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A Study into DNA Recombination Proteins and Novel Plasmid Recombination Sites from *Acinetobacter baumannii*

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Statement of Originality

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Abstract

The discovery of multiple, inverted, *dif*-like recombination sites (p*dif*) flanking antibiotic resistance genes (*dif* modules), has prompted great interest in the area. Many of these recombination sites are located on plasmids originating from clinical isolates of the serious pathogen *Acinetobacter baumannii*. The study hypothesied inverted p*dif* sites on either side of a gene could function as a novel gene transfer and / or gene shuffling system via Xer site-specific recombination. Xer site-specific recombination is a process where the recombination proteins XerC and XerD bind to *dif* sites to catalyse two rounds of DNA strand cleavage, exchange and ligation. The recombination of *dif* sites on a plasmid can lead to the excision or inversion of the internal DNA, depending on the orientation of the recombination sites. Understanding this process could uncover mechanisms by which antibiotic resistance genes can mobilise and disseminate throughout bacterial populations.

The current study evaluated the binding interaction between two p*dif* sites and the *A. baumannii* recombinases XerC and XerD. A series of electrophoretic mobility shift assays (EMSA) demonstrated XerC and XerD cooperatively bind to the p*dif* sites, a crucial step preceding the catalysis of Xer site-specific recombination.

The study then predicted potential cross-species binding interactions between *A. baumannii* $FtsK_{\gamma}$ ($FtsK_{\gamma\_AB}$) and *E. coli* XerD ($XerD_{EC}$), an interaction necessary to activate catalysis of Xer site-specific recombination. A combination of predictive structural software, and hydrophobic and electrostatic protein profiles, revealed the

possibility of a cross-species binding interaction. Subsequent *in vivo* recombination assays of the *A. baumannii* chromosome *dif* site, involving $FtsK\gamma_{\_AB}$ and $XerD_{EC}$ resulted in weak levels of recombination, a likely indication $FtsK\gamma_{\_AB}$ and $XerD_{EC}$ do interact to allow recombination to proceed.

Other *in vivo* recombination assays involved the inverted p*dif* sites, which were able to undergo recombination to generate inversion products. The recombination assay was performed within a host *E. coli* cell, which demonstrated the ability of *E. coli* Xer proteins to catalyse recombination events at variant *dif* sites. The ability of non-native Xer recombinases to recognise and catalyse variant *dif* sites could be prevalent in other bacterial species, which could aid the mobilisation of *dif* modules harbouring antibiotic resistance genes.

The study also investigated the *A. baumannii* DNA translocase FtsK ($FtsK_{AB}$), which could serve as a future therapeutic target. $FtsK_{AB}$ was confirmed to be a strong DNA-dependent ATPase. However, the identification of an FtsK orientating polar sequence (KOPS) on the *A. baumannii* chromosome remains elusive. The study suggested several octomers that could potentially function as the KOPS motif on the *A. baumannii* chromosome. Yet, none of the listed octomers were as highly skewed or over-represented as the well-established KOPS motif GGGNAGGG on the *E. coli* chromosome. Nevertheless, it is likely the KOPS motif on the *A. baumannii* chromosome differs from that on the *E. coli* chromosome. The difference in motif sequence could indicate key FtsK proteins folds responsible for recognising the

substrate DNA differs between the two species, which could be beneficial in developing new antibiotics specifically targeting FtsK within pathogenic strains of Acinetobacter.

Overall, the study demonstrated the novel p*dif* sites can be recognised and cooperatively bound by the *A. baumannii* Xer recombinases. Additionally, the p*dif* sites can undergo recombination within *E. coli*, which serves as an early indication that the novel p*dif* sites could function as a gene transfer and / or gene shuffling system, in support of the hypothesis.

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DNA	deoxyribonucleic acid
AA	amino acid
AAA+	ATPases associated with various cellular activities
ADP	adenosine diphosphate
ADPnP	ATP analog 6'-adenylyl-/3,y-imidodiphosphate
AMR	antimicrobial resistance
ATP	adenosine triphosphate
ATPγS	adenosine 5'-O-(3-thio)triphosphate
BLAST	Basic Local Alignment Search Tool
CRAb	Carbapenem resistant *Acinetobacter baumannii*
Cryo-EM	cryogenic electron microscopy
dif	deletion induced filamentation region
dsDNA	double stranded DNA
EcFtsK$_C$	*E. coli* FtsK C-terminal domain
EM	electron microscopy
EMSA	electrophoretic mobility shift assay
FtsK	filamenting temperature sensitive (protein) K
FtsK$_{50C}$	*E. coli* FtsK C-terminal domain, with 50 amino acid linker region
FtsK$_C$	FtsK C-terminal domain
FtsK$_\gamma$	FtsK gamma subdomain
FtsK$_N$	N – terminal domain of FtsK
FtsK$_{\alpha\beta}$	alpha – beta (motor) subdomain of FtsK
GFP	green fluorescent protein

HJ	Holliday junction
HR	homologous recombination
IS	insertion sequence
KOPS	FtsK orienting polar sequence
MCS	Multiple cloning site
MW	Molecular weight
oriC	origin of chromosome replication
PaFtsK$_{C}$	*P. aeruginosa* FtsK C-terminal domain
PaFtsK$_{C\Delta\gamma}$	*P. aeruginosa* FtsK C-termina domain, without gamma subdomain
PaFtsK$_{\alpha\beta}$	*P. aerugnisa* FtsK C-terminal domain, with alpha and beta subdomains
PCR	polymerase chain reaction
Pi	inorganic phosphate
RI	resistance island
RNA	ribonucleic acid
RS	restriction site
SEC	size exclusion chromatography
ssRNA	single stranded ribonucleic acid
ter	terminus region
Xer	chromosomal encoded recombinase
XerC$_{AB}$	*A. baumannii* XerC
XerC$_{EC}$	*E. coli* XerC
XerD$_{AB}$	*A. baumannii* XerD
XerD$_{EC}$	*E. coli* XerD