1,2-dioxetane
AMP~D	Unstable dephosphorylated intermediate of AMPPD degradation
AP	Alkaline phosphatase
Ap	Ampicillin
ATP	Adenine triphosphate
BCIP	5-Bromo-4-chloro-2-indolylphosphate
bp	Base pair
CCC	Covalently closed circular
Cm	Chloramphenicol
CsCl	Cesium chloride
CTAB	Hexadecyltrimethyl ammonium bromide
DIG	Digoxigenin
DM	Distance migrated
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
EDTA	(Ethylenedinitrilo) tetra-acetic acid
EtBr	Ethidium bromide
IE	Inside end (of IS*50* and Tn*5*)
IncF	Incompatibility group F plasmid
IncP	Incompatibility group P plasmid
IS	Insertion sequence
kb	Kilobase pairs
Km	Kanamycin
LB	Luria-Bertani medium
NBT	4-Nitro blue tetrazolium chloride
Nm	Neomycin
nt	Nucleotide
OC	Open circular
OE	Outside end (of IS*50* and Tn*5*)
oriT	Origin of transfer
RM	Relative electrophoretic mobility
Sp	Spectinomycin
SSC	Standard sodium citrate
ssDNA	single-stranded DNA
TBE	Tris-borate-EDTA
Tc	Tetracycline
TE	Tris-EDTA buffer
tra	Transfer genes
Tris	2-Amino-2-(hydroxymethyl)- 1,3-propanediol acetate
Tn	Transposon
TY	Tryptone Yeast extract medium