Université de Montréal

Cloning and characterization of *xerC* gene of *Streptococcus suis*

par

Fuli Jia

Département de microbiologie et immunologie
Faculté de Médecine

Mémoire présenté à la Faculté des études supérieures
en vue de l'obtention du grade de Maître ès sciences (M. Sc.)
en microbiologie et immunologie

Décembre, 2005

Ce mémoire intitulée :

Cloning and characterization of *xerC* gene of *Streptococcus suis*

présentée par :

Fuli Jia

a été évalué par un jury composé des personnes suivantes :

.....Dr France Daigle.....
président-rapporteur

.....Dr George Szatmari.....
Directeur de recherche
.....Dr Josée Harel.....
Membre du jury

Résumé

XerC et XerD, deux recombinases impliquées dans la recombinaison site spécifique, résolvent des plasmides multimères en monomères. Les multimères générés lors de la réplication du chromosome circulaire chez les bactéries, sont en général très instables et peuvent engendrer des pertes de matériel génétique. Le rôle important de XerC et de XerD est donc de veiller à la stabilité chromosomique chez les bactéries. Membres de la famille des tyrosines recombinases, ont retrouvent plusieurs homologues de ces protéines chez les bactéries. Chez *Staphylococcus aureus*, *Streptococcus pneumoniae* et *Pseudomonas fluorescens*, des mutants Xer atténuent l'infection et la pathogénicité chez la souris. Chez *Streptococcus suis*, une bactérie gram positive impliquée dans de nombreuses maladies chez l'animal et l'humain, une forte homologie de séquences a été trouvée entre un gène et celui de *xer* chez les bactéries *Streptococcus*. Pour déterminer l'implication de Xer dans la pathogénicité chez *Streptococcus suis*, nous avons cloné, surexprimé et purifié XerC dans le but de réaliser des études de fonctionnalité avec cette protéine. Comme c'est le cas chez *Bacillus subtilis*, les résultats montrent que XerC s'attache sur l'ADN au site *dif*. D'autre part, nous montrons qu'une mutation dans XerC affecte la croissance et cause d'importants changements morphologiques.

Mots-clés : Recombinasion spécifique de site /tyrosine recombinase/XerC/*dif*/ *Streptococcus suis*

Summary

XerC and XerD mediated site-specific recombination contributes to the stability of circular chromosomes in bacteria by resolving plasmid mutlimers and chromosome dimers to monomers prior to cell division. The XerC and XerD proteins are members of the tyrosine recombinase family. Homologues of *xerC/xerD* genes have been found in many bacteria. Recently, *xer* mutants in *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas fluorescens* have demonstrated reduced pathogencity suggesting a possible relationship between Xer proteins and the pathogenicity of these bacteria. *Streptococcus suis* is a Gram-positive bacterium, which is a leading cause of a wide range of diseases in animals and is also implicated in human diseases. The analysis of the *S. suis* genomic sequence demonstrated the presence of an open reading frame (ORF) that shows strong homology to the *xer* genes of streptococcal bacteria. In our project, we cloned, overexpressed and purified the *xerC* gene and its product as a maltose binding protein fusion. The function of XerC protein was characterized and showed DNA binding activity at *dif* site of *Bacillus subtilis.* The *dif* site of *S. suis* was also discovered during this work and the XerC protein of *S. suis* showed specific binding to this site. A *S. suis xerC* mutant showed a slower growth rate and displayed significant morphological differences.

Keywords: *Streptococcus suis*/Site-specific recombination/tyrosine recombinase/XerC/*dif*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

ARTICLE

APPENDIX

List of Symbols and Abreviations

AMINO ACIDS

A: alanini

C: cystein

D: aspartic acid

E: glutamic acid

F: phenylalanine

G: glycine

H: histidine

I: isoleucine

K: lysine

L: leucine

M: methionine

N : asparagine

P: proline

Q: glutamine

R: arginine

S: serine

T: threonin

V: valine

W: tryptophane

MEASUREMENT UNITS

Å: angstrom unit

bp: base pair

cm: centimetre

Da: Dalton

g: gram

h: hour

kb: kilobase

kDa: kilodalton

µg: microgram

µl: microlitre

µM: micromole

min: minute

ml : millilitre

mM : millimole

ng: nanogram

s: second

×g: centrifugation speed

°C: Degree celsius

v/cm: volt per centimeter

OTHERS

Ap: ampicillin

ATP: adenosine triphosphate

ATPase: adenosine triphosphatase

BSA: bovine serum albumin

BsArgR: ArgR of *Bacillus subtilis*

CodVBs: CodV of *Bacillus subtilis*

C-terminal: carboxyl-terminal

DAPI: 4', 6-diamidino-2-phenylindole

difBs: *dif* site of *Bacillus subtilis*

difEc: *dif* site of *Escherichia coli*

difSs: *dif* site of *Streptococcus suis*

DIG: digoxygenine

DNA: deoxyribonucleic acid

EDTA:ethylenedinitrolotetraacetic acid

HJ: Holliday junction

IPTG: isopropyl B-D thigalactoside

LB: Luria-Bertani

MBP: maltose binding protein

NaCl: sodium chloride

NEB: New England Biolabs

OD600nm: optical density at 600 nanometre

ORF: open reading frame

PAGE: polyacrylamide gel electroporesis

PBS: phosphate bufferred saline

PCR: polymerase chain reaction

SDS: sodium dodecyl sulfate

TBE: tris-borate EDTA buffer

THA: Todd-Hewitt broth with agar

Ts: thermosensitive

α: alpha β: beta λ: lambda

THY: Todd-Hewitt broth with 1% yeast extract

UV: ultraviolet

XerCEc: XerC of *Escherichia coli*

XerDEc: XerD of *Escherichia coli*

XerCSs: XerC of *Streptococcus suis*

Y: tyrosine

λ Int: λ phage integrase

3'OH: three prime hydroxyl

3'PO4: three prime phosphate

Chapter I

Introduction

1. Site-Specific Recombination

Recombination is a ubiquitous process where organisms reshuffle their genetic information. This genetic exchange occurs between DNA molecules from the two parents or between two DNA segments within the same molecule. Such recombination may be general, occurring between two DNA substrates with extensive homology, which is called general homologous recombination, or site-specific, occurring between two specific, relatively short DNA targets, which is designated site-specific recombination.

In DNA rearrangements mediated by site-specific recombination, four DNA strands are broken, exchanged and resealed at specific positions of two separate recombination sites (Stark *et al.,* 1992; Landy, 1993; Nash, 1996; Jayaram, 1994). Outcomes of a recombination event depend on the relative disposition of the two sites. Intramolecular recombination between inverted or directly repeated sites will invert or excise respectively the intervening DNA segment. Recombination between sites on separate DNA molecules will integrate one molecule into the other (Figure 1).

These different structural consequences of site-specific recombination lead to various biological functions. It includes integration and excision of bacteriophages into and out of bacterial chromosomes, inversion gene switches that provides alternative gene expression of bacterial cell surface proteins and of phage tail fibers, conversion of initial products of intermolecular genetic transposition into transposition end products, copy number control and stable inheritance of microbial plasmids, and normal partition of the *Escherichia coli* chromosome (reviewed by Sadowski, 1986; Stark *et al.*, 1992).

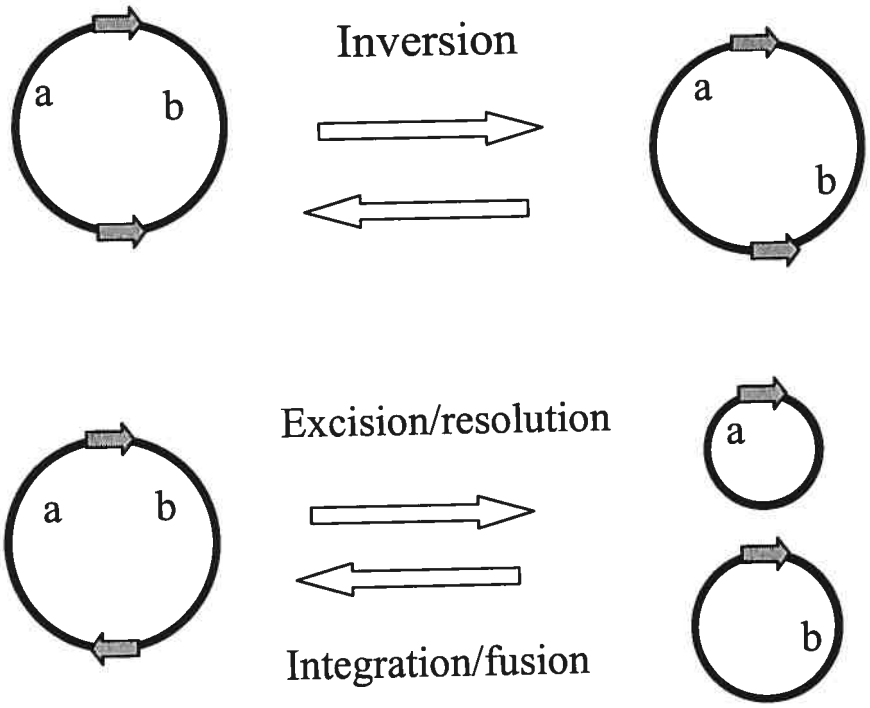


Figure 1 Outcomes from site-specific recombination. Triangles show the orientation of the recombination sites. a and b indicate the position of distinct genetic markers and the recombination loci. 'Excision' and 'integration' refer to recombination events involving genetic entities of different size and /or function (e.g., the bacterial chromosome and a phage genome), whereas 'resolution' and 'fusion' apply to equivalent DNA molecules, (e.g., two plasmids) (Adapted from Hallet and Sherratt, 1997).

Site-specific recombinases utilise a topoismerase I-like mechanism, cleaving and rejoining one strand of DNA per promoter. A complete recombination event therefore requires at least four molecules of the recombinase, two on each DNA recombination partner. DNA strand exchange is conservative in two ways: there are no deletions or additions of nucleotides at the site of exchange and there is no need for high- energy factors. A transient 3'-phosphotyrosine/phosphoserine linkage between protein and DNA conserves the energy of the cleaved phosphodiester bond (Nunes-Düby *et al.,* 1998).

Site-specific recombinases fall into one of two unrelated families, the resolvase/DNA invertase family and the lambda integrase family (Hatfull and Grindley, 1988; Argos *et al.,* 1986; Sadowski, 1986; Stark *et al.,* 1992). Enzymes of both families catalyze conservative DNA break-join reactions that proceed by two-step transesterifications in which protein phosphodiesters act as reaction intermediates.

1.1 The Resolvase/Invertase Family

The resolvase/invertase family, of which there are currently approximately 40 different members, forms a rather homogenous group of related proteins in which a conserved serine residue plays a key catalytic role (Hatfull *et al.,* 1988; Leschziner *et al.,* 1995). The best-characterized recombinases of this family are the invertases Gin from bacteriophage Mu and Hin from *Salmonella sp.* and the resolvases of Tn*3* and γδ transposons (Stark *et al.,* 1992; Van de Putte *et al.*, 1992; Grindley *et al.,* 1994; Stark *et al.*, 1995; Johnson, 1995; Johnson, 1991).

In a recombination catalyzed by resolvases or invertases, double strand breaks staggered by 2 bp occur at the middle of the two paired core sites, giving rise to recessed 5' ends and 3'-OH overhangs. One recombinase subunit is linked to each of the 5' ends through the conserved the serine residue of the family (Reed *et al.,* 1984; Klippel *et al.,* 1988). This serine presumably provides the primary nucleophile hydroxyl group in the cleavage reaction (Leschziner *et al.,* 1995). The ligation step that follows strand exchange can be viewed as the converse of the cleavage: the protein-DNA phosphoseryl bond of one strand is attacked by the 3'-OH end of the partner to release the enzyme and reseal the DNA backbone in the recombinant configuration (Figure 2). Thus, recombination by a resolvase/invertase family occurs by a mechanism in which four DNA strands are broken and rejoined in a concerted manner.

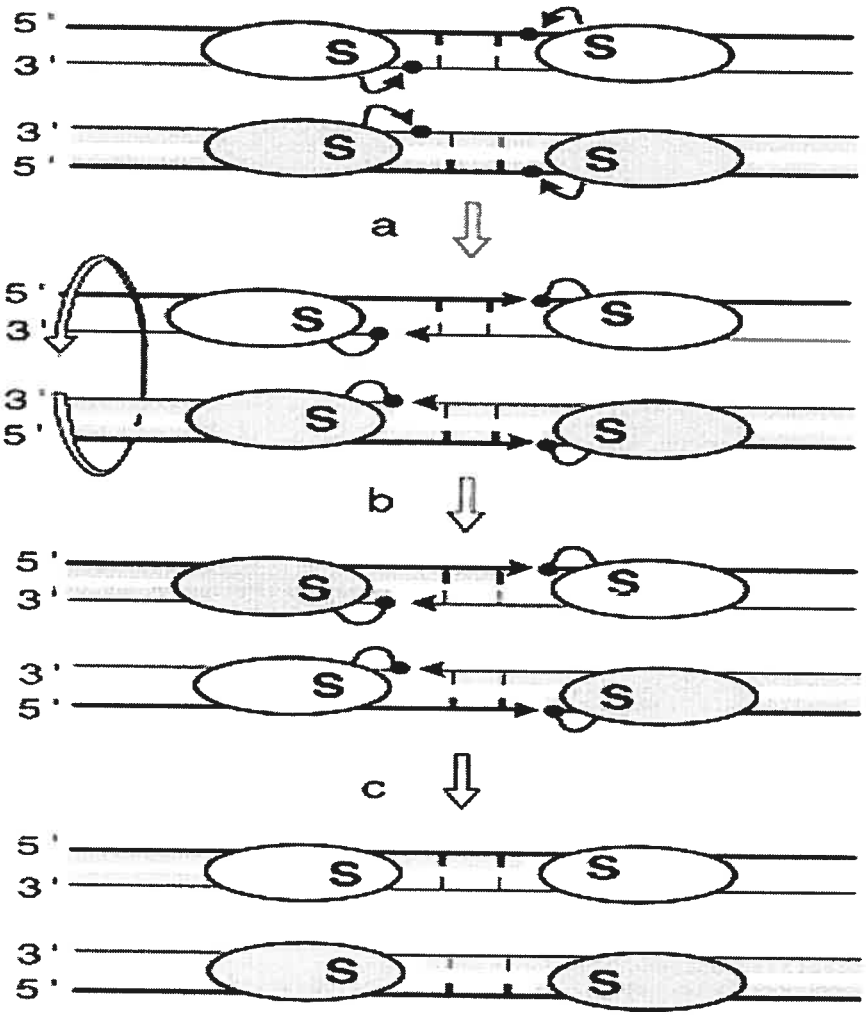


Figure 2 Model of the action of the resolvases/invertases. The subunit rotation model is shown. The ovals represent recombinase subunits with the conserved catalytic serine'S'. Thick and thin lines are the top and bottom strands of the recombination sites, respectively. The short vertical bars are the 2 bp of the overlap region between the two cleavage points. Black arrows represent the nucleophilic attacks of phosphates (black dots) by hydroxyl groups (arrowheads). The four DNA strands are cleaved (a), exchanged by 180° rotation of the half-site bound subunits (b) and religated in the recombinant configuration (c) (Hallet and Sherratt, 1997).

1.2 Lambda Integrase Family

1.2.1 Generalities

The lambda integrase or 'tyrosine recombinase' family includes over 130 members identified according to sequence similarity (Nunes-Düby *et al.,* 1998). Most biochemical studies of this family of enzymes have focused on the integrase from bacteria phage λ (Int) (Landy, 1989), Flp recombinase from yeast 2μ plasmid (Sadowski, 1995), Cre recombinase from bacteria phage P1 (Hoess *et al.,* 1985) and the XerC and XerD recombinases from *Escherichia coli* (Sherratt *et al.,* 1995). These proteins share only limited sequence similarity and are much more divergent, with only four completely invariant residues intimately involved in catalysis: the RHRY tetrad (Argos *et al.*, 1986; Abremski *et al.,* 1992; Blakely *et al.,* 1996). However, these recombinases carry out site-specific recombination using a common mechanism that involves the formation of a Holliday junction (HJ) intermediate (Craig, 1988). Moreover, unlike the recombinases of the resolvase/invertase family, site-specific recombinases related to λ Int exchange the two pairs of DNA strands separately and sequentially.

1.2.2 The Recombination Reaction

To initiate the first strand exchange, the tyrosine residue of the conserved catalytic motif RHRY attacks a specific scissile phosphate in one strand (defined here after as the top strand) of each recombination core sites, thereby forming a 3'phosphotyrosyl-linked-recombinase-DNA complex and generating a free 5'-OH end (Figure 3). The polarity of

this cleavage reaction is thus reversed when compared to that of the resolvase/invertase-mediated cleavages. In a second step, the recombinase-DNA phosphotyrosyl bond is attacked by the 5'-OH end from the partner duplex to generate a four-way branched structure, or 'Holiday junction' intermediate, in which only two DNA strands have recombined. To resolve this intermediate and complete the recombination reaction, the two other (bottom) strands are exchanged by repeating the cleavage/religation process 6-8 bp downstream of the first strand cleavage position (Hallet and Sherratt, 1997).

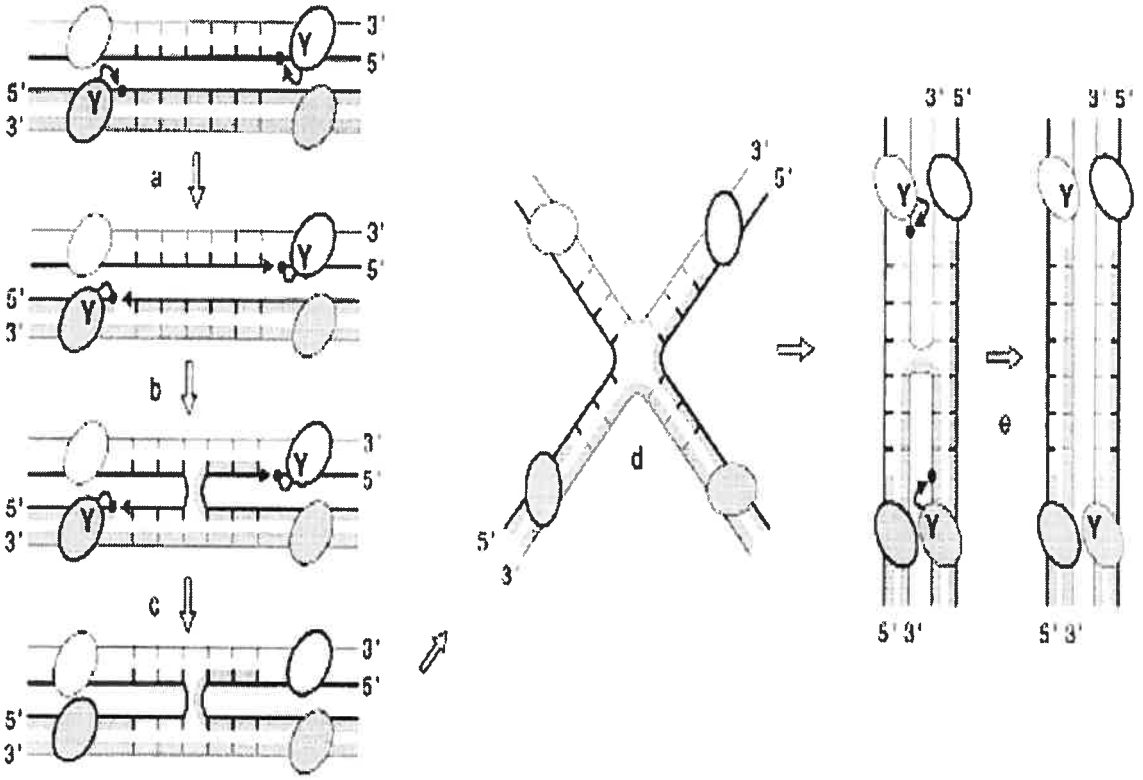


Figure 3. Sequential strand exchange by the λ Int family site-specific recombinases. The DNA strand swapping /isomerisation model is presented. The letter 'Y' refers to the conserved catalytic tyrosine. The ovals represent recombinase subunits. Thick and thin lines are the top and bottom strands of the recombination sites, respectively. Black arrows represent the nucleophilic attacks of phosphates (black dots) by hydroxyl groups (arrowheads). The top strands (thick lines) are cleaved first (a), swapped between the two partners (b), and then religated (c). The branch point of the generated Holliday junction intermediate is positioned at the middle of the (6-bp) overlap region and the top strands are crossed. Isomerisation of the Holliday junction to a recombination configuration in which the bottom strands are crossed requires the reorganization of the DNA helices and the four half-sites-bound recombinase subunits within the complex (d). The resulting Holliday junction isoform is resolved by repeating steps a to c in order to exchange the bottom strands (e) (Hallet and Sherratt, 1997).

1.2.3 The Conserved Motifs

The proteins of the tyrosine recombinase family are very divergent and share limted similarity in the amino acid sequence, but how they can carry out site-specific recombination using a common mechanism? Alignments of this integrase family of proteins identified some conserved motifs, which are related to their catalytic function. All proteins harbor two conserved regions, Box I and Box II, with marked sequence similarity, originally identified from the alignment of only eight recombinases (Argos *et al.*, 1986). Box I includes the fourth conserved residue R, and Box II contains other three conserved residues, the triad H-R-Y, which includes the active site tyrosine (Abremski and Hoess, 1992; Nunes Düby, 1998). The conservation of Box I is striking in prokaryotic recombinases and it extends with some variation to eukaryotic recombinases. Box II is also relatively strongly conserved among the prokaryotic recombinases, but less so between prokaryotic and eukaryotic proteins. Whereas the active tyrosine is absolutely conserved, the surrounding residues are rather divergent, allowing for quite different secondary structures. Furthermore, the crystal structure of the λ Int catalytic domain revealed an additional pattern of conserved hydrophobic residues that forms the core of the globular structure. It suggests that all members of the integrase family adopt similar folds for the region spanning Box I, the interval region and Box II (Nunes-Düby *et al.*, 1998).

In addition to the highly conserved Box I and Box II motifs and the pattern of core hydrophobic residues, three patches of conserved sequence were identified in the extensive alignment of the prokaryotic recombinases (Nunes-Düby *et al.*, 1997). Patch I is located within the

short N-terminal region upstream of Box I, consensus sequence LT-EEV—LL. Patch II contains a lysine (K235) flanked on both sides by serine or threonine in one subgroup of proteins and by glycine or methionine in another subgroup. For example, Lambda integrase (S234, K235, T236) belongs to the first subgroup, whereas XerD (G234, K235, G236) belongs to the second one. Patch III consists of a hydrophobic cluster rich in phenylalanines, preceded by acidic and followed by polar residues in the majority of proteins: [D, E]-[F, Y, W, V, L, I, A] 3–6 [S, T]. It is located in the divergent region between Box II and I, and is an important stabilizer of the native folds of integrase family recombinases (Nunes-Düby *et al.*, 1998).

2. Xer Site-Specific Recombination

2.1 Generalities

The physical state of circular chromosomes, unlike linear chromosomes, can be changed by homologous recombination. Odd numbers of homologous recombination events between circular replicons during or after replication, produce dimers that need to be converted to monomers before they can be segregated normally at cell division (Austin *et al.*, 1981; Blakely *et al.*, 1991; Kuempel *et al.*, 1991). Plasmid dimers can also arise as a consequence of rolling circle replication during conjugal transfer (Warren and Clark, 1980; Erickson and Meyer, 1993). The Xer site-specific recombination system, initially discovered for its role in plasmid ColE1 stable inheritance, also functions in the normal inheritance of the *Escherichia coli* chromosome and the stable inheritance of other multicopy plasmids. It is encoded by the circular chromosomes of many bacteria and functions to ensure that both circular chromosomes and multicopy plasmids are monomeric

before their segregation to daughter cells at cell division (reviewed in Sherratt *et al.*, 1995). Xer recombination is mediated by enzymes that belong to the lambda integrase family of site-specific recombinases (the 'tyrosine recombinases'), which are structurally and mechanistically related to the type IB topoisomerases of eukaryotes (reviewed in Sherratt and Wigley, 1998).

However, Xer site-specific recombination exhibits three features that distinguish it from other well-characterized members of the lambda integrase family. First, it uses two related recombinases, XerC and XerD, each of which catalyses one specific pair of strand exchanges (Blakely *et al.*, 1993, 1997; Arciszewska and Sherratt, 1995; Colloms *et al.*, 1996, 1997; Arciszewska *et al.*, 1997). The use of two recombinases potentially allows each pair of strand exchanges to occur separately and could direct the order of strand exchanges (Colloms *et al.* 1996). Second, the recombination reaction has different requirements and outcomes depending on whether it occurs at plasmid or chromosomal recombination sites. Recombination at natural plasmid sites is preferentially intramolecular and requires the two recombinases and the 28-30 bp recombination core site, as well as additional accessory proteins and adjacent accessory DNA sequences. Interaction of the accessory proteins and accessory sequences promotes the formation of a synaptic complex of precise topology, that can form efficiently on directly repeated recombination sites in the same molecule (Colloms *et al.*, 1996, 1997). In contrast, the recombination at the *E. coli* chromosome site, *dif*, requires only a 28 bp recombination core site at which the two recombinases act. Recombination *in vivo* at *dif*, present in multicopy plasmids, occurs both intermolecularly and intramolecularly (Blakely *et al.*, 1991, Leslie and Sherratt, 1995;

Tecklenberg *et al.*, 1995). Third, despite the sequence divergence of integrase family recombinases, Xer-like recombinase sequences are present in the majority of eubacteria, suggesting that the mechanism of dimer resolution used by *E. coli* is highly conserved (Table 1) (Recchia and Sherratt, 1999).

Table 1 The Xer recombinases and FtsK homologues in eubacteria and archaeabacteria. For FtsK, '+' means having homologues. '–' means no homologue found [adapted from Recchia and Sherratt, 1999].

Organism	**Recombinase Gene number**	**FtsK Homologue**
Eubacteria		
Escherichia coli	2, *xerC/xerD*	+
Bacillus subtilis	2, *ripX/codV*	+
Mycobacterium tuberculosis	2, *xer1/xer2*	+
Haemophilus influenzae	2, *xerC/xerD*	+
Helicobacter pylori J99	2, *xer1/xer2*	+
Chlamydia pneumoniae	2, *xer1/xer2*	+
Treponema pallidum	2, *xer1/xer2*	+
Thermotoga maritima	1	–
Synechocystic PCC6803	1	–
Mycoplasma genitalium	0	–
Mycoplasm pneumoniae	0	–
Borrelia burgdorferi	0	+
Archaeabacteria		
Pyrococcus horikoshii	1	–
Aeropyrum pernix	1	–
Methanococcus jannaschii	0	–

2.2 XerC and XerD

XerC and XerD are encoded at 4024 kb and 3050 kb on the *E.coli* chromosome respectively. Each recombinase is expressed with at least two other proteins that don't appear to have a role in Xer recombination (Colloms *et al.*, 1990; Blakely *et al.*, 1993). XerC and XerD belong to the large tyrosine recombinase family and possess the characteristic RHRY signature of active site residues of this family (Esposito *et al.*, 1997; Sherratt *et al.*, 1998). They show 37% identity and bind to separate halves of the recombination site (Blakely *et al.*, 1993).

2.2.1 The Conserved Genes

The alignment of the amino acid sequence of the tyrosine recombinases firstly reveals that the complete genomes of 16 eubacteria and 5 archaebacteria contain proteins homologous to XerC and XerD in bacteria with circular chromosome as shown in Table 1 (Recchia and Sherratt, 1999). Now, There are more Xer recombinase have been found such as *Caulobacter crescentus* (Jouan and Szatmari, 2003), *Lactobacillus leishmannii*Becker and Brendel, 1996), *Proteus mirabilis* (Manuela and Szatmari, 1998) *Streptococcus pneumoniae*(Reichmann et al., 2002), *Vibrio cholerea*(Huber and Waldor, 2002). Moreover, most eubacteria with only partial genome sequences are also possess two Xer protein including *Pseudomonas, Vibrio*, *Bordetella, Neisseria, Staphylococcus* and *Enterococcus* species (Recchia *et al.*, unpublished). Secondly, the majority of eubacteria possess two putative Xer recombinases, suggesting that the mechanism of dimer resolution

used by *E. coli* is highly conserved. However, two eubacterial species appear to contain only one Xer homologue. In these cases, either one Xer protein has been lost or, assuming that *xerC* and *xerD* genes arose from a single ancestral gene, these organisms diverged from other bacterial lineages prior to this duplication. Likewise, organisms in which no Xer-like sequences were identified may have either lost both sequences or separated from other bacterial lineages prior to the evolution of Xer. Thirdly, the bacteria with a linear chromosome such as the spirocheaete *Borrelia burgdorferi* lack any identifiable Xer homologues, which may indicate the Xer recombination only occurs in bacteria with a circular chromosome. Furthermore, species such as *Mycoplasma genitalium* and *Mycoplasma pneumoniae* are deficient in homologous recombination genes and also lack in the Xer genes. These correlations are consistent with the functional inter-relationship between homologous recombination and Xer recombination. Finally, most eubacteria that possess Xer recombinases also possess FtsK homologues, whereas *M. genitalium* and *M. pneumoniae*, which lack identifiable Xer homologues, also appear to lack an FtsK homologue (Table1). This suggests that the functional interaction between Xer and FtsK proteins in controlling chromosome dimer resolution is highly conserved (Recchia and Sherratt, 1999).

2.2.2 XerC

2.2.2.1 Generalities

By sequence analogy, XerC appears to be a member of the bacteriophage lambda integrase family of recombinase (Argos *et al.,* 1986; Colloms *et al.*, 1990). The *xerC* gene

maps close to the *E. coli* origin of replication, *oriC*, at 85 min (3700 kb). It is expressed as the third gene of a four-gene multicistronic unit that contains *dapF, orf235, xerC* and *orf238.* The *orf235* and *orf238* are unknown in their function but appear to be translated at levels similar to those of *dapF and xerC* (Kohara *et al.*, 1987; Richaud *et al.*, 1987; Richaud and Printz, 1988; Colloms *et al.*, 1990).

The *xerC* gene encodes a protein with a calculated molecular mass of 33.8 kDa. The translated protein sequence of XerC contains two regions, which are homologous to the two conserved domains of the lambda integrase family of site-specific recombinases (Argos *et al.,* 1986; Colloms *et al.* 1990). Domain 2 of the XerC sequence has three totally conserved amino acids, histidine (H), arginine (R), and tyrosine (Y), as well as other less conserved amino acids. The XerC sequence has 32% amino acid identity to the *E. coli* proteins FimB and FimE in an alignment covering about 160 amino acids. These two proteins are involved in inverting a segment of the *E.coli* chromosome to switch fimbrial antigen (Klemm *et al.*, 1986). Within conserved domain 2, the XerC sequence shows considerable similarity (66% identity) to an integrase-like inferred protein sequence from plasmid R46 (Hall *et al.*, 1987).

2.2.2.2 Function

The stable inheritance of natural muticopy plasmids related to ColE1 requires the function of the Xer site-specific recombination system (for example, *cer* in ColE1; Summers and Sherratt, 1984). The recombination occurs only intramolecularly and resolves plasmid multimers, which arise by intermolecular homologous recombination, to

monomers. Three unlinked *E.coli* genes whose products are required for recombination at *cer* and its natural plasmid homologs have already been discribed and characterized. XerC has been shown to bind to recombination sites (Colloms *et al.,* 1990). ArgR (originally XerA) and PepA (originally XerB) are required for recombination at *cer*, and have an accessory role participating in the resolution selecticity process (Stirling *et al.,* 1988, 1989; Summers, 1989).

In addition to its role in converting multimers of plasmid ColE1 to monomers, XerC also has a role in the segregation of replicated chromosome at cell division. *xerC* mutants form filaments with aberrant nucleoids that appear unable to partition properly. A DNA segment (*dif*) from the replication terminus region of the *E. coli* binds XerC and acts as a substrate for Xer-mediated site-specific recombination when inserted into multicopy plasmids. This *dif* segment contains a region of 28 bp with sequence similarity to the cross over region of ColE1 *cer* (Blakely *et al.*, 1991). Therefore, XerC not only functions in maintaining ColE1-like plasmids in the monomeric state, but also has a role in normal *E. coli* chromosomal metabolism, which resolve chromosome dimers to monomers prior to cell division.

2.2.3 XerD

2.2.3.1 Generalities

During the characterization of the RecJ exonuclease of *E. coli*, an open reading frame was reported and showed sequence similarity to the integrase family of site-specific recombination (Lovett and Kolodner, 1991). This open reading frame was designated *xerD*

(Blakely *et al.,* 1993). The predicted amino acid sequence of the XerD protein showed a 37% amino acid identity to XerC. Both XerC and XerD are predicted to have 298 amino acids. A high degree of sequence conservation between XerC and XerD is present in domain I and II, regions highly conserved in all integrase family recombinases (Blakely *et al.,* 1993). Note the presence of the invariant four amino acids (R...H...R...Y): Mutations at each of these four positions lead to loss of normal recombination activity in FLP recombinase (Lee *et al.,* 1992; Chen *et al.,* 1992). The conserved tyrosine in domain II of FLP is required for the nucleophilic attack that initiates the first strand exchange (Prasad *et al.*, 1987; Pargellis *et al.*, 1988). It has been proposed that the three other conserved residues of FLP form part of the active site that is involved in activation of the phosphodiester targets prior to nucleophilic attack during each of the transesterification steps (Lee *et al.*, 1992).

2.2.3.2 Function

XerD is also required in addition to XerC for site-specific recombination at *cer* and *dif* (Blakely *et al.,* 1993). The *xerD* gene is cotranscribed with two other genes, *xprA* and *recJ.* Insertion of Tn*10-9* into *xprA* and *recJ* did not generate a Xer^- phenotype. In contrast, an insertion at *xerD* gene gave a Xer^- phenotype suggested XerD is transcribed from its own promoter. A plasmid containing a deletion that removes most of the *xerD* gene fail to complemented the *xerD2* mutation, whereas a plasmid deleted for more than half of the *xprA* gene complemented the *xerD2* defect. Therefore, the two related recombinases XerC and XerD are required for site-specific recombination at *cer* and *dif* and

the two genes that are coexpressed with *xerD (xprA* and *recJ*) have no apparent role in Xer site-specific recombination. The putative catalytic active sites of both XerC and XerD are required for normal Xer site-specific recombination *in vivo*. XerC and XerD bind separately and cooperatively to the *dif* and *cer* sites *in vitro*. XerC binds the *dif* left-half site and XerD binds the *dif* right-half site (Blakely *et al.*, 1993).

2.2.3.3 Structure

The structure of XerD has been solved at 2.5Å resolution and reveals that the protein comprises two domains (Subramanya *et al.*, 1997). Domain 1 consists of residues 1-107, while domain 2 comprises residues 108-298. Domain 1 contains four α-helices, arranged such that there are two parallel helix hairpins arranged at 90° to each other. Domain 2 is also mainly α-helical, but with a three–stranded antiparalled β-sheet along one edge (Figure 4). The fold of this domain is similar to that determined for λ and HP1 integrase (Hickman *et al.*, 1997; Kwon *et al.*, 1997). Domain 1 and Domain 2 of XerD correspond to domains of λ Int, HP1 Int and FLP identified by limited proteolysis (Moitoso de Vargas *et al.*, 1988; Evans *et al.*, 1990; Chen *et al.*, 1991; Pan and Sadowski, 1993; Sadowski, 1995; Hickman *et al.*, 1997; Kwon *et al.*, 1997).

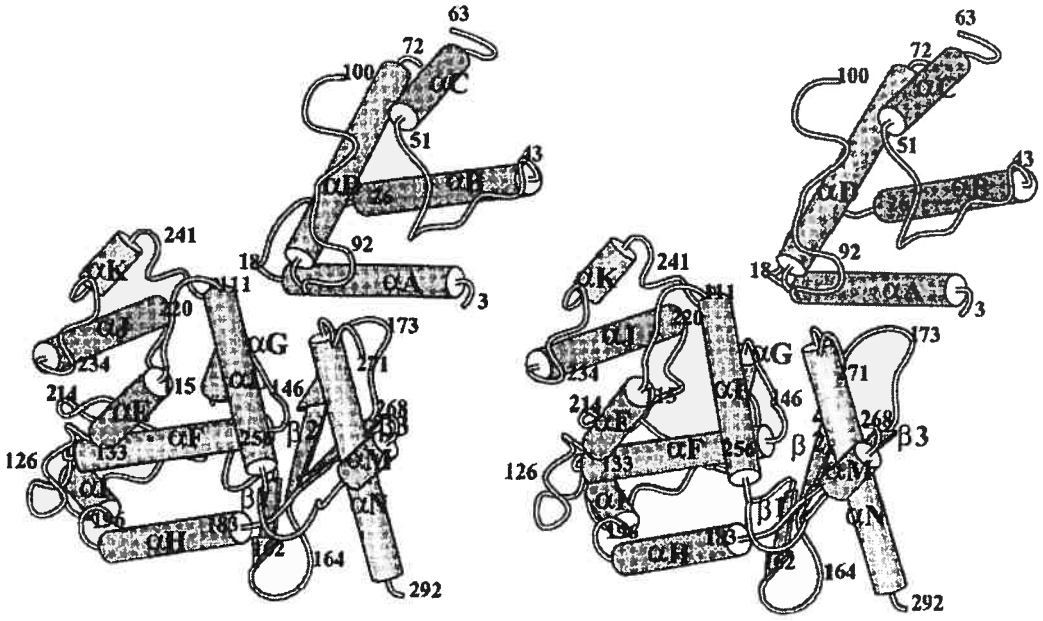


Figure 4. Overall structure of the XerD protein. The numbering refers to the beginning and end of secondary structural elements. Residues that are not defined are located at the N- and C-termini and in three disordered loops (residues 64–70, 101–110 and 269-270). (Subramanya *et al.*, 1997).

The region of structural homology within the C-terminal domains of XerD, λ Int and HP1 Int spans ~170 residues (Figure 5). Two conserved sequence motifs are located in domain 2 of XerD. The locations of motif I and the N-terminal portion of motif II are similar in the structure of XerD (residues 145–159 and 244–281, respectively) and those of λ and HP1 integrases (Hickman *et al.*, 1997; Kwon *et al.*, 1997). However, the extreme C-terminal portions of these proteins, which include the C-terminal portion of motif II, are quite different (Figure 5). In λ Int, these C-terminal residues (334–356) form a flexible loop that is disordered in one of the two molecules in the asymmetric unit, but is more

ordered in the other, where the final 15 residues form two additional β-strands along one edge of the antiparallel sheet. By contrast, in XerD, this region (residues 271–298) forms a turn followed by a long a-helix, containing the active site tyrosine, which extends almost to the C-terminus) (Subramanya *et al.*, 1997).

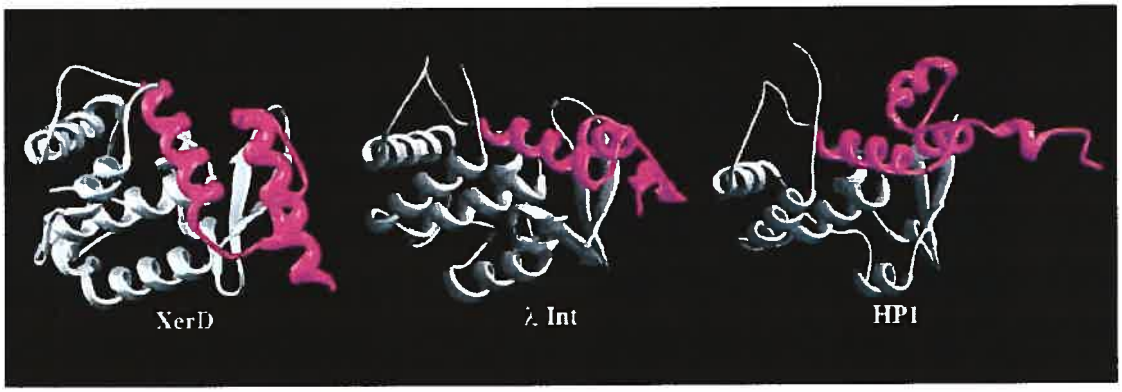


Figure 5. Comparison of the structures of the C-terminal domains of XerD, λ Int and HP1 Int. Regions of the C-terminal domains of the proteins that show the greatest structural similarity are shown in grey. The major structural differences (shown in magenta) are located in the polypeptide segments that extend from conserved motif II (Argos *et al.*, 1986) to the C-terminus of the proteins. (Adapted from Subramanya *et al.*, 1997).

2.2.4 The Catalytic Mechanism of XerC and XerD

XerC and XerD are related 298-amino-acid site-specific recombinases, each of which is responsible for the exchange of one pair of strands in Xer recombination. Both recombinases encode functions necessary for sequence-specific DNA-binding, co-operative XerC/XerD interactions, synapsis and catalysis.

In recombination mediated by XerC and XerD, DNA strands are cleaved and rejoined through the formation of a transient DNA–protein covalent intermediate involving a conserved tyrosine as the catalytic nucleophile. The same mechanism is used by the related type IB topoisomerases (reviewed in Sherratt and Wigley, 1998). However, type IB topoisomerases break and reseal the same phosphodiester bond to remove supercoils in DNA, whereas XerC and XerD catalyze two consecutive pairs of strand exchanges, with the formation of a Holliday junction (HJ) as a recombination intermediate. Each reciprocal strand exchange reaction is a concerted two-step process in which the 3' phosphotyrosyl DNA–protein bonds generated by cleavage of one DNA strand in each recombination site are subsequently attacked by the free 5' OH ends of the partner sites. DNA strands are exchanged by swapping of a few central region nucleotides (Nunes-Düby *et al.*, 1995; reviewed in Guo *et al*, 1999). This mechanism implies that specific pairs of active sites are sequentially switched on and off in the recombinase tetramer to ensure that appropriate DNA strands will be exchanged at both reaction steps. It has been demonstrated that the catalytic activity of XerC and XerD is controlled by an interaction involving the extreme C-terminal donor region of each protein and an internal acceptor region adjacent to the active site [Figure 6]. The donor–acceptor region interactions between adjacent recombinase molecules act as molecular springs in the switch that leads to sequential and synchronized activation/inactivation of pairs of recombinase subunits during recombination (Hallet *et al.*, 1999).

XerC and XerD cleave DNA by providing all catalytic residues in *cis* (Arciszewska and Sherratt, 1995; Blakely *et al.*, 1997). Consistent with this, the crystal structure of XerD

shows that tyrosine and the other active site residues are clustered together (Subramanya *et al.*, 1997). The integrases of phages lambda and HP1 and the recombinase Cre from bacteriophage P1 can also cleave DNA in *cis* (Nunes-Düby *et al.*, 1994; Guo *et al.*, 1997; Hickman *et al.*, 1997; Kwon *et al.,* 1997). However, the yeast recombinase FLP cleaves DNA in *trans* (Chen *et al.*, 1992; Lee *et al.*, 1999).

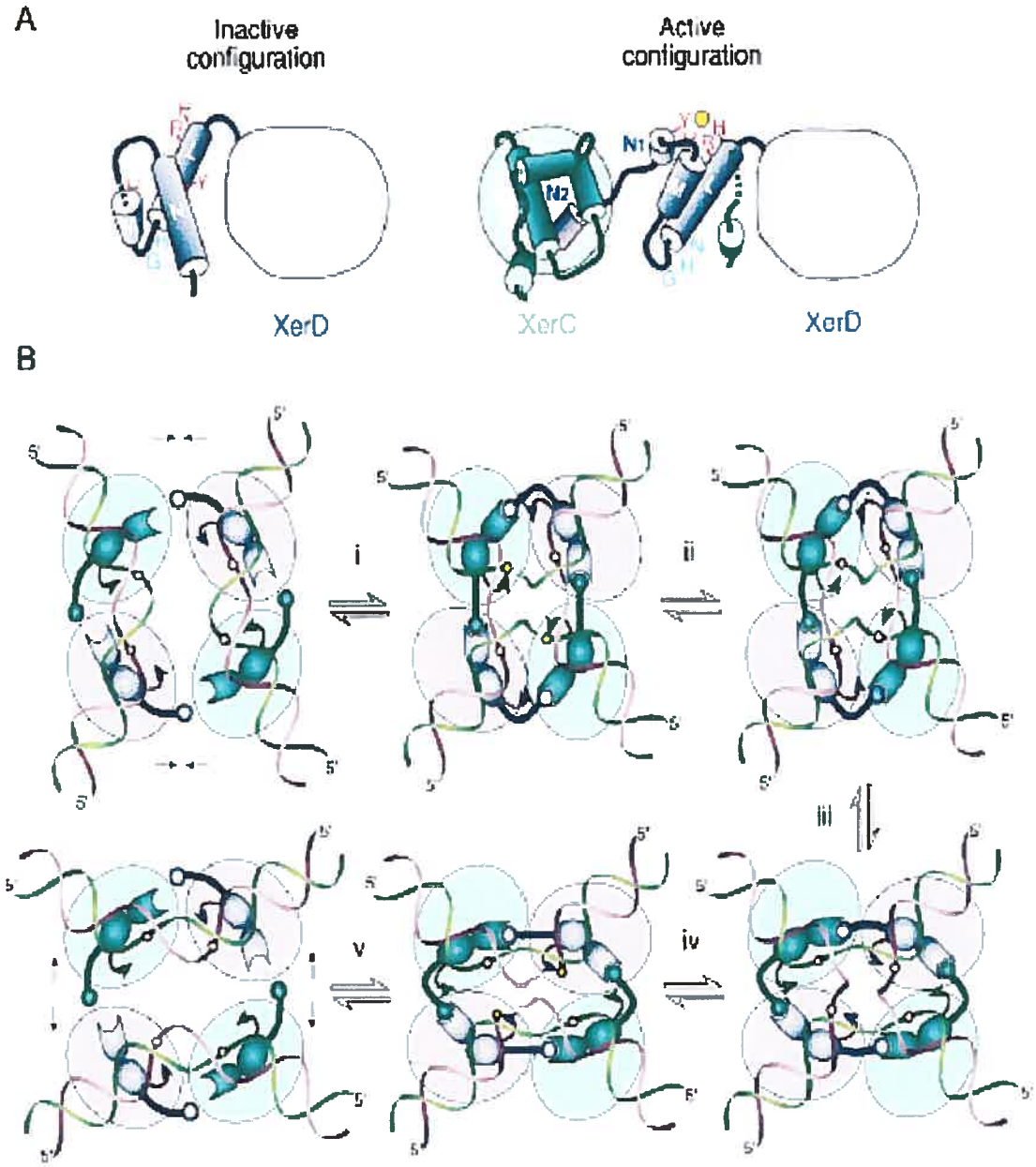


Figure 6. Control of Catalysis in Xer Recombination. (A) Proposed reconfiguration of XerD C-terminus upon assembly of the recombination complex on DNA. (B) A model for the reciprocal control of catalysis by XerC and XerD

Color code is as in (A). The ball-and-socket joint depicts the interaction between the donor and acceptor regions of adjacent subunits. Step i to step v is the recombination pathway in which XerC strand exchange occurs first. (i) Interactions between XerC and XerD molecules bound on a same duplex, possibly coupled with additional interpromoter interactions across the synapse, force the DNA to bend in a configuration where the top (green) strand of the recombination site central region is exposed toward the outside of the duplex. The torsion energy stored in the bent DNA may act on the XerC–XerD donor–acceptor interaction so as to activate XerC catalysis by repositioning of the tyrosine nucleophile (arrowhead), and possibly other catalytic residues with respect to the DNA target phosphate (circle). DNA torsion strains released upon cleavage may also promote the unwinding and extrusion of the cleaved strands in order to orient the 5′ OH ends for the rejoining step. (ii) Completion of the strand exchange reaction generates a 2-fold symmetric HJ intermediate in which the top strands are crossing. (iii) Coupled protein and DNA conformation changes convert the complex into a configuration in which the bottom strands (purple) are crossing. (iv) This leads to synchronized inactivation of the XerC subunits and concomitant activation of the XerD subunits. (v) The recombinant duplexes are bent in the opposite direction to that of the initial recombination sites. This inversion of the DNA bending strains may promote the restacking of the DNA helices and the dissociation of the resealed molecules from the complex (Hallet *et al.*, 1999).

2.3 The Site of Action of the Xer Recombinases

Xer recombinase mediated recombination occurs in two different recombination substrates and has different biological functions. One is at chromosome recombination sites called *dif*, originally found in *Escherichia coli*. The Xer site-specific recombination ensures that dimeric chromosomes are converted monomers prior to cell division (Blakely *et al.*, 1991; Kuempel *et al.*, 1991). Another is at plasmid sites such as ColE1 *cer* and pSC101 *psi*. The Xer site-specific recombination system is involved in the stability of naturally occurring plasmids by ensuring that plasmid multimers are converted to monomers (Summers and Sherratt, 1984; Cornet et *al.*, 1994). The Xer site-specific recombination is conserved in most eubacteria (Recchia and Sherratt, 1999). The alignment of 19 naturally occurring plasmids and some eubacterial chromosomes revealed that the wide existence of the homologues of Xer recombination core site (Table 2) (Hayes *et al.*, 1997; Lesterlin *et al.*, 2004). XerC binding sites are more variable whereas XerD binding sites are well conserved. The central region of the Xer sites, which displays no consensus and separates XerCD binding sites by a 6 (chromosome site) or 8 bp (plasmid site) spacer, is a key determinant of the Xer recombination pathway. It determines the requirements for accessory proteins and accessory sequences on the plasmid recombination site (e.g. ColE1 *cer* site or pSC101 *psi* site). It also determines the presence of FtsK in chromosome dimer resolution (Barre *et al.*, 2001). Several sets of data, obtained on the Xer systems and other tyrosine recombinase system, indicated that this region is an important determinant of the comformation of the recombinase-core sequence complexes (Azaro and

Landy, 1997; Gopaul *et al.*, 1998; Arciszewska *et al.*, 2000; Lee and Sadowski, 2001; Capiaux *et al.*, 2002).

Table 2 Alignment of *dif* sites from different bacteria and core sequences of plasmid-borne Xer sites (Adapted from Hayes *et al.*, 1997 and Lesterin *et al.*, 2004)

Origin	XerC binding site	Central region	XerD binding site
Plasmids Sites			
ColE1 *cer*	GGTGCGTACAA	TTAAGGGA	TTATGGTAAAT
ColA *car*	GGTGCGTACAA	----CGGATG	TTATGGTAAAT
CloDF13 *parB*	GGTACCGATAA	----GGGATG	TTATGGTAAAT
ColK *ckr*	GGTGCGTACAA	TTAAGGGA	TTATGGTAAAT
ColN	GGTGCGTACAA	--TAAGGGA	TTATGGTAAAT
NPT16	GGTGCGCGTAA	--TGAGACG	TTATGGTAAAT
pMB1	GGTGCGTACAA	TTAAGGGA	TTATGGTAAAT
pSC101 *psi*	GGTGCGCGCAA	----GATCCA	TTATGTTAAAT
ColE2	GGGGCGTACAA	----CGGGAG	TTATGGTAAAT
ColE3	GGTGCGTACAA	----CGGGAG	TTATGGTAAAT
ColE4-CT9	GGTGCGTACAA	----CGGGAA	TTATGGTAAAT
ColE5-099	GGTACGTACAA	----CGGGAG	TTATGGTAAAT
ColE6-CT14	GGTGCGTACAA	----CGGGAG	TTATGGTAAAT
ColE7-K317	GGTGCGTACAA	----CGGGAG	TTATGGTAAAT
ColE8-J	GGTACGTACAA	----CGGGAA	TTATGGTAAAT
ColE9-J	GGTACGTACAA	----CGGGAG	TTATGGTAAAT
ChromosomeSites(*dif*)			
E. coli	GGTGCGCATAA	-----TGTATA	TTATGTTAAAT
S. typhimurium	GGTGCGCATAA	-----TGTATA	TTATGGTAAAT
S. typi	GGTGCGCATAA	-----TGTATA	TTATGGTAAAT
V. cholerae chrI	ATGGCGCATTA	-----TGTATG	TTATGGTAAAT
V. cholerae chrII	AATGCGCATTA	-----CGTGCG	TTATGGTAAAT
H. influenzae	ATTTCGCATAA	-----TATAAA	TTATGGTAAAT
B. subtilis	ACTTCCTAGAA	-----TATATA	TTATGTAAACT

2.3.1 Chromosome Recombination Site

2.3.1.1 *Escherichia coli dif*

2.3.1.1.1 Position and Polarity

Two main sites of the circular *E. coli* chromosome are implicated in the cell cycle: *oriC*, where replisomes are assembled for bidirectional replication (Messer *et al.*, 1996), and the diametrically opposite *dif* site, where chromosome dimers are resolved (Blakely *et al.*, 1991; De Massy *et al.*, 1987). The *dif* is located in the replication terminus region at min 33.6 of the genetic map, kilobase 1608 of the physical map, between the innermost terminators *terA* and *terC* (Figure 7) (Kuempel *et al.*, 1991). Recently, it has been discovered that this position is crucial for dimer resolution (Leslie and Sherratt, 1995; Tecklenburg *et al.*, 1995; Cornet *et al.*, 1996; Kuempel *et al.*, 1996). To be active, *dif* must be inserted within a narrow zone around its natural position, the DAZ (*dif* activity zone). The DAZ is the scene of specific recombination between *dif* sites that occurs only in cells that are able to form chromosome dimers (i.e. proficient for homologous recombination) (Pérals *et al.*, 2000; 2001).

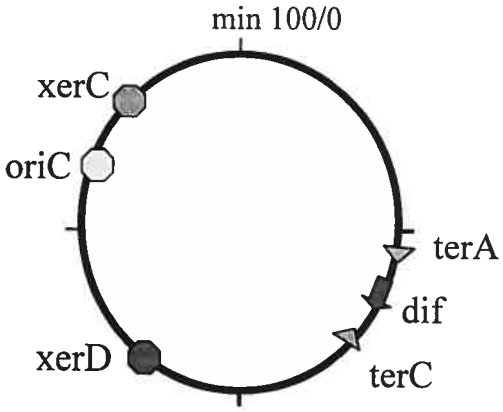


Figure 7. Map of the *E. coli* chromosome, showing the position of the *xerC* and *xerD* genes, the position of *oriC* and *dif*, and the position of two replication terminator sites [from Barre and Sherrat, 2002]

The sequences surrounding *dif* appear to be intrinsically polarized along the *oriC-dif* axis and their relative orientation is the main determinant of DAZ positioning. Notably, the deletion of sequences surrounding *dif* is harmless, whereas inversion of the same sequences inhibits dimer resolution (Tecklenburg *et al.*, 1995; Cornet *et al.*, 1996; Pérals *et al.*, 2000). The data suggest that the polarization determinants are present throughout a large terminal domain (more than 200 kb around *dif*) and are highly repeated. Chromosome sequences are oriented following the *oriC/ter* axis, defining the two replichores (Blattner *et al.*, 1997). Several types of short-sequence elements showing a strongly biased orientation following the *oriC-dif* axis exist. This results from the intrinsic biased orientation of chromosome sequences that define its replichore organization: strongly expressed genes, G/C skew, Chi

sites and numerous other oligomers (Salzberg *et al.*, 1998; Lobry and Louarn, 2003). Among these, short degenerate motifs, termed RAG, have been proposed as good candidate based on their highly biased orientation (Lobry and Louarn, 2003). However, previous attempts to show that the RAG motif controls another FtsK activity or colocalized other active elements were unfruitful (Perals *et al*, 2000; Massey *et al.*, 2004; Saleh *et al.*, 2004). However, DNA motifs, named FtsK orienting polar sequences (KOPS), have been identified which direct the movement of the *E. coli* FtsK translocase (Levy *et al.*, 2005; Bigot *et al.*, 2005). Levy group (2005) identified the GNGNAGGG motif, its complement, or both as the best candidate to specify FtsK directionality. They found that a GNGNAGGG sequence efficiently reverses FtsK translocation. Bigot group (2005) used a functional approach and also identified this motif, displaying a high biased orientation and over-represented on the whole chromosome. *In vitro*, these motifs display KOPS activity: they inhibit Xer recombination activation by FtsK in an orientation-dependent manner; they also stop FtsK from dissociating branched DNA structures depending on their orientation; additionally, single molecule data suggest that they block FtsK translocation. Their effect on FtsK translocation is stochastic; the presence of two or three motifs is required to observe a strong effect.

2.3.1.1.2 Structure

The minimal *dif* site sufficient for chromosome monomerization activity and for recombination in a plasmid substrate is 28 bp in length (Leslie & Sherratt, 1995; Tecklenburg *et al.*, 1995). This site (also called the core recombination site) consists of two 11 bp XerC and XerD

binding sites separated by a 6 bp central region at the boundaries of which strand cleavage and exchange occur. The XerC and XerD binding sites are partial palindromes, but the two halves of *dif* are recognized specifically by the XerC and XerD recombinases, which themselves share 59% similarity (Blakely *et al.*, 1993). The XerC and XerD binding sites are partial palindromes at six of 11 positions, but other five positions are never palindromes which determine the specifically binding of XerC and XerD, as shown in Figure 8.

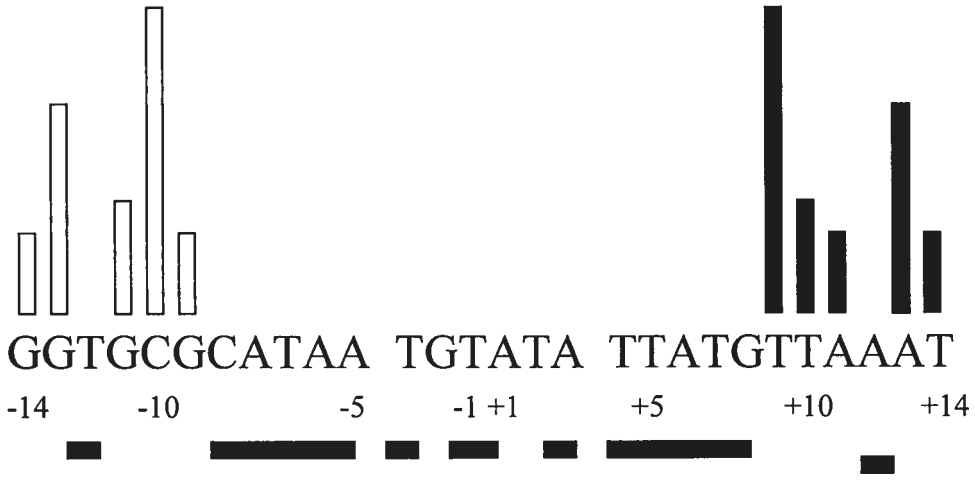


Figure 8. Hierarchy of specificity determinants in the XerC and XerD binding sites of *dif.* Shaded boxes below the sequence denote positions that are palindromic between the XerC and XerD binding sites. Bars above the sequence indicate the relative contributions of particular nucleotides to XerC or XerD binding specificity. The longest bars identify bases that are most significant for specificity and the shortest bars denote nucleotides whose contribution is least critical. Bars of intermediate length indicate positions of intermediate importance. Note that, while the T → C substitution at position +9 had a strong affect on XerD binding and recombination in *vivo*, all plasmid sites examined to date (except *psi)* have a G nucleotide at this position (Adapted from Hayes *et al.* 1997).

2.3.1.2 *Bacillus subtilis dif*

Homologues of *E. coli dif* site have found in other bacterial chromosomes (Table 2). The *Bsdif* site is located at approximately 166° on the *B. subtilis* chromosome, 6° counterclockwise from the *B. subtilis* terminus of replication (Kunst *et al.*, 1997). There are six different nucleotides in the CodV (XerC) binding region when compared to the *dif* site, which may be required for the specific binding of CodV. The RipX (XerD) binding site is more conserved and has only one divergent nucleotide. Experimental evidence has been provided to substantiate the authenticity of this site. First, integration of nonautonomously replicating plasmids carrying either cloned *Bsdif* DNA or a synthesized *Bsdif*-oligomer occurs at a high frequency in *recA* backgrounds. The integration of *Bsdif*-containing plasmids was dependent on the presence of RipX, CodV, and the chromosomal *dif* site. Second, deletion of the *Bsdif* site from the chromosome resulted in the development of a subpopulation of cells with aberrantly partitioned nucleoids that closely resembled in appearance and frequency those seen in *ripX* mutants. Third, the RipX and CodV proteins demonstrated specific binding to, and cleavage of, synthetic *Bsdif* DNA *in vitro*. Therefore, *Bsdif* is utilized by the CodV and RipX recombinases to ensure that normal chromosome partitioning occurs in advance of the completion of cell division (Sciochetti *et al.*, 2001).

2.3.2 Plasmid Recombination Site

Unlike the 28 bp *dif* site, which does not require accessory sequences, the plasmid-borne sites contain accessory sequences and require additional accessory proteins. They include a core site, to which XerC and XerD bind, and ~180 bp of adjacent upstream accessory sequence to which additional accessory proteins bind (Stirling *et al.*, 1988; Colloms *et al.*, 1997). The function of accessory proteins and accessory DNA sequences impose a 'topological filter' on Xer recombination, which ensures that the recombination is preferentially intramolecular (Alen *et al.*, 1997; Colloms *et al.*, 1997).

2.3.2.1 ColE1 *cer*

The *cer* locus of the ColE1 plasmid is the archetype of sites displaying a strong bias towards intramolecular exchanges and multimer resolution (Summers and Sherratt, 1984). Its presence improves plasmid stability in *E. coli* by maximizing the number of segregation units at division (Summers *et al.*, 1993; Summers and Sherratt, 1984). Importantly, the core sequence alone is inactive, and the presence of accessory sequences and factors is required not only for directionality of exchanges but also for the overall recombination activity of the site. The core 30 bp sequence, which is recognized by XerC and XerD (Blakely *et al.*, 1993) and contains the site of strand exchange (Summers *et al.*, 1985; Summers, 1989), is embedded in a longer sequence of 280 bp (Summers and Sherratt, 1988) which binds accessory factors required for full activity. These accessory factors are ArgR and PepA. The structure of the *cer* site is different from the *dif* site of *E. coli* in two aspects: one is containing the accessory sequences; another is that the binding sites of

XerC/D are separated by 8 bp spacer. Recombination at *cer* sites is preferentially intramolecular. This selectivity is correlated with the requirement for accessory protein and ~180 bp of accessory sequences (Summers and Sherratt 1984; Summers, 1989; Sherratt *et al.*, 1995). What determines whether recombination will be preferentially intramolecular and require accessory factors, or will be both intermolecular and intramolecular, requiring only recombinases and a recombination core site? Summers (1989) demonstrated that the central region size difference could determine recombination requirements and outcomes. Moreover, Blakely and Sherratt (1996) set up a model system to explore the selectivity for intramolecular recombination. They found that the requirement for accessory factors could arise by increasing the spacing between XerC- and XerD-binding sites from 6 to 8 bp. This reduces the affinity of the recombinases for the core site and changes the geometry of the recombinase/DNA complex. These changes are correlated with the altered interactions of the recombinases with the core site and a reduced efficiency of the XerC-mediated cleavage. The accessory sequences and proteins compensate for these changes and provide a nucleoprotein structure of fixed geometry that can only form and function effectively on circular molecules containing directly repeated sites (Blakely and Sherratt, 1996).

2.3.2.2 pSC101 *psi*

In pSC101, the *cer/dif* homolog, *psi*, is located between positions 6783 and 6810 (Bernardi *et al.,* 1984), downstream from the essential replication gene *repA* and just beyond an unknown open reading frame *orfX.* The *psi* and *dif* sequence are very similar in the two 11-bp flanking elements, especially the right-hand one. The XerC-binding site has

a two-nucleotide difference, whereas the XerD-binding site only has one divergent nucleotide. The central region is also 6 bp like the *dif* site but the sequence is different (Cornet *et al.*, 1994). Deletions of *psi* and its surrounding region resulted in the reduction of stability compared with that of the parental pSC101 plasmid. The role of the *psi* sequence in site-specific recombination has been explored in two contexts. It was cloned in a derivative of plasmid p15A and inserted into the chromosome in place of *dif*. In the first situation, *psi* activity required accessory sequences and resulted in multimer resolution and recombination was intramolecular; in the second situation, it suppressed the effects of the *dif* deletion and promoted intermolecular exchanges. Thus, *psi* is a site whose recombination activity (intramolecular or intermolecular) depends on the context, the first in the *cer/dif* family known to exhibit such flexibility (Cornet *et al.*, 1994).

Although the *psi* recombination site is similar to the *cer* site, differences between *cer* and *psi* site recombination *in vivo* and *in vitro* have been observed. First, recombination between *psi* sites *in vivo* requires PepA, XerC and XerD, but not ArgR, whereas *cer* sites recombination requires both PepA and ArgR. Second, in *in vitro* reactions, recombination at *psi* occurs by XerC-mediated top-strand exchange followed by XerD-mediated bottom-strand exchange, to produce a fully recombinant product via a Holliday junction intermediate. However, recombination at *cer* stops after XerC-mediated top-strand exchange, producing a Holliday junction-containing product. Third, *in vitro*, *cer* produces Holliday junctions whereas *psi* produces catenanes. *In vivo*, *cer* also produces Holliday junctions early in the reaction which persist for quite some time, but recombination at *psi in vivo* goes by XerC-mediated Holliday junction formation followed by XerD-mediated

Holliday junction resolution (Colloms *et al.*, 1996). Moreover, in addition to PepA as an accessory protein for recombination at the *psi* site, another protein, ArcA, is required for effecient recombination *in vivo* at *psi.* The DNA-binding protein ArcA and the sensor kinase ArcB constitute a two-component regulatory system that regulates gene expression in *E.coli* in response to anaerobic growth conditions. ArcA is an accessory protein for recombination at *psi* in that ArcA-P binds to the accessory sequences of *psi* and stimulates recombination. ArcB is not absolutely required for recombination *in vivo*. ArcA plays a similar role at *psi* to that played by ArgR at *cer* (Stirling *et al.*, 1988; Colloms *et al.,* 1998).

2.4 Accessory Factors

In the Xer recombination system, accessory factors are required to complete the recombination reaction. For example, a complete dimer resolution reaction during recombination at *dif* requires the action of the C-terminal domain of FtsK (FtsKc). The *cer* and *psi* sites require accessory factors (ArgR/PepA, ArcA/PepA) to convert multimers to monomers. These accessory factors don't directly participate in the strand exchange reaction, but are thought to activate (FtsK) or bring sites together in the correct conformation (ArgR, ArcA and PepA).

Firstly, for the FtsK accessory factor, FtsK has been implicated in positioning the terminus regions of chromosome dimers at mid-cell and synapsing their *dif* sites (Capiaux *et al.,* 2002; Corre and Louarn, 2002). Moreover, FtsK is directly involved in Xer recombination and in locally promoting XerD strand exchanges after synapse formation (Aussel *et al.*, 2002). Secondly, recombination at *cer* is exclusively intramolecular and

occurs only between directly repeated sites, so that it resolves but does not generate plasmid multimers. ArgR, PepA and the accessory sequences of the *cer* have been implicated in ensuring this resolution selectivity. Evidence for this comes from the study of a number of conditionally constrained *cer* variants which recombine exclusively intramolecularly in the presence of ArgR, PepA and the accessory sequences, but recombine inter- and intra- molecularly when any one of these factors is removed (Summers, 1989; Guhathakurta and Summers, 1995; Guhathakurta *et al.*, 1996).

2.4.1 ArgR/PepA

2.4.1.1 ArgR

ArgR, originally identified as a repressor of genes for arginine biosynthesis, is also essential for *cer*-mediated multimer resolution (Stirling *et al.*, 1988). The ArgR protein is 156 amino acids long and is a 100 kDa hexamer of identical 17 kDa subunits (Lim *et al.*, 1987; Lu *et al.*, 1992). The polypeptide forms a very stable hexamer in the presence of arginine.

ArgR possesses at least two functions. Firstly, ArgR represses transcription of the chromosomal *arg* regulon by binding to two 18 bp inverted repeated sequence (ARG boxes) separated by 2 or 3 bp (Cunin *et al.*, 1986). Binding appears to be co-operative, as the affinity for binding two boxes is about 100-fold higher than binding to a single box. ArgR binding introduces a bend of about 70-90° in the DNA helix axis (Tian *et al.*, 1992; Burke *et al.*, 1994). Secondly, it is required for *cer* recombination. The *cer* site contains a

single binding box for ArgR approximately 100 bases to the left of the XerC binding site. ArgR binds to the single ARG box within *cer*, ~110 bp from the point of strand exchange, and induces a bend of ~65° (Burke *et al.*, 1994). It seems likely that during recombination at *cer*, a single ArgR hexamer binds to one ARG box from each participating *cer* site, helping to synapse two *cer* sites and/or introducing a structurally important bend within the accessory sequences. Miller group (1997) have proposed that AhrC, the *Bacillus subtilis* homologue of ArgR, binds to a single ARG box bending the DNA around itself so that one sepecific and one non-specific set of protein-DNA interactions are made. This model might also be appropriate for the ArgR-*cer* interaction (Hodgman *et al.*, 1998). It is interesting to note that AhrC can substitute for ArgR in *cer* recombination (Smith *et al.*, 1989).

Mutagenesis results have shown that the ArgR subunit is made up of two functional regions: a basic N-terminal half responsible for DNA binding and an acidic C-terminal half responsible for oligomerization and arginine binding (Burke *et al.*, 1994; Tian and Maas, 1994). The N-terminal domain (residues 1–70) is a member of the winged helix-turn-helix family and adopts the same fold as shown for this region of *Bacillus stearothermophilus* (Ni et *al.*, 1999). The X-ray structure of the hexameric C-terminal oligomerization domain shows that ArgR forms a 32-symmetric hexamer in which the subunits are organized into two trimers, each with tightly packed hydrophobic cores. Each subunit has a α/β fold composed of a four-stranded antiparallel β-sheet and two antiparallel a-helices. β-strands 3 and 4 from each of three subunits contribute side-chains to form the hydrophobic core of a trimer and the hexamer is formed by two dyad-related trimers (Figure 9A.) (van Duyne et

al., 1996). The X-ray structure of the entire ArgR protein from *B. stearothermophilus* has also been solved. It proposed a model, in which the arginine-bound ArgR interacts with ArgR box (Figure 9B) (Ni *et al.*, 1999).

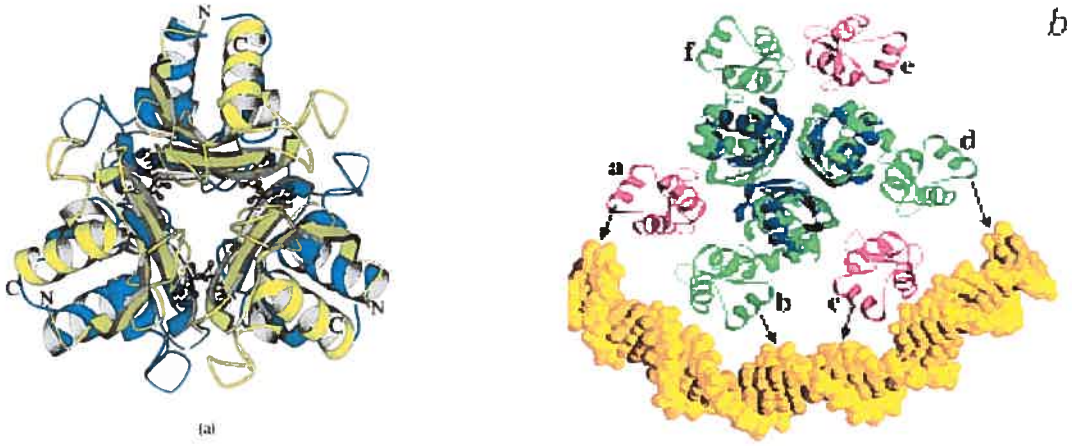


Figure 9 (A) the structure of the ArgR C-terminal domain in *E. coli* [Adapted from van Duyne *et al.*, 1996]. (B) The model of *B. stearothermophilus* ArgR binding to DNA (Adapted from Ni *et al.*, 1999).

2.4.1.2 PepA

PepA, originally designated as XerB, is an aminopeptidase and has strong similarity to bovine lens leucine aminopeptidase (LAP) (Vogt, 1970; Stirling *et al.*, 1989). It is a hexamer in solution, consisting of six identical 55 kDa monomers, each comprising 503 amino acids. It is an Mn^{2+}-dependent aminopeptidase (McCulloch *et al.*, 1994). The structure of PepA has been determined at 2.5Å resolution. PepA comprises two domains, which have similar folds to the two domains of LAP. The smaller N-terminal domain

(residues 1-166) probably plays a significant role in DNA binding and is rotated by 19° compared with its position in LAP. The larger C-terminal domain (residues 93-503) contains the aminopeptidase active site. Both domains have a mixed α/β structure. A long α-helix links the N-terminal and C-terminal domain (Sträter *et al.*, 1999).

PepA is a multifunctional protein. Firstly, it is an aminopeptidase and cleaves a broad range of peptide substrates. It belongs to the widespread family of leucine aminopeptidases, which are present in mammals, plants and bacteria (Cuypers *et al.*, 1982; Bartling and Weiler, 1992; Burley *et al.*, 1992; Wood *et al.*, 1993). Secondly, it is also involved in pyrimidine-specific transcriptional regulation of the *carAB* operon. This operon encodes the genes for carbamoylphosphate synthetase, which catalyses a common step in the biosynthesis of arginine and pyrimidines (Charlier *et al.*, 1995). Thirdly, it has been found that PepA is required for Xer site-specific recombination (Stirling *et al.*, 1989). It might play a structural role and could involve direct interactions between PepA and the recombination site DNA and/or protein-protein interactions with ArgR and recombinases (Guhathakurta *et al.*, 1995). It has been demonstrated that the peptidase activity is not required in the pyrimidine-specific regulation of *car*AB or in Xer site-specific recombination (McCulloch *et al.*, 1994; Charlier *et al.*, 1995). PepA appears to act as an architectural protein, bending and wrapping DNA so as to allow interaction between other proteins bound at distant sites on the DNA.

2. 4. 1. 3 Xer Synaptic Complex

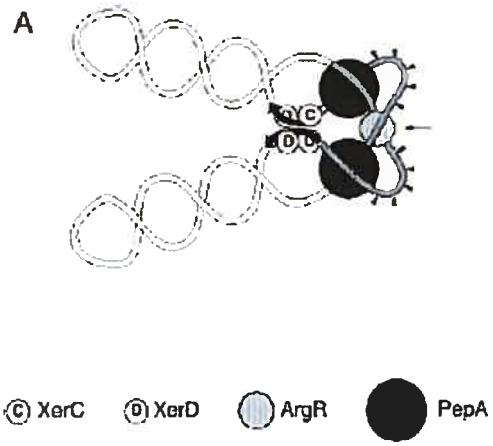
Since PepA and ArgR are required for Xer site-specific recombination at ColE1 *cer* site, how they can assemble into a specific structure for completing the recombination reaction. Two alternative models have been proposed for the Xer complex, in which either one or two PepA molecules, ArgR and the recombinases interact with the two *cer* sites (Figure 10A) (Alén *et al.*, 1997). Both types of complex are proposed to contain 2-fold molecular axis, such that each *cer* site makes equivalent interactions with PepA and ArgR. PepA makes contacts with *cer* adjacent to the recombinase-binding sites and adjacent to the ARG box distal to the recombinases-binding sites. It also shows a highly curved ~60 bp loop of DNA between the ARG box and the recombinase-binding sites. Furthermore, based on the structural and biochemical data of PepA, a model for the *cer* synaptic complex was presented (Figure 10B) (Sträter *et al.*, 1999). The most striking feature of this type of molecular sandwich is that the presumed DNA-binding grooves of PepA form right-handed helical paths, about which two *cer* sites could be interwrapped to form a –3 synapse. Two *cer* sites are wrapped around the common 3-fold axis of PepA and ArgR and PepA again by way of the PEP1, ARG and PEP2 sequences. This leaves two vacant DNA-binding grooves, which can bind to the third sequence (PEP3) of each *cer* site in order to juxtapose the two recombination core sites and allow Xer recombination. Each *cer* site therefore interacts with the proteins in the order PEP1-ARG-PEP2-60bp LOOP-PEP3-XERC-XERD.

The model proposed by Sträter *et al* is two hexamers of PepA and one hexamer of ArgR are aligned alone their threefold axes. The DNA is bound by the C-terminal grooves of PepA and does not contact the N-terminal domains extensicvely. All three grooves are

occupied by DNA in both PepA hexamers. However, Reijns *et al.* (2005) proposed different model according to their results of mutagenesis of PepA. They selected PepA mutants that were unable to support efficient Xer recombination. These mutants were defective in DNA-binding and in transcriptional regulation of *carAB*, but had normal peptidase activity. The mutations define extended patches of basic residues on the surface of the N-terminal domain of PepA that flank a previously proposed DNA-binding groove in the C-terminal domain of PepA. Based on their data, they propose a new model for the Xer synaptic complex, in which two recombination sites are wrapped around a single hexamer of PepA, bringing the cross-over sites together for strand exchange by the Xer recombinases. In this model, PepA stabilizes negative plectonemic interwrapping between two segments of DNA by passing one segment through the C-terminal groove while the other is held in place in a loop over the groove. In this new model for the synaptic complex, two DNA crossings are trapped on two faces of the triangular PepA hexamer, and the recombination core complex occupies the third face. ArgR and ArcA serve only to bend the DNA in the overpassing loops and it is easy to see how PepA alone could define the entire structure and topology of the synapse. PepA is the major determinant of the interwrapped synapse structure, whereas in Sträter model, much of the interwrapping of the two sites is around ArgR rather than PepA.

The accessory sequences of both *cer* and *psi* are thought to form a specific interwrapped synaptic complex with the accessory proteins before strand exchange at these sites. This complex can only be formed easily between two sites in directly repeat on a

supercoiled molecule (Alén *et al.*, 1997; Colloms *et al.*, 1997). The requirement for this complex ensures that recombination occurs only between directly repeated sites on the same molecule. This ensures the biologically important directionality of the recombination, so that multimers are converted to monomers and not vice versa.



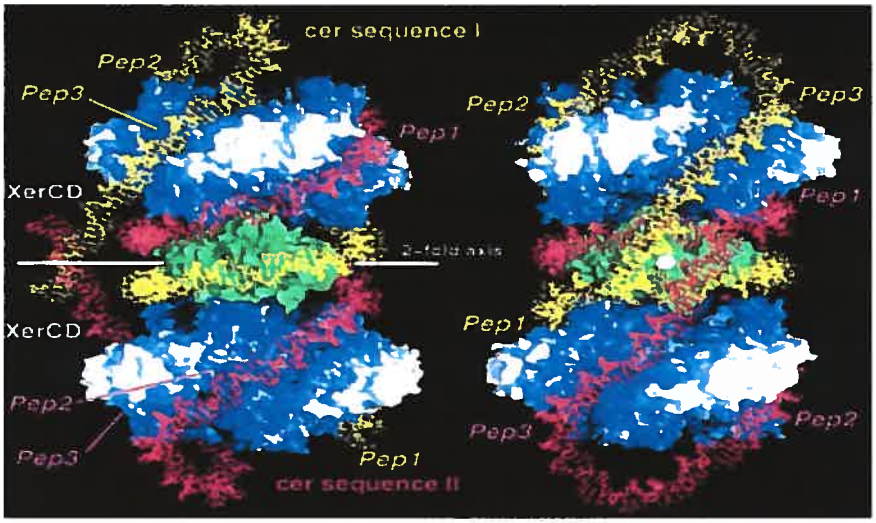


Figure 10 (A) Model for complex formed between two *cer* sites in the presence of PepA and ArgR (Adapted from Alén *et al.*, 1997). (B) Model for the Xer Complex. PepA and ArgR are represented by their molecular surfaces coloured in blue and green, respectively. The two-cer sites are coloured in yellow and red (Sträter et al. 1999).

2.4.2 FtsK

Chromosome dimer resolution requires the XerC and XerD recombinases; it also requires FtsK, a large, multifunctional, integral membrane protein, which coordinates chromosome segregation and cell division (Liu *et al,* 1998; Capiaux *et al*, 2001). FtsK can be divided into three domains: a membrane-spanning 200 aa N-terminal domain (FtsKN), containing four transmembrane regions, which localizes to the division septum and is essential for cell division (Draper *et al*, 1998); a long linker (600 amino acids) of unknown function; and a 500 aa C-terminal AAA ATPase domain (FtsKc) (Yu *et al*, 1998; Barre *et al*, 2000; Aussel *et al*, 2002). It is necessary for normal chromosome segregation (Liu *et al.* 1998; Yu *et al.* 1998b), at least in part because it is necessary for Xer recombination at *dif* (Recchia *et al.* 1999; Steiner *et al.* 1999). Cells lacking FtsKc form septate chains and filaments with aberrant and mispositioned nucleoids. FtsKc is homologous with the C-terminal domain of SpoIIIE, a protein involved in DNA transfer from the mother cell to the prespore in *Bacillus subtilis* (Wu *et al.* 1995). To date, two roles have been assigned to FtsK in **c**hromosome **d**imer **r**esolution (**CDR**).

First, FtsK has been implicated in positioning the terminus regions of chromosome dimers at mid-cell and synapsing their *dif* sites (Capiaux *et al*, 2002; Corre and Louarn, 2002). FtsK mobilized the DNA stretches that cross the septum to bring the two *dif* sites together (Corre and Louarn, 2002; Bigot *et al.*, 2004). This may allow synapsis of the *dif* sites in or near the septum and FtsK-dependent activation of XerCD catalysis. FtsK may thus be a major actor of the positional control exerted on *dif* activity. Consistent with such a role, FtsK50C, an active derivative of FtsKC, was shown to be an ATP-dependent DNA

translocase *in vitro* (Aussel *et al*, 2002). FtsK must operate not just quickly but in the right orientation; FtsK activity would be counterproductive if it pushed the two *dif* sites away from each other. The single-molecule work with purified FtsK50C demonstrated that the DNA sequence directs the translocase (Pease *et al*., 2005). Bigot *et a.l*(2005) and Levy *et al.* (2005) identified a specific instance of the GNGNAGGG motif, its complement, or both are effective in specifying FtsK's directionality and that the skew of GNGNAGGG well explains FtsK's action *in vivo*. DNA motifs provide FtsK with the necessary information to faithfully distribute chromosomal DNA to either side of the septum, thereby bringing the *dif* sites together at the end of this process.

Second, FtsKC is directly involved in Xer recombination. In the absence of FtsK, Holliday junctions (HJ) formed at *dif in vitro* are the result of catalysis by XerC (Barre *et al*., 2000). In contrast, in the presence of FtsK, XerD catalyzes HJ formation *in vitro* and *in vivo* (Aussel *et al*., 2002). Moreover, a low level of HJ formation *in vivo* by XerD is also reported when using a very sensitive detection assay in *E. coli* (Hallet *et al.*, 1999). Based on the above data, Aussel *et al.* (2002) proposed two alternative pathways (FtsK-dependent/independent pathway) of Xer recombination at *dif*, one initiated by XerC and the other by XerD. The role of FtsK in promoting chromosome dimer resolution is to switch the activity of the XerCD recombinases in the synaptic complex, so that Xer recombination follows one pathway in which XerD mediates the first pair of strand exchanges to form HJ intermediates that are resolved to products by XerC (FtsK-dependent pathway). On the contrary, the HJ intermediates that are formed by XerC-strand exchanges in the absence of FtsK are part of an abortive pathway as far as dimer resolution is concerned; the HJs are

rapidly converted back to substrates in cycles of XerC-mediated strand exchanges (FtsK-independent pathway) (Figure 11) Consistent with this pathway, synthetic *dif* HJs are resolved efficiently by XerC (Arciszewska and Sherratt, 1995), while synthetic *dif* HJs or plasmid HJs formed by XerC are not resolved by wildtype XerD under any of the conditions that have been tried, despite the presence of $FtsK_{50C}$ (Aussel *et al.*, 2002).

Therefore, the C-terminal domain of FtsK (FtsKc) is a DNA translocase implicated in helping synapsis of the *dif* sites and in locally promoting XerD strand exchanges after synapse formation. Furthermore, it has been shown that FtsKc ATPase activity is directly involved in the local activation of the Xer recombination and activation only occurs with a DNA segment adjacent to the XerD-binding site. This suggests that FtsK needs to contact the XerD recombinase to switch its activity on using ATP hydrolysis (Massey *et al.*, 2004).

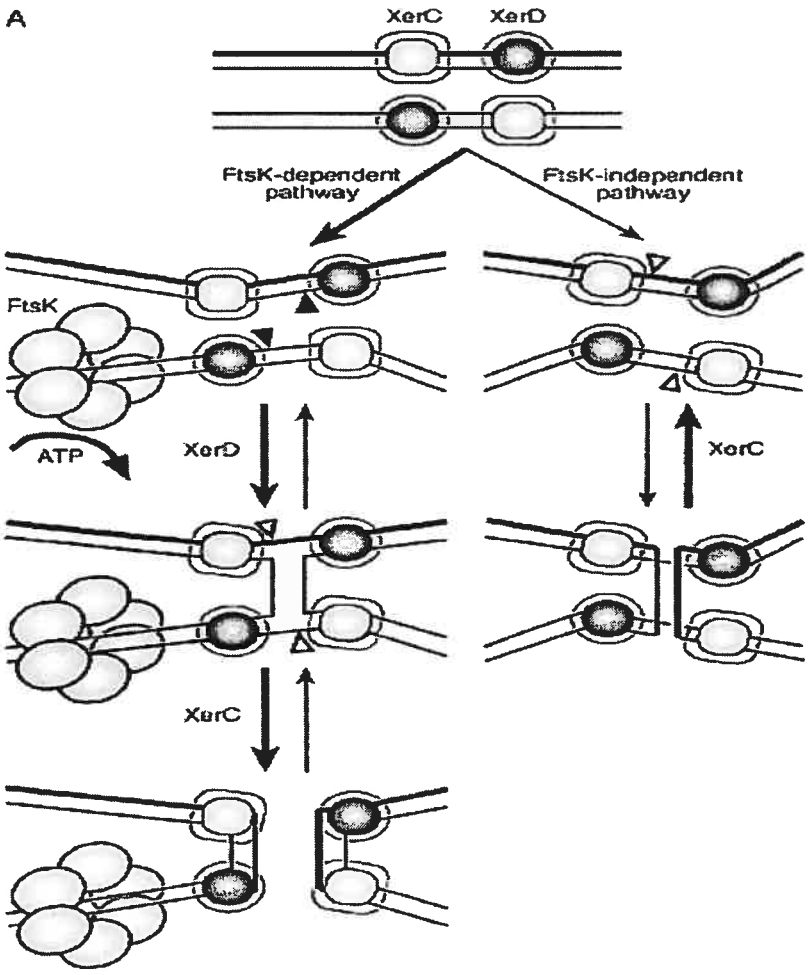


Figure 11 FtsK-Dependent and independent Pathways of Xer Recombination at *dif.* In the absence of FtsK, the Xer synaptic complex adopts a conformation suitable for XerC-mediated strand exchanges. FtsK can use the energy of ATP to switch the Xer synaptic complex to a conformation suitable for XerD-strand exchanges (Adapted from Aussel *et al.*, 2002).

2.5 Regulation of Xer Recombination

Xer recombination is subject to at least three different but interacting levels of control: the location of the *dif* site, homologous recombination, and the presence of the division septum-associated protein FtsK. By their interacting control, *dif* recombination can only occur on dimer chromosomes but not on monomer chromosomes, which demonstrates the directionality of *dif* recombination. Therefore, Xer recombination is regulated temporally (i.e. by time, just before cell division) and spatially (i.e. at the *dif* site).

2.5.1 DAZ and FtsK Control

How the Xer system acts specifically to resolve chromosome dimers and not to create them has been a long-standing question since the discovery that *dif* is devoid of accessory sequences and exhibits no directionality when inserted in multicopy plasmids (Blakely *et al.*, 1991; Kuempel *et al.*, 1991). The first evidence for directionality of *dif* site recombination came from the work of Steiner and Kuempel (1998). According to their observations, it was suggested that *dif* recombination only occurs on dimeric chromosomes. Moreover, it was found that efficient dimer resolution is dependents on the position of *dif* on the chromosome. For full activity, the *dif* site must be located in a 30-kb zone, the *dif* activity zone (DAZ) (Cornet *et al.*, 1996; Kuempel *et al.*, 1996; Pérals et al., 2000; Tecklenburg *et al.*, 1995). Specific DAZ induction only operates on dimeric chromosomes, restricting *dif* recombination to dimers and preventing recombination between monomers (Pérals *et al.*, 2001). This result solved the first question but arises another question. How

is *dif* recombination restricted to chromosome dimers? Recent data has shown it was controlled by the position of *dif* on the chromosome and the septum-located protein FtsK.

The location of *dif* on the chromosome is crucial factor for its activity. To recombine efficiently and resolve chromosome dimers, *dif* must be located in a 15-20kb region surrounding its normal position, called the DAZ region. Transition between the DAZ and the bulk of the chromosome occurs progressively along about 20-kb-long regions on either side of *dif* [Perals *et al.*, 2000]. The transition regions are dispensable for *dif* activity. However, they contain polarization signals that condition the formation of the DAZ at their junction. It is proposed that the DAZ-mediated control of CDR (Chromosome Dimer Resolution) is achieved via positioning of the *dif* sites at division septum (Pérals *et al.*, 2000).

FtsK is another crucial factor for *dif* recombination. As mentioned before, FtsK has been assigned to two roles in the chromosome dimer resolution. One is directly involved in Xer recombination catalysis by activating XerD; another is positioning the terminus regions of chromosome dimers at mid-cell and synapsing their *dif* sites (Capiaux *et al.,* 2002; Corre and Louarn, 2002; Aussel *et al.,* 2002; Yates *et al.*, 2003). How does the FtsK position the chromosome for dimer resolution? Lesterlin *et al* (2004) proposed a model in which septum-associated FtsK would load onto chromosomes and mobilize DNA according to its intrinsic polarization (Figure12). This process would stop when encountering XerCD-bound *dif* sites, thereby ensuring a proper sorting of chromosome DNA in the sister cells and synapse of the *dif* sites. Then a physical contact between two XerCD/*dif* complexes and septum-borne FtsK allows resolution of dimers to occur. When a dimer is present, the

XerCD/*dif* complexes and FtsK colocalize at the division septum at the time of septation. This restricts of *dif* recombination to the septum region.

Localization of FtsK is ensured by its N-terminal domain and localization of the *dif* sites by a chromosome polarization-dependent process. Since a polarization-dependent process is required for precise positioning of *dif* sites, it suggests FtsK may read chromosome polarization (Lesterin *et al.,* 2004). The chromosome polarity was revealed during the searching the determents of DAZ positioning. The sequences surrounding *dif* appear to be intrinsically polarized along the *ori-dif* axis and their relative orientation is the main determinant of DAZ positioning. The polarization determinants are present throughout a large terminal domain (more than 200 kb around *dif*) (Lesterin *et al.,* 2004). Thus, how can DNA polarity affect FtsK-dependent positioning of *dif* sites? Monitoring DNA translocation by $FtsK_{50C}$ at the single molecule level did not reveal any direct influence of the DNA sequence, suggesting that the control effected by DNA polarity on *dif* positioning is a complex phenomenon that implies the activities of other protein *in vivo* and/or of other domains of the FtsK protein (Saleh *et al.*, 2004). Interestingly, the C-terminal domain of *H. influenzae* FtsK can replace its *E. coli* counterpart for the *in vivo* processing of DNA polarity inside *E. coli,* which indicates conservation of the mechanism of polarity reading (Bigot *et al.*, 2004). Furhtermore, a specific instance of the GNGNAGGG motif rescently has been identified. This motif, its complement, or both are effective in specifying FtsK's directionality. It provides FtsK with the necessary information to faithfully distribute chromosomal DNA to either side of the septum, thereby bringing the *dif* sites together at the end of this process (Bigot *et al.*, 2005; Levy *et al.*,

2005).

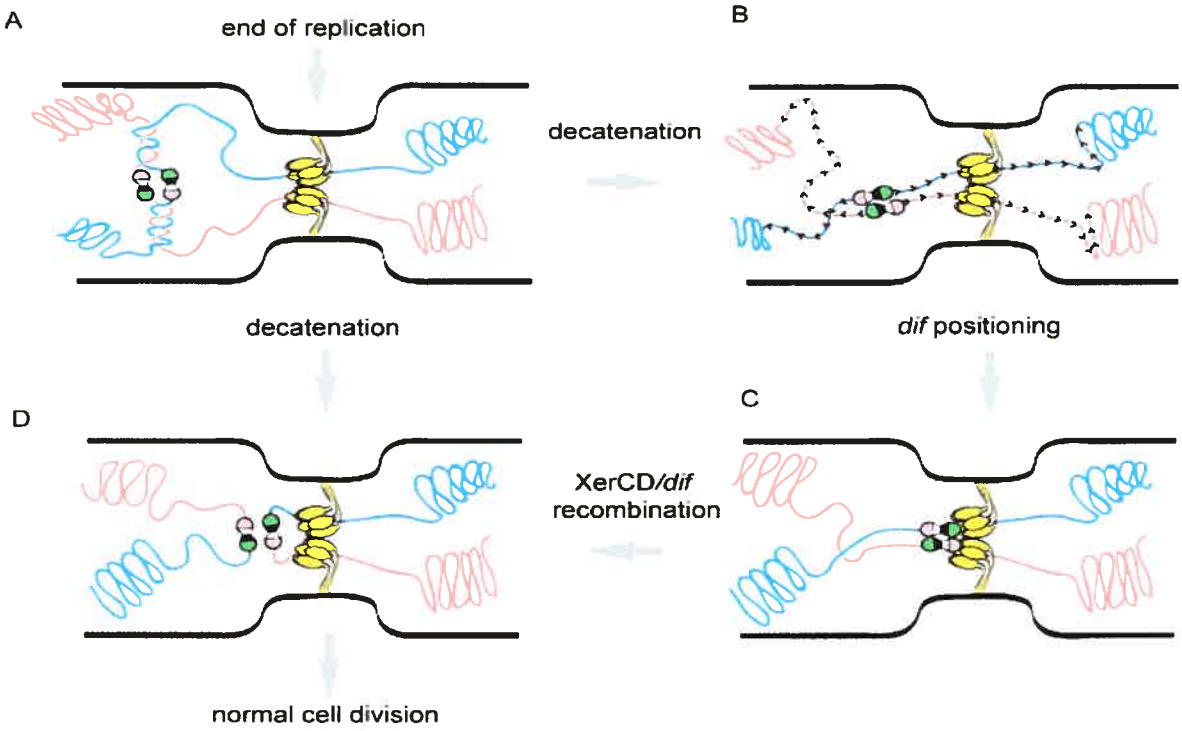


Figure 12 Model for segregation of the Ter domains and chromosome dimer resolution (Adapted from Lesterin *et al.*, 2004). The cartoon represents the central part of a dividing cell. The yellow bouquet represents hexamers of FtsK bound at the constricting septum. The chromosomes are shown as red and blue lanes, the *dif* sites as black and white dumbbells and the recombinases as the rose and green circles.

2.5.2 Homologous Recombination Control

Complete Xer recombination product at chromosomal or plasmid *dif* also depends on a functional homologous recombination system, which is necessary to generate dimeric chromosomes. Furthermore, the levels of Xer recombinational exchanges at *dif* seem to be proportional to the amounts of ongoing homologous recombination (Recchia and Sherratt, 1999; Steiner and Kuempel, 1998a, 1998b).

The major role of homologous recombination is to allow the reassembly of functional replication forks that have broken or stalled, either as a consequence of DNA breaks or lesions (Cox *et al.,* 2000) or because of stalled transcription machinery (McGlynn *et al.*, 2000). The recombination process can either exchange the flanking sequence (referred to as sister chromatid exchange (SCE) or 'crossing over') to produce dimer, or noncrossover, leaving monomeric chromosomes.

The rate of dimer formation depends on the frequency of recombination between sister chromosomes and on the frequency at which recombination events lead to sister chromatid exchange. There are two major Rec-dependent recombination pathways in *E. coli,* the RecFOR and the RecBCD pathways. Both pathways produce a Holliday junction, which is normally resolved by the RuvABC complex, although it maybe processed by other means in the absence of Ruv (Van Gool *et al.*, 1999; Cromie and leach, 2000; Michel *et al.,* 2000). On the basis of the assumption that all chromosome dimmers are resolved at *dif*, chromosome dimer formation has been indirectly quantified by monitoring Xer recombinational exchanges at *dif* with a density label assay. Dimer formation reaches 15% in wild-type cells and depends on homologous recombination (Figure 13) (Steiner and

Kuempel, 1998a, 1998b). Mutations in either of these pathways leads to about a 50% decrease in the number of Xer recombinational exchanges at *dif*, whereas mutational ablation of both pathways almost abolishes Xer recombination exchanges at *dif*. This estimate of the frequency of SCEs that lead to dimers fits well with the general phenotype of Xer mutants is consistent with 15% of divisions giving no viable progeny (Pérals *et al.*, 2000).

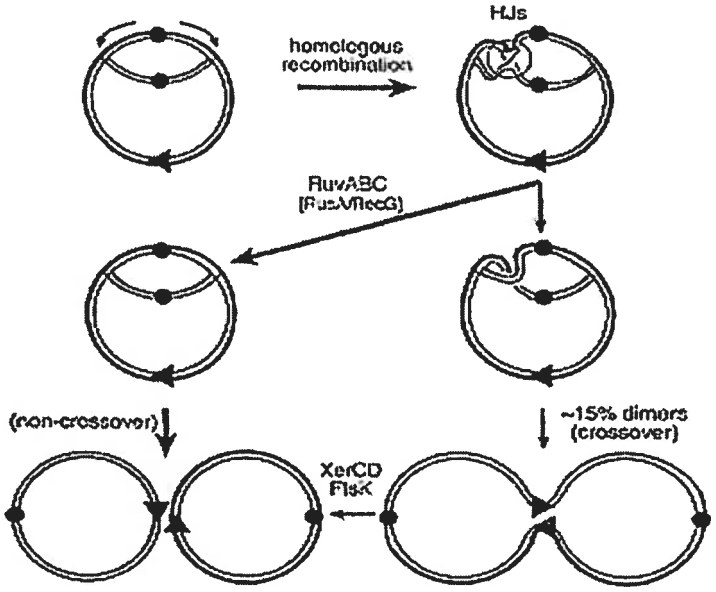


Figure 13 Dimer formation by homologous recombination. (Adapted from Barre and Sherratt, 2002). HJs made by homologous recombination can be resolved to crossover or noncrossover events. RuvABC (RusA/RecG) preferentially resolves them to noncrossover events. However, crossover events still occur. Consequently, dimers are formed. The Xer recombination system ensures their conversion to monomers.

2. 6. Xer System and Pathogenicity

Xer recombination is catalysed by two site-specific recombinases of the tyrosine recombinase family, XerC and XerD (Blakely *et al.*, 1993; Azaro and Landy, 2002). Orthologues of XerC and XerD are found in most eubacteria that harbour circular chromosomes (Recchia and Sherratt, 1999; Chalker *et al.*, 2000) and have been shown to be required for faithful segregation of the chromosome in *Bacillus subtilis* (Sciochetti *et al.*, 1999) and *V. cholerae* (Huber and Waldor, 2002). However, Xer mutants sometimes display intriguing and unexplained phenotypes, that Xer recombinases may function in processes indicating other than chromosome dimer resolution, such as pathogenicity.

2.6.1 *Pseudomonas fluorescens*

The first indication about the relativity between Xer recombinases and pathogenecity came from the biocontrol agent *Pseudomonas fluorescens*. The pseudomonads possess orthologues of genes encoding site-specific recombinases. A gene from *Pseudomonas aeruginosa* affecting pyoverdin production, and named *sss*, was shown to be homologous to the *E. coli xerC* gene (Hofte *et al.*, 1994). An *sss* mutant of *P. fluorescens* WCS365 was affected in competitive rhizosphere colonization, and was displaced by the wild type from the root tip of a variety of plants (Dekkers *et al.,* 1998). It has also been shown that introduction of extra copies of the *sss* gene can improve rhizosphere colonization (Dekkers *et al.,* 2000) and biocontrol abilities of different pseudomonads (Chin-a-Woeng *et al.,* 2000). Lately, a second recombinase encoded by the *xerD* gene was found and also implicated in phenotypic variation in *P. fluorescens* (Martinez-Granero *et al.,* 2005).

Mutants affected in the *sss* or *xerD* genes produced a very low quantity of phenotypic variants compared to the wild-type strain, both under prolonged cultivation in the laboratory and after rhizosphere colonization, and they were severely impaired in competitive root colonization. Both site-specific recombinases are involved in phenotypic variation (Martinez-Granero *et al.,* 2005).

2.6.2 *Staphylococcus aureus*

Homologs of the XerCD enzymes have been identified in the genomes of S*taphylococcus aureus*. A *S. aureus xerC* null mutant displayed *in vitro* characteristics consistent with the segregation defect reported for *E.coli* mutants, and was also found to be significantly attenuated in a murine infection model, suggesting that even a small impairment in the cell's ability to segregate efficiently may be highly deleterious to the successful establishment of infection. Strikingly, the *S. aureus xerD* gene appears to be absolutely required for viability and may therefore be the first example of an essential gene of the lambda integrase family. A *S. aureus xerD* mutant in allelic replacement mutagenesis studies could not be obtained. All 24 transductants carrying the correct allelic replacement were found to also contain a copy of the wild-type *xerD* gene, suggesting that only *xerD* mutants contain a functional second gene copy are viable. The absolute requirement of *S. aureus* XerD cannot be explained by a segregation deficiency alone. Hence it seems likely that this gene product has a separate function, in addition to or instead of a chromosome resolvase activity (Chalker *et al.,* 2000).

2.6.3 *Streptococcus pneumoniae*

It was also found that a *Streptococcus pneumoniae xer1* (putative *xerC*) null mutant was highly attenuated in a murine respiratory infection model, suggesting that it may control processes that affect virulence (Chalker *et al.,* 2000). Moreover, Reichmann and Hakenbeck (2002) also found that S. *pneumoniae* mutants in a putative *xerD* gene appeared to have several growth defects, such as longer generation time and longer chains of misshaped cells.

Taken together, Xer recombinases may encode other functions, involved in the pathogenicity of some species, in addition to chromosome dimer resolution. The results of Chalker *et al* (2000) demonstrated that XerD is a potential target for a novel anti-*S. aureus* inhibitor. In addition, the behaviour in their murine infection models of *S.aureus xerC* mutants and *S. pneumoniae xer* mutants demonstrated that targeting additional members of the lambda integrase family could extend the spectrum of pleiotropic effects on virulence. Further investigations of the function of these enzymes in different bacterial species will help to clarify their potential as antibacterial targets.

3. Streptococcus suis

Streptococcus suis is a world-wide causative agent of infections in swine and humans. Infections in pigs range from severe clinical forms, such as meningitis, septicaemia, arthritis and bronchopneumonia, to subclinical forms resulting in asymptomatic carriers (Chanter *et al.*, 1993; Reams *et al.*, 1994; Gottschalk and Segura,

2000). In humans, *S. suis* can cause meningitis, especially persons exposed to animals infected by *S.suis* as an occupation (Arends and Zanen, 1988; Kopic *et al.*, 2002).

The control of infection is hampered by the presence of many serotypes and limited knowledge on pathogenesis and virulence factors. To date 35 serotypes have been described based on capsular antigens (Higgins *et al.*, 1995). In addition, a substantial number of non-typeable strains exist (Wisselink *et al.*, 2000). Serotype 2 strains are considered the most important ones, because of the fact that they are frequently isolated from diseased animals. However, in some geographical regions other serotypes show higher prevalence, and it is now generally accepted that virulence of *S. suis* is not solely associated with the serotype. A number of putative virulence factors have been identified in recent years, such as the capsular polysaccharides (CPS), which protect bacteria against phagocytosis (Smith *et al.*, 1999); the muramidase released protein (MRP) and extracellular protein factor (EF), both of which are virulence associated proteins (Vecht *et al.*, 1991), and the cytolytic toxin suilysin (Jacobs *et al.*, 1994), of which the role in virulence is unclear. Further putative virulence associated factors are the Gala 1–4 Gal adhesin (Haataja *et al.*, 1993; Tikkanen *et al.*, 1996), and the AdiS protein, a temperature induced surface protein that is possibly involved in survival under acidic and anaerobic conditions (Winterhoff *et al.*, 2002). However, general conclusions from these studies are limited as most were restricted to serotype 2 strains, which are not necessarily representative for virulent *S. suis* as outlined above. So far, the CPS is the only proven critical virulence factor since unencapsulated isogenic mutants were shown to be completely avirulent and rapidly cleared from the circulation in both pig and mouse models of infection (Charland *et al.*,

1998; Smith *et al.*, 1999). However, natural nonvirulent *S. suis* serotype 2 strains are also encapsulated and have an amount of sialic acid in the CPS similar to the amount found in virulent strains (Charland *et al.*, 1996). On the other hand, suilysin, MRP, and EF protein have been associated with the virulent phenotype of European strains, but they are absent in most virulent North American strains (Gottschalk *et al.*, 1999). The exact roles of MRP and EF protein in *S. suis* pathogenesis are unknown.

Up to now, highly efficient treatment and control of diseases caused by *S. suis* are still not available. Attempts to prevent introduction of carrier pigs from endemic herds are not completely reliable because the infection can be subclinical, and there are no reliable tests to monitor its presence. Furthermore, it may be introduced in other ways (e. g. by flies). Another possibility when new outbreaks occur is that mild strains already endemic have mutated to become more virulent. Once in a herd, it tends to remain endemic; neither vaccination nor therapy of all animals will eliminate it. Although killed vaccines are used, their efficacy is unproved. Good husbandry reduces environmental stress and decreases clinical disease. Prophylactic or strategic medication is commonly used, usually in feed or water but sometimes by injection of long-acting antibiotics. The organism tends to become resistant to tetracyclines and sulfonamides. Most isolates are sensitive to penicillin, but it is rapidly inactivated in feed and, therefore, may fail to control disease.

4. The Master's Project

There are three reasons to focus on *Streptococcus suis* in our project. Firstly, *S. suis* is a Gram-positive bacterium, which is the leading cause of a wide range of diseases in

animals and is also implicated in human diseases. The genome of this organism has been partially sequenced by the Sanger Institute and Joint Genome Institute (JGI) (http://genome.jgi-psf.org/draft_microbes/strsu/strsu.home.html). The analysis of the partial sequence demonstrated the presence of open reading frames (ORF) that show strong homology to the *xer* genes of *S. aureus* and *S. pyogenes*. Therefore, one objective of our project was to clone and characterize the potential *S. suis xerC* gene. Secondly, *xer* mutants in *S.pneumoniae, S.aureus* and *P. fluorescens* have shown that a slight defect in the segregation of chromosomes can affect the growth and pathogenicity of these bacteria. It would be significant to inactive *xerC* gene of *S. suis* and to observe its effect on growth and pathogenicity. Thirdly, many strains of *S. suis* are poorly transformable by plasmids containing a Gram-negative replication origin, due to a lack of suitable shuttle vectors. Fortunately, we were able to obtain an *E. coli-S. suis* shuttle vector fot this work. The vector possesses both thermosensitive (Ts) replicons and gram-negative replication origin and can be used as either a normal plasmid vector or as a suicide vector for transposon delivery of gene inactivation studies. Takamatsu group and St-Hyacinthe group have succefully inactivate *S. suis* genes by introducing this vector into *S. suis*. (Takamatsu *et al.* 2001; Harel and Gottschalk, personal communication). Therefore, another objective of our project is to use this thermosensitive vector to inactivate the *xerC* gene and explore the role of this gene in the viability and pathogenicity of *S. suis.*

Chapter II

Article

The cloning and characterization of the *xerC* gene from *Streptococcus suis*

Fuli Jia and George Szatmari*

Département de microbiologie et immunologie

Université de Montréal

Keywords: *Streptococcus suis*/Site-specific recombination/

tyrosine recombinase/XerC/*dif*

*Address for correspondence:

CP 6128 succursale centre-ville

Montréal, Québec, Canada, H3C 3J7

Phone : 514-343-5767

Fax : 514-343-5701

Abstract

XerC and XerD are members of the tyrosine recombinase family and mediate site-specific recombination which contributes to the stability of circular chromosomes in bacteria by resolving plasmid multimers and chromosome dimers to monomers prior to cell division. Homologues of *xerC/xerD* genes have been found in many bacteria. In this paper, an open reading frame in *Streptococcus suis* with a strong homology to *xer* genes found in streptococcal bacteria was discovered. The gene, designated *xerC*, was cloned, overexpressed and purified as a maltose binding protein fusion. The purified XerC-MBP fusion showed specific DNA binding activity to the *dif* site of *Bacillus subtilis*, as well as *dif* site of *S. suis*. *xerC* mutants of *S. suis* showed significant growth and morphological changes.

1. Introduction

The Xer site-specific recombination system, initially discovered for its role in the stable maintenance of plasmid ColE1, also functions in the normal inheritance of the *Escherichia coli* chromosome. It is encoded by the circular chromosomes of many bacteria and functions to ensure that both circular chromosomes and multicopy plasmids are monomeric before their segregation to daughter cells at cell division (Sherratt *et al.*, 1995). Recombinations mediated by XerC/XerD are performed by the tyrosine recombinase family of site-specific recombinases. All members of this family contain invariant residues which are R...H-X-X-R...Y (Nunës-Duby *et al.*, 1998). Homologues of the *xerC/xerD* genes have been found in many bacteria (Recchia and Sherratt, 1999). They have been functionally characterized in gram-negative bacteria, such as *Pseudomonas aeruginosa, E. coli, Salmonella typhimurium , Haemophilus influenzae, Vibrio cholerae, Caulobacter crescentus,* and *Proteus mirabilis* (Höfte *et al.*, 1994; Sirois and Szatmari, 1995; Hayes *et al.*, 1997; Neilson *et al.*, 1999; Huber and Waldor, 2002; Jouan and Szatmari, 2003; Villion and Szatmari, 2003). They have also been characterized in gram-positive bacteria, such as *Bacillus subtilis, Lactobacillus leichmanni, Staphylococcus aureus* and *Streptococcus pneumoniae* (Slack *et al.*, 1995; Sciochetti *et al.*, 1999; Becker and Brendel, 1996; Chalker *et al.*, 2000; Reichmann and Hackenbeck, 2002).

Xer recombination occurs at two different sites and produces different biological outcomes. On chromosomal sites, like the *dif* site of *E. coli*, Xer recombination ensures that the chromosome is converted into two monomers prior to cell division (Blakely *et al.*, 1991; Kuempel *et al.*, 1991). On plasmid sites, such as *cer* (ColE1 plasmid) and *psi*

(plasmid pSC101), recombination is involved in the monomerization of plasmids, increasing their stability. All these sites contain the recombination core site and many homologues have been found in eubacteria and naturally occurring plasmids (Hayes and Sherratt, 1997; Lesterlin *et al.,* 2004). In all the Xer recombination sites, XerC and XerD cooperatively bind to specific 11 bp consensus sequences that are separated by a 6- to 8-bp central region at the borders of which the DNA strands are cleaved and exchanged (Blakely *et al.,* 1993). In recombination mediated by tyrosine recombinases, DNA strands are cleaved and rejoined through the formation of a transient DNA-protein covalent intermediate involving a conserved tyrosine as the catalytic nucleophile. The mechanism is the same as that found in the related type IB topoisomerases (Sherratt and Wigley, 1998). On the chromosomal site *dif*, the minimal 28 bp recombination core site is sufficient to promote both intra- and intermolecular recombination (Blakely *et al.,* 1991; Blakely *et al.*, 1993; Neilson *et al.,* 1999). However, to ensure that the correct order of strand exchanges occur at the right time and location, the *dif* site must be located at the terminus of the *E. coli* chromosome, and the C-terminal region of the FtsK protein is required (Steiner *et al.,* 1999; Barre *et al.,* 2000). Recombination at natural plasmids also requires additional accessory factors such as PepA, ArgR and ArcA (Stirling *et al.,*1988; 1989; Colloms *et al.,* 1998).

Homologues to *xer* genes have been found in *Staphylococcus aureus* and *Streptococcus pneumoniae* and *xer* mutations in these bacteria displayed attenuation in a murine infection model (Chalker *et al.,* 2000; Reichmann and Hackenbeck, 2002). Moreover, *Pseudomonas fluorescens*, the biocontrol agent, also possesses XerC and XerD tyrosine recombinases and strains with *xer* mutations displayed reduced pathogenicity (Dekkers *et al.,* 1998; Martinez *et al.,* 2005). It suggests Xer

recombinases may also function in processes other than chromosome dimer resolution (CDR), or that deficiencies in CDR may have far-reaching consequences on other cellular processes.

Streptococcus suis is a Gram-positive bacterium, which is leading cause of a wide range of diseases in animals (Staats *et al.,* 1997) and has also been implicated in human diseases (Arends and Zanen, 1988). By analyzing the available sequence data of *S. suis*, an open reading frame (ORF) was found that showed a strong homology to *xer* genes of *S. aureus* and *S. pneumoniae*. In this paper, we report the cloning of the *S. suis xerC* gene and characterization of its product by DNA binding assays. The *S. suis xerC* gene was also inactivated, and the growth and morphology of the *xerC* mutant was characterized.

2. Materials and methods

2.1 Bacterial strains and plasmids

The *S. suis* strains used in this study were the virulent strains P1/7, S735 and 31533 of serotype 2. *E. coli* strain AG1 (*recA1 endA1 gyrA96 thi-1 hsdR17* [R_K- M_K^+] *supE44*) was used for cloning and plasmid purification. For overexpression of maltose-binding protein (MBP)-fused genes, strain DS981 (*thr1 leuB6 hisG4 thi1 ara14 Δ(gpt-proA)62 argE3 galK2 supE44 xyl5 mtl1 tsx33 lacY1 rpsL31* (Str^r) *recF143 lacI* ^{q} *Δ(lacZ)M15 xerC2*) was used (Colloms *et al.,* 1990).

Plasmid pQE30 (Qiagen) was used to clone the PCR-amplified *xerC* gene of *S. suis*. For overexpression and purification, the *xerC* gene was subcloned as a *Bam*HI-*Sac*I fragment into plasmid pMalC2 (New England Biolabs, NEB). The thermosensitive

suicide plasmid pBEA756 was used for S. *suis* mutagenesis (gift from M. Gottschalk and J. Harel). This plasmid is derivative of pCIV2 (Okada *et al.*, 1993). An internal fragment of the *xerC* gene of *S. suis* was amplified by PCR and cloned into the *Eco*RI site of pBEA756.

2.2 Growth conditions and DNA manipulations

E. coli strains were routinely grown in LB broth or plated on LB agar, containing the appropriate antibiotics when required. Ampicillin was used at 100 μg mL^{-1}, kanamycin at 50 μg mL^{-1}. *S. suis* was grown in Todd-Hewitt broth (THY, Oxoid) or agar (THA) with 1% yeast extract (Difco) and kanamycin (400 μg mL^{-1}) was supplied when required. Restriction enzymes, Taq DNA polymerase, Vent DNA polymerase and T4 DNA ligase were obtained from NEB and used according to the supplier's conditions. All routine DNA manipulations were performed as described by Jouan and Szatmari (2003). DNA fragments were extracted from agarose gels using the QIAquick gel extraction kit or QIAEXII gel extraction kit (Qiagen). Genomic DNA of *S. suis* was prepared using the DNeasy Tissue Kit (Qiagen). Plasmids were extracted from *S. suis* using the QIAquick miniprep kit with the following modification: cell pellets were suspended in P1 buffer; 1mg/ml lysozyme was added and incubated for 30 min at 37° C. Southern hybridizations were done according to Sirois and Szatmari (1995).

2.3 PCR conditions

PCR reactions were performed using a CyclePro Thermocycler (Bio-Can) with either Taq DNA polymerase or Vent DNA polymerase (NEB). For the amplification of

the *S. suis xerC* gene, the cycling conditions were: 15 s at 95°C followed by 30 s at 54°C and 1min 7s at 72°C for 30 cycles, a final extension at 72°C for 5 min. Reactions were carried out in 50 µl reactions using chromosomal DNA from *S. suis* using Vent DNA polymerase. Primers SsuisXerCFwd (5'GATGAGACGCGAGTTATTATTGG3') and SsuisXerCRev (5'TCACAACTGATCCAGAGCAT3') were used. The PCR product was sequenced by the CHUM sequencing facility of the Université de Montréal using the following primers: pQE (5'GATTCAATTGTGAGCGGA3'), and SsuisXerCRev (5'TCACAACTGATCCAGAGCAT3'). Primers were synthesized by BioCorp Inc.

2.4 Protein overexpression and purification

The *S. suis xerC* gene was amplified by PCR using Vent polymerase with primers SsuisXerCFwd and SsuisXerCRev using the cycling conditions described previously and cloned into *Sma*I-cleaved pQE30. The cloned *xerC* gene was then transformed into an *E. coli xerC* $^{-}$ mutant strain DS981. The overexpressed histidine-tagged XerC protein was insoluble when overexpressed. In order to overcome this problem, the pQE30 *Bam*HI-*Sac*I fragment containing *xerC* was subcloned into pMalC2 and was transformed into DS981. Cells were incubated at 37°C to an OD_{600} of 0.4-0.6 and then induced with 0.2 mM IPTG for 2h. Once harvested, pellets were resuspended in column buffer (20mM Tris-HCl, pH 7.4, 200mM NaCl, 1mM EDTA) and were freeze-thawed and sonicated followed by centrifugation at 13,000 ×g at 4°C. Supernatants were passed through an amylose column prepared according to the manufacturer's directions. Elution steps were performed according to the same protocol, except an additional step of washing in column buffer was performed before elution with 10 mM maltose in column buffer.

Most of the protein eluted in the first 1 ml fraction. Proteins were separated by SDS-PAGE on 12.5% gels and visualized by Coomassie blue staining. Protein concentrations were estimated by the Bradford method using the Bio-Rad Protein assay (Bio-Rad).

The *E. coli* XerD and $XerC_{MBP}$ (*E. coli* XerC fused to the maltose binding protein (MBP)) proteins were used as controls and also for the cooperative binding experiments to test different combinations of Xer proteins from *E. coli* and *S. suis*. XerDEc (*E. coli* XerD) was induced from pRM130 in BL21 pREP4 with 0.2 or 0.5 mM IPTG for 2 hr at 37°C. It was then purified as described by (Arciszewska *et al.*, 1997) on a His-trap column from Amersham Pharmacia Biotech. $XerC_{MBP}$ was induced from pGB500 in DS9029 and purified as for XerCSs-MBP protein.

2.5 DNA-binding assay

Specific DNA binding was determined by the gel retardation assay (Jouan and Szatmari, 2003) using specific fragments labeled with digoxygenin (DIG) by PCR. For the *E.coli dif* (*difEc*) fragement, the following primers were used: difF22 (5' CAGAAAAGCACTTCGCATCAC3') and difR4 (5'CAATCATGACCGCCAACGAC3') using the following conditions: 15s at 95°C followed by 30s at 58°C and 20s at 72°C for 30 cycles, a final extension at 72°C for 5 min. For labeling *B. subtilis dif* (*difBs*) fragment, the following primers were used: BsdifF (5' GCGCATATGGCGGATATGACGCTGG3') and BsdifR (5' CTGAATTGGCGATTTTCCGATTGGG3') using the following conditions: 15s at 95°C followed by 30s at 68°C and 40s at 72°C for 30 cycles, a final extension at 72°C for 5 min. The Dig-labeled fragments were extracted from polyacrylamide gel by using the QIAEXII gel extraction kit (Qiagen). For labeling the *S. suis dif* (*difBs*) fragment, the

following primers were used: difSsuisF (5'AGTCTTTCCGACACCGAA3') and difSsuisR (TCATCAGTGTCCTAGTATTGTACGTTT3'), under the following conditions: 15s at 95°C followed by 30s at 51°C and 30s at 72°C for 29 cycles, a final extension at 72°C for 5 min. The *difSs* fragment was purified by using QIAquick Gel Extraction kit (Qiagen).

DNA binding assays were performed using TENg buffer (20mM Tris-HCl, pH 7.5, 1mM EDTA, 50mM NaCl and 5% glycerol) with 1 μg polydIdC (Amersham Pharmacia Biotech) and DIG-labeled *dif* sites. Reactions were incubated at 37°C for 30 min and electrophoresed at room temperature on a 6% polyacrylamide gel in 1×TBE buffer at 8V/cm. Gels were then transferred onto positively charged nylon membranes (Hybond^{TM}-N^{+}, Amersham) and UV-crosslinked. Final detections were done with CDP-Star (NEB), according to the standard digoxigenin detection methods and followed by exposure to Fuji SuperRX-Xray film.

2.6 Preparation of *S.suis* Electrocompetent cell

An overnight culture of *S.suis* was diluted 1:40 into 200 ml of fresh THY medium and grown at 37°C until OD_{600} reached 0.4-0.5, corresponding to early-logarithmic growth phase. The cells were collected by centrifugation at 8000 rpm at 4°C for 15 minutes and obtained cell pellets. The cells were washed four times with pre-chilled electroporation medium (0.5 M sucrose) at 9000 rpm at 4°C for 15 minutes, resuspended in 2 ml chilled 0.5 M sucrose, aliquoted 50 μl in each tube and stored at -70°C until use.

2.7 Electroporation and Inactivation of *S. suis xerC*

The thermosensitive plasmid pBEA756 was used to inactivate the *xerC* gene of *S. suis*. This plasmid is a derivative of pCIV2 (Okada *et al.,* 1993) and carries a kanamycin resistance element and an *E. coli* ColE1 origin and a thermosentitive (Ts) gram-positive replication origin from plasmid pVE6007 (Maguin *et al.,* 1992). An internal sequence of the *xerC* gene was amplified by PCR using the primers: SSXerCinF (CTA TGA ATT CGG GAG CGT CCC TTG CT) and SSXerCinR (CTT CGA ATT CGG CAG ACC ACG GTA TTC G), using the following conditions: 15s at 95°C followed by 30s at 57°C and 1min at 72°C for 30 cycles, a final extension at 72°C for 5 min. The 580 bp PCR product was purified and ligated into the PCR cloning vector pDrive (Qiagen). The *Eco*RI-cleaved fragment from pDrive vector was subcloned into pBEA756 and transformed into AG1, forming the plasmid pBEAXerCint. This plasmid was mixed well with *S.suis* electrocompetent cells and transferred into chilled electroporation cuvettes (inter-electrode distance = 0.2 cm), then electroporated into *S. suis* using a BioRad gene pulser with a setting of 25 μF, 2.5 kV and 200Ω. This setting resulted in atime course ranging from 4.6 to 5.2ms. Immediately after the pulse, 950 μl of THY medium was added and the samples were incubated at 28°C for 3h, and spread on a THA plate and incubated at 28°C (permissive temperature for the Ts origin). The resulting transformants were obtained after prolonged incubation (3 days) after electroporation, and were then grown in THY broth overnight with kanamycin selection at 28°C. Small aliquots of overnight cultures were spread on selective THA plates and incubated at 37°C (non-permissive temperature) to inactivate the Gram-positive origin. Cells, which remained kanamycin-resistant, presumably had integrated the plasmid into the

chromosome by homologous recombination at the *xerC* locus, inactivating the gene. This was confirmed by Southern blot analysis.

2.8 Phenotypic analysis/microscopy

Because of the increased length of time required for *xerC* mutant cells to grow on solid media, the generation time of wild type and *xerC* mutant strains was analyzed in broth cultures. Overnight cultures were diluted in fresh 25 ml THY medium for both *xerC*⁻ and wild type *S. suis* strains. Cultures were incubated at 37°C with vigorous shaking. The optical density at 600 nm was measured at various time intervals using a Bausch and Lomb Spec 20 spectrophotometer.

To examine the morphology of both wild type and *xerC* mutants, cells from overnight cultures were fixed in a mixture of 100 µl 16% formaldehyde and 0.4 µl 25% gluteraldehyde followed by a 5min centrifugation at 5,000 rpm and washed 2-3 times in 1 ml PBS. Twenty-five microliter samples were dropped on the slides and covered with poly-lysine-treated coverslips, and were examined by DIC (differential interferential contrast, also named Nomarski) microscopy using a Nikon TE2000U fluorescence inverted microscope with a Nikon Plan Apo NA 1.4 100 x objective. Images were captured using a Photometics CoolSnap HQ 12-bit CCD black and white camera and were analysed using Metamorph ver6.3 (Universal Imaging Corporation).

3. Results

3.1 Sequence analysis

The *xerC* gene of *S. suis* encodes a 356 aa protein, which shows typical features of the tyrosine recombinase family (Figure 1). It possesses all the RKHRH conserved residues and includes the catalytic tyrosine residue (Y) close to the carboxy-terminus. Analysis of the sequence displays a very high degree of similarity with the Xer proteins in other streptococcal bacteria. It shows 79% identity and 88% similarity with XerC protein of *S. pneumoniae*, but only 27% identity and 45% similarity to XerC (CodV) of *B. subtilis* (Figure 1). This protein was designated as a XerC recombinase by the diagnostic residues XR_{282} for XerC recombinase (Subramanya *et al.,* 1997). It also contains the tripeptide LGH, which is well conserved among integrase family members (Cao *et al.,* 1997; Blakely and Sherratt, 1996).

```
XerCSs          ERIADIPLDVLEHLTKKDMEAFILYLRERPLLNANTTQNGVSQTTINRTLSALSSLFKYL 120
XerCSpn         DKISDIPLSVLENMSKKDMESFILYLRERPLLNANTTKQGVSQTTINRTLSALSSLYKYL 120
XerCBs          -------INGFEEAAYQDTRIFLTEAYEK----------GLSRRTISKKISALRSFYKFL 87
                       :. :*. : :* . *:    *:          *:*: **.:.:*** *::*:*

XerCSs          TEEVENEQGEPYFYRNVMKKVSTKKKKETLAARAENIKQKLFLGDETMEFLDYVDKEYQV 180
XerCSpn         TEEVENDQGEPYFYRNVMKKVSTKKKKETLAARAENIKQKLFLGDETEGFLTYIDQEHPQ 180
XerCBs          MREKLIEE-------NPFQLVHLPKQ--------EKRIPKFLYQKELEELFEVSDISQPA 132
                 .*   ::       * :: *   *:        *:   *::  .*   ::   * .

                                        I                                III
XerCSs          NLSKRALSSFQKNKERDLAILALLLASGVRLSEAVNLDLRDVNLNMMIIEVTRKGGKRDS 240
XerCSpn         QLSNRALSSFNKNKERDLAIIALLLASGVRLSEAVNLDLRDLNLKMMVIDVTRKGCKRDS 240
XerCBs          GMR-------------DQALLELLYATGMRVSECCSITINDVDLFMDTVLVHGKGKKQRY 179
                 :              * *:: ** *:*:*:**. .: :.*::* *  : *  ** *:

XerCSs          VNVAGFAKLYLEAYMGIRQQ--RYKAEKTDTAFFLSEYRG-LPNRIDASSIEKMVAKYSA 297
XerCSpn         VNVAAFAKPYLENYLAIRNQ--RYKTEKTDTALFLTLYRG-VPNRIDASSVEKMVAKYSE 297
XerCBs          IPFGSYAREALKVYMNSGRQCLLMKAKEPHDLLFVNQRGGPLTARGIRHILSGLVQKASS 239
                : ...:*:  *: *:   .*    *:::..  :*:.   * :. *     :. :* * *
                                       II
XerCSs          DFKIRVTPHKLRHTLATRLYDATKSQVLVSHQLGHANTQVTDLYTHIVNDEQKNALDQL- 356
XerCSpn         DFKVRVTPHKLRHTLATRLYDATKSQVLVSHQLGHASTQVTDLYTHIVSDEQKNALDSL- 356
XerCBs          --TLHIHPHMLRHTFATHLLNEGADLRSVQELLGHSNLSSTQIYTHVSKEMLRNTYMSHH 297
                  .::: ** ****:**:* :   .   *.. ***:. . *::***: .:  :*:  .

XerCSs          -------
XerCSpn         -------
XerCBs          PRAFKKN 304
```

Figure 1. Alignment of XerC proteins of *S. suis, S. pneumoniae* and *B. subtilis*. Alignments were done using: ClustalW from the European Bioinformatics Institute sequence analysis server: http://www.ebi.ac.uk/clustalw/index.html. *S. suis* shares 79% and 27% identities with *S. pneumoniae* and *B. subtilis*, respectively. Motifs I, II, III are described by Cao *et al.* (1997). The catalytic residue (Y) and conserved residue (R, H, R,) are indicated in bold. (*) corresponds to identity, (:) to a high degree of similarity and (.) to a lower degree of similarity between amino acids.

3.2 The *S. suis* XerC protein binds to the *B. subtilis dif* site

There are three well-characterized *dif* sites from the circular chromosomes of *E. coli, H. influenzae* and *B. subtilis*. Xer proteins bind specifically to *dif* sites and carry out their catalytic activities in the presence of the C-terminus of FtsK. The Xer recombinases of several species like *H. influenzae, C. crescentus*, *P. mirabilis* and *B. btilis* have been shown to bind to the *E. coli dif* site (Neilson *et al.,* 1999; Jouan and Szatmari, 2003; Villion and Szatmari, 2003; Sciochetti *et al.,* 1999). Moreover, the XerC and XerD proteins of *E. coli* could also bind to the *B.subtilis dif* sites (Sciochetti *et al.,* 2001). The *S. suis* XerC protein was cloned and expressed as an 82 kDa N-terminal MBP fusion, which optimized the solubility of this protein when overexpressed in *E. oli*. Previous work had shown that the MBP fusion did not affect the protein's binding ability (Sciochetti *et al.,* 1999).

The *dif* site from *B. subtilis (difBs)* was first chosen to analyze the binding activity of *S. suis* XerC-MBP fusion protein (XerCSs-MBP), since it is the only well characterized *dif* site from gram-positive bacteria. To ascertain if XerCSs-MBP could specifically bind *difBs*, gel retardation analysis was performed using DIG-labeled *difBs* with the purified XerCSs-MBP (Figure 2A). Two protein-DNA complexes that migrated with reduced mobility were observed at a concentration of 1 μM XerCSs-MBP (Figure 2A). The retarded bands were observed in the presence of 600 ng polydIdC competitors.

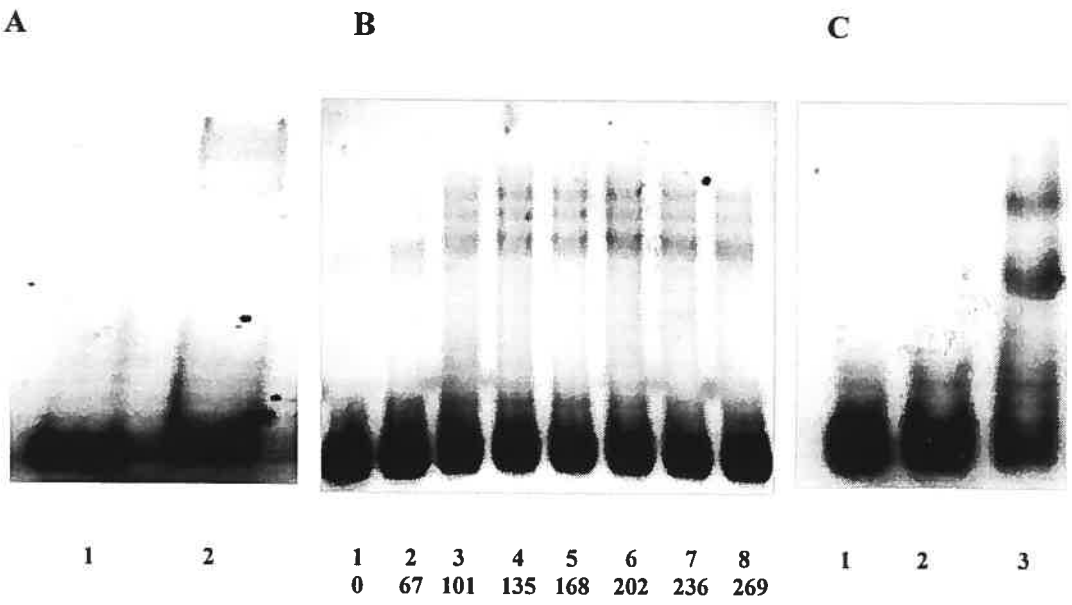


Figure 2. DNA binding assays with Xer proteins from *S. suis* and *E. coli*. **A**. XerCSs-MBP protein binding to *difBs*. The binding reactions were performed with 600 ng poly dIdC competitor. Lane 1, no added protein; lane 2, 1 mM XerCSs-MBP. **B**. XerCSs-MBP binding to *difSs*. The amount of XerCSs-MBP added from lanes 1-8 are 0, 67, 101, 135, 168, 202, 236 and 269 nM, respectively. Each reaction contained 600 ng poly dIdC. **C.** *E. coli* XerC/ XerD binding to potential *difSs*. One microgram dIdC was added to each reaction. Lane 1, no protein; lane 2, 298 nM XerCEc-MBP; lane 3, 1.5 mM XerDEc.

3.3 Cooperative Binding studies

Since the *S. suis* XerC protein possesses all conserved residues of site-specific recombinases and displays some similarity with XerC protein from *E. coli*, the *dif* site of *E. coli* (*difEc*) was initially used to analyze its binding activity by gel retardation assay. However, no specific binding to *difEc* was detected in our assays (data not shown). In

E. coli, XerC and XerD bind cooperatively (Blakely *et al.,* 1993); and it is possible that *S. suis* XerC might require its potential partner such as XerD for binding to *difEc*, but the addition of XerDEc to the binding reaction did not affect the binding of XerCSs to *difEc* in our assay (data not shown). However, it was interesting to note that stronger retardated bands were observed for XerDEc binding to *difEc* in the presence of XerCSs-MBP (Figure 3). This suggests that *S. suis* XerC protein stimulated XerDEc binding to *difEc*. XerCEc and XerDEc have been shown to bind cooperatively *dif* site (Blakely *et al.*, 1993). Our observation suggests that these two proteins may cooperate in binding to *difEc*, but do not form additional complexes as observed in Blakely *et al.* (1993).

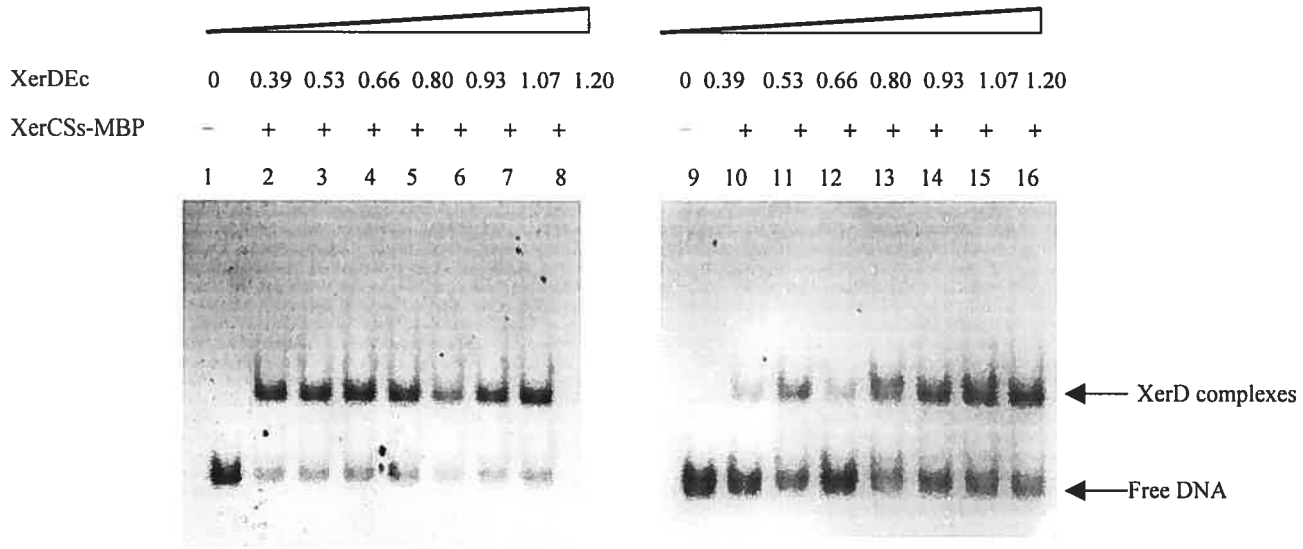


Figure 3. XerC *S. suis* stimulates XerDEc binding to *difEc*. Lanes 1-8, increasing amounts of XerDEc (μM) added to *difEc* in the presence of XerCSs-MBP (0.67μM). Lanes 9-16, XerDEc binding to *difEc* without XerCSs-MBP. One microgram dIdC competitor was added in each binding reaction.

3.4 Discovery of a putative *S. suis dif* site

Since XerCSs-MBP showed specific binding activity to *difBs*, it was quite likely that a specific DNA sequence similar to *difBs* may be found on the *S .suis* chromosome. By homology searching, a potential *dif* site of *S. suis* was found (Figure 4). The site, named *difSs,* is defined by two 11-bp half-sites separated by a 6-bp central region. An alignment of *difSs* with *dif* sites from *B. subtilis, H. influenzae* and *E. coli* demonstrates that the right half-site sequence is highly conserved (11-bp matches to *difBs*, 9-bp matches to *difEc* and *difHin*), while the left half-site is more divergent (6-bp matches to *difBs* and *difEc*, 7-bp matches to *difHin*) (Figure 4).

```
difSs      5′  AGT TTA CAC AA  AA TA AA  TTA TGT AAA CT
difBs      5′  ACT TCC TAG AA  TA TA TA  TTA TGT AAA CT
difEc      5′  GGT GCG CAT AA  TG TA TA  TTA TGT TAA AT
difHin     5′  ATT TCG CAT AA  TA TA AA  TTA TGT TAA AT
                *   *          *          *     *
```

Figure 4. Alignment of chromosomal recombination site sequences. *difSs, difBs* and *difEc* refer to recombination sites from *S. suis, B. subtilis* and *E.coli*, respectively. Central region sequences are in bold face type.

The binding activity of XerCSs-MBP to *difSs* was analysed by gel retardation assays. In binding reaction mixtures, increasing quantities of XerCSs-MBP were added to a fixed concentration of DNA and 600 ng polydIdC competitors. A retarded band was observed at 67nM protein (Figure 2B, lane 2), with additional retarded bands observed with increasing concentrations of XerCSs-MBP (Figure 2B, Lanes 2-8).

Since *difSs* site also shows similarity with *difEc*, we speculated that XerC and XerD of *E. coli* might also bind to this site. A gel retardation assay was performed to detect this possibility using purified XerC-MBP and XerD of *E.coli.* Addition of *E. coli* XerC-MBP to *difSs* did not produce a detectable complex; however, addition of 1.5 μM *E. coli* XerD alone gave rise to two complexes (Figure 2C lane 3).

3.5 Inactivation of the *S. suis xerC* gene

The vector pBEA756 possesses both Gram-positive thermosensitive (Ts) and ColE1 replication origins. An internal fragment of the *S. suis xerC* gene was generated by PCR and cloned into this vector, generating the plasmid pBEA756XerCint. This plasmid was then successfully introduced into *S. suis* by electroporation and was extracted as described in section 2.2. At the restrictive temperature (37°C), homologous recombination events were selected for by maintaining growth in the presence of kanamycin. A single crossover event between the cloned *xerC* gene on the plasmid and the chromosome copy of *xerC* results in the inactivation of the *xerC* gene, which was first confirmed by PCR (data not shown), and by Southern blot (Figure 5).

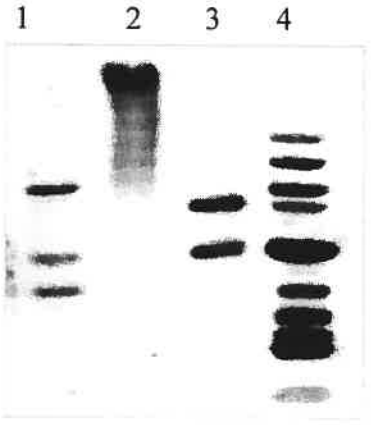


Figure 5. Southern blot analysis of wild type and mutant *S. suis*. *Hin*dIII-digested total genomic DNAs from wild-type and *xerC* mutant strains of *S. suis* were separated by electrophoresis through 0.7% agarose gel, transferred and hybridized with a DIG-labeled *xerC* gene probe. Lane 1, mutant; lane 2, mutant genomic DNA control (non digested); lane 3, wild-type; Lane 4, molecular weight marker (2-log DNA ladder).

3.6 Phenotypic analysis of an *xerC* mutant of *S. suis*

The *xerC* mutant of *S. suis* was viable, but showed a slower growth rate when compared to wild-type (Figure 6A). The mutant culture showed an extended lag phase of up to one hour in THY medium, resulting in a 40-60% reduction in cell numbers at each time point compared with wild-type control. It also extended by about two hours the time required to reach stationary phase.

Microscopic analysis of *xerC* mutant cells showed a significant increase in average chain length, averaging 6-18 compared to 2-7 cells per chain in wild-type cells (Figure 6B). Most of wild-type cells are 2 or 3 cells long, while mutants are more than 6 cells

long, and extremely long chains, containing more than 30 cells, were also observed (Table 1).

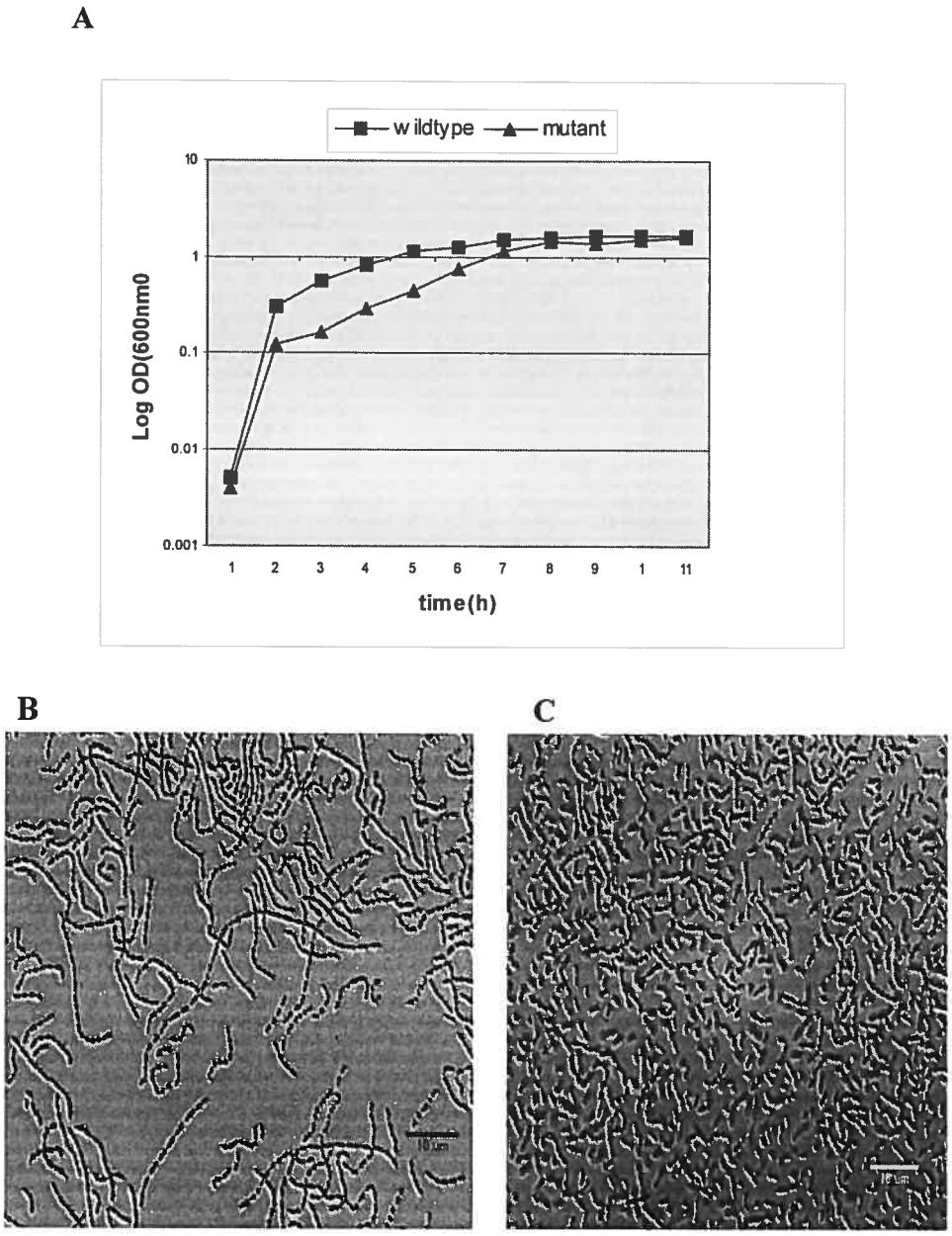


Figure 6. Growth curves and morphology of S. *suis xerC* mutant. **A.** Growth curves of *xerC* mutant (triangles) and wild type (squares) *S. suis*. **B.** Micrographs of cells of *xerC* mutant (left) and wild-type *S. suis* (right).

Table 1. Chain length comparison between wild type and *xerC* mutant of *S. suis*. Chain lengths were enumerated from micrographs in Figure 5. "Chain length" 1 refers to the number of cocci per chain and "number" 2 refers to the number of chains observed for each respective length.

Wild-type		Mutant	
chains length[1]	chains number[2]	chains length	chains number
2-3	99	2-3	6
4	36	4	25
5	19	5	15
6-7	15	6-7	49
9	1	9	11
over 10	0	10-20	37
Total	170	Total	143

4. Discussion

In this report, we described the cloning, overexpression, purification and inactivation of the *S. suis xerC* gene and its MBP-fused product. The deduced amino acid sequence of the *S. suis* XerC protein displays all the conserved triad residues RHR and catalytic tyrosine residue (Y) of site-specific recombinase family. It especially displays strong similarity to XerC proteins of other streptococcal bacteria. For example, it displays 79% identity and 88% similarity with the *S. pneumoniae* XerC protein. However, it only displays 27% identity with XerC (CodV) *B. subtilis* (Figure 1). The same low identity was also observed when comparing with XerC/XerD of *E. coli*. This may be explained by the great evolutionary divergence of the two organisms from which

the proteins were derived. According to the phylogenetic tree of Reichmann and Hackenbeck (2002), it was proposed that two distinct groups might exist in the XerD family, one of which is represented by the streptococcal protein. These proteins show lower identity when they compared to the Xer proteins of *E. coli or B. subtilis*.

The *S. suis* XerC recombinase was overexpressed and purified as a maltose binding protein fusion. The purified fusion protein displayed specific binding to the *dif* site from *B. subtilis*. Addition of XerCSs-MBP to *difBs* gave rise to two protein-DNA complexes that migrated with reduced mobility (Figure 2). This result differs from what has been observed with CodV binding to the *difBs* site (Sciochetti *et al.*, 2001), which only gave rise to a single protein-DNA complex that migrated with mobility consistent with a single recombinase monomer binding to the recombination site (Sciochetti *et al.*, 2001; Blakely *et al.*, 1997). It is possible that XerCSc-MBP might bind to both halves of *difBs*.

Since the *S. suis* XerC protein possesses all conserved residues of site-specific recombinases and displays some similarity with XerC protein from *E. coli*, the *dif* site of *E. coli* (*difEc*) was initially used to analyze its binding activity by gel retardation assay. However, no specific binding to *difEc* was detected in our assays (data not shown). We speculated several possibilities of the failure to detect binding. Firstly, the failure to detect binding to *difEc* might be due to differences between XerC proteins of *E. coli* and *S. suis* in the residues implicated in base and phosphate contacts that determine binding specificity (Subramanya *et al.*, 1997). Secondly, the binding might be of such low affinity that our assays are unable to detect it.

In *E. coli*, XerC and XerD bind cooperatively; it is possible that XerCSs-MBP might require its XerD partner for binding, but the addition of XerDEc to the binding

reaction did not have any effect on XerCSs-MBP binding to *difEc* (data not shown). It suggests that XerDEc could not cooperatively stimulate XerC *S. suis* binding to *difEc*. It is possible that the high degree of divergence between these proteins may not allow for cooperative binding at *difEc* or at *difSs*. We also attempted to find the *xerD* gene of *S. suis*. An open reading frame was identified that displayed a strong similarity with *S. aureus* XerD, but this protein was truncated by about 100 aa at its amino terminus. With the completion of *S.suis* genomic sequencing, the putative *xerD* gene might be explored and the two proteins cooperatively binding might be examined in the future study. Alternatively, it is possible that this truncated XerD protein may still be able to interact with XerCSs for binding cooperativity and to catalyze site-specific recombination. Future studies will look at this possibility.

However, it was interesting to note that stronger retardated bands were observed for XerDEc binding to *difEc* in the presence of XerCSs-MBP (Figure 3). This suggests that *S. suis* XerC protein stimulated XerDEc binding to *difEc*. XerCEc and XerDEc have been shown to bind cooperatively *dif* site (Blakely *et al.*, 1993). Our observation suggests that these two proteins may cooperate in binding to *difEc*, but do not form additional complexes as observed in Blakely *et al.*, 1993.

A potential *dif* site from the *S. suis* chromosome was found by homology searching, which demonstrated a high degree of homology with *difBs*. The *dif* sites from *E. coli, H. influenzae* and *B. subtilis* have been characterized by the presence of two 11-bp half-sites that contain partial dyad symmetry separated by 6-bp central region (5'-ATAA N6 TTAT or 5'AGAA N6 TTAT) that delineates the positions of strand cleavage and exchange (Blakely *et al.*, 1997; Neilson *et al.*, 1999; Sciochetti *et al.*, 2001). The right half-site of potential *difSs* shows a high degree of conservation when compared to the

Gram-negative bacterial sites *difEc* and *difHi* and Gram-positive bacterial site *difBs* (former 9 of 11-bp match, latter complete 11-bp match) considering their great evolutionary divergence (Figure 4). The left half-site of *difSs* is much more divergent compared to other XerC binding sites. It is important to note that for the nucleotides at those important positions for binding specificity, such as –9 and –13, mutations at these positions of *difEc* site abolished XerC binding (Hayes and Sherratt, 1997), which might be one possible explanation why we were unable to detect specific binding of XerCSs-MBP to *difEc*. We also note that the central region sequence of *difSs* is very similar to the *H. influenzae dif* site (5-bp matches) and suggests that maintenance of this sequence may be of major functional importance.

Specific binding of *difSs* was detected at XerCSs-MBP concentrations of 67 nM and above, in the presence of poly dIdC competitor (Figure 2B). Additional retarded bands were observed with increasing concentrations of protein. XerCSs-MBP displayed a higher affinity for the *difSs* site (binding observed at 67 nM protein concentration, Figure 2B) than for *difBs* (binding observed at 1 μM protein concentration, Figure 2A). In the Xer recombination site, different nucleotides at the left half-site DNA sequence determine the binding specificity of XerC (Hayes and Sherratt, 1997). More than one DNA-protein complex was observed with *difSs* binding which is similar to results observed with XerCSs-MBP binding to *difBs* (Figure 2A). It has been shown that *B. subtilis* RipX (XerD) could bind both half-sites of *difBs*, but CodV (XerC) could only bind to one half-site (Sciochetti *et al.*, 2001). The observation of more than one complex may suggest that XerCSs-MBP is binding to both half-sites of *difSs*. Since a strong candidate *xerD* gene of *S. suis* has not yet been discovered, we also speculate the

possibility that *S. suis* may only use one recombinase and in vitro experiments are currently underway to explore this possibility.

Despite the strong of similarity between *difEc* and *difSs*, no cooperative binding between XerCSs-MBP and the *E. coli* XerD (XerDEc) proteins was observed. But our results showed XerDEc alone could bind to *difSs* (Figure 2C). XerDEc binding to *difSs* is most likely due to the high conservation of right-half sites between *difSs* and *difEc*. The failure to detect *E. coli* XerC-MBP binding to *difSs* is similar to what has been observed with its binding behavior to *difBs*. Even the cooperative interactions between XerC and XerD of *E. coli* are not sufficiently strong to overcome the low affinity of XerC for the *difBs* (Sciochetti *et al.*, 2001). It might be the similar case for *difSs* and is probably related to the divergence of left-half site between two recombination core sites.

It has been observed that *xer* mutations in *S. pneumoniae, S. aueus* and *P. fluorescens* also affected the pathogenicity of these bacteria (Chalker *et al.*, 2000; Reichmann and Hackenbeck, 2002; Martinez-Granero *et al.*, 2005). This suggests that Xer recombinases may function in processes other than chromosome dimer resolution or that affects in chromosome segregation may have far reaching consequences for the bacterial cells.

Inactivation of the *S. suis xerC* gene generated mutants that were viable but grew with a considerably longer generation time compared with wild-type *S. suis* (Figure 6A). Mutants also had a tendency to grow in longer chains of cells (Figure 6B). Similar phenomena were observed with an *xerD* mutant of *S. pneumoniae* and an *xerC* mutant of *S. aureus* (Chalker *et al.*, 2000; Reichmann and Hackenbeck, 2002). In order to investigate the phentype change of chains length that might come from the mutant XerC protein, rule out the possibility of the interruption of the downstream gene expression of

xerC S.suis, the *in vivo* complementations assay of *S. sui xerC* mutant will be done in the future study. It is quite possible that the lower growth rate of the *xer* mutants might be related to a defect in chromosome segregation. Nucleoid morphology was investigated by DAPI-staining wild-type and mutant cells, but no significant morphological changes were observed by this method (data not shown). In coccus bacteria, dimensional changes resulting from perturbation of chromosome segregation may be rather subtle as they have the potential to occur in more than one plane, and this may explain why microscope was insufficiently sensitive to detect the morphological changes.

Our report is the first work which characterizes the XerC protein in streptococcal bacteria. In the future, further investigations of the catalytic activity of XerC and its interaction between other cellular proteins and DNA will allow us to determine how Xer recombination functions in these medically-important bacteria. Future work with animal infection models will help to show the effect of *xerC* on pathogenicity in *S. suis*.

Acknowledgements

We wish to thank Drs Josée Harel and Marcelo Gottschalk for *S. suis* p1/7 and 31533 genomic DNA, *S. suis* strain S735 and plasmid pBEA756; Manuela Villion for providing us with purified XerC-MBP and XerD proteins from *E. coli*; Monique Vasseur for technical assistance with fluorescence and DIC microscopy and image analysis; Manuela Villion, Hélène Sénéchal, Yong Xiao and Imad Baaklini for their assistance and advice. This work was supported by grants from the National Science and Engineering Research Council of Canada and the Université de Montréal.

References:

Arends, J. P. and Zanen, H.C. (1988). Meningitis caused by *Streptococcus suis* in humans, *Rev. Infect. Dis.* **10**:131–137

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1987). *Current Protocols in Molecular Biology*. Wiley, New York..

Barre, F.-X., Aroyo, M., Colloms, S.D., Helfrich, A., Cornet, F. and Sherratt, D.J. (2000). FtsK functions in the processing of a Holliday junction intermediate during bacterial chromosome segregation. *Genes Dev.*, **14**: 2976-2988.

Becker, J. and Brendel, M. (1996). Molecular characterization of the *xerC* gene of *Lactobacillus leichmannii* encoding a site-specific recombinase and two adjacent heat shock genes. *Curr. Microbiol.* **32**: 232-236.

Blakely, G., S. Colloms, G. May, M. Burke, and Sherratt, D. (1991). *Escherichia coli* XerC recombinase is required for chromosomal segregation at cell division. *New Biol.* **3**:789-798

Blakely, G.W., Davison, A.O. and Sherratt, D.J.. (1997). Binding and cleavage of nicked substrate by site-specific recombinases XerC and XerD. *J. Mol. Biol.* **265**:30-39.

Blakely, G., May, G., McCulloch, R., Arciszewska, L.K., Burke, M., Lovett, S.T. and Sherratt, D.J. (1993). Two related recombinases are required for site-specific recombination at *dif* and *cer* in *E. coli* K12. *Cell* **75**: 351-361.

Blakely, G.W. and Sherratt, D.J. (1996). Cis and trans in site-specific recombination. *Mol. Microbiol.* **20**: 233-238.

Chalker, A.F., Lupas, A., Ingraham, K., So, C.Y., Lunsford, R.D., Li, T., Bryant, A., Holmes, D.J., Marra, A., Pearson, S.C., Ray, J., Burnham, M.K.R., Palmer, L.M., Biswas, S. and Zalacain, M. (2000). Genetic characterisation of Gram-positive homologs of the XerCD site-specific recombinases. *J. Mol. Microbiol. Biotechnol.* **2**: 225-233.

Cao, Y., Hallet, B., Sherratt, D.J., and Hayes, F. (1997). Structure function correlations in the XerD site-specific recombinase revealed by pentapeptide scanning mutagenesis. *J.Mol.Biol.* **274**: 39-53.

Colloms, S.D., Alèn, C., and Sherratt, D.J. (1998). The ArcA/ArcB two-component regulatory system of *Escherichia coli* is essential for Xer site-specific recombination at psi. *Mol. Microbiol.* **28**: 521-30.

Colloms, S.D., Sykora, P., Szatmari, G., Sherratt, D.J. (1990). Recombination at *ColE1* requires the *Escherichia coli xerC* gene product, a member of the lambda integrase family of site-specific recombinases. *J. Bacteriol.* **172**:6973-6980.

Dekkers, L.C., Phoelich, C.C., Van Der Fits, L. & Lugtenberg, B.J.J. (1998). A site-specific recombinase is required for competitive root colonization by *Pseudomonas fluorescens WCS365*. *Proc. Natl. Acad. Sci. USA* **95**:7051–7056.

Hayes, F., Lubetzki, S.A., and Sherratt, D.J. (1997). *Salmonella typhimurium* specifies a circular chromosome dimer resolution system which is homologous to the Xer site-specific recombination system of *Escherichia coli*. *Gene* **198**:105-110.

Hayes, F. and Sherratt, D.J. (1997). Recombinase binding specificity at the chromosome dimer resolution site *dif* of *Escherichia coli*. *J. Mol.Biol* **266**:525-537.

Höfte, M., Dong, Q., Kourambas, S., Krishnapillai, V., Sherratt, D. and Mergeay, M. (1994).The *sss* product, which affects pyoverdin production in *Pseudomonas aeruginosa 7NSK2*, is a site-specific recombinase. *Mol. Microbiol.* **14:**1011-1020.

Huber, K.E. and Waldor, M.K. (2002) Filamentous phage integration requires the host recombinases XerC and XerD. *Nature* **417**: 56-659.

Jouan, L. and Szatmari, G. (2003) Interactions of the *Caulobacter crescentus* XerC and XerD recombinases with the *E. coli dif* site. *FEMS Microbiol Lett.* **222**: 257-262.

Kuempel, P.L., Henson, J.M., Dircks, L. Tecklenburg, M., and Lim, D.F. (1991). *dif*, a recA-independent recombination site in the terminus region of the chromosome of *Escherichia coli*. *New Biol.* **3**:799-811.

Lesterlin, C., Barre, F-X, Cornet, F. (2004). Genetic recombination and the cell cycle: what we have learned from chromosome dimers. *Mol. Microbiol.* **54**:1151-1160.

Maguin, E., Duwat, P. Hege, T. Ehrlich, D. and Gruss, A. (1992). New thermosensitive plasmid for gram-positive bacteria. *J. Bacteriol.* **174**:5633-5638.

Martınez-Granero, F., Capdevila, S., Sanchez-Contreras, M., Martın, M. and Rivilla, R. (2005). Two site-specific recombinases are implicated in phenotypic variation and competitive rhizosphere colonization in *Pseudomonas fluorescens*. *Microbiology* **151**: 975–983.

Neilson, L., Blakely, G. and Sherratt, D.J. (1999). Site-specific recombination at *dif* by *Haemophilus infuenzae* XerC. *Mol. Microbiol.* **31**: 915-926.

Nunes-Düby, S.E., Kwon, H.J., Tirumalai, R.S., Ellenberg, T. and Landy, A. (1998) Similarities and differences among 105 members of the Int family of site-specific recombinases. *Nucleic Acids Res.* **26**: 331-391.

Okada, N., Geist, R. T. and Caparon, M. G. (1993) Positive transcription control of *mry* regulates virulence in the group A streptococcus. *Mol. Microbiol.* **7**: 893-903

Recchia, G.D. and Sherratt, D.J. (1999). Conservation of *xer* site-specific recombination genes in bacteria. *Mol. Microbiol.* **34**: 1146-1148.

Reichmann, P. and Hakenbeck, R. (2002). XerD recombinase with unusual active site motifs in *Streptococcus pneumoniae*. *J. Mol. Microbiol. Biotechnol.* **4**:101-110.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual, 2nd ed.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sciochetti, S., Piggot, P and Blakely, G.W. (2001). Identification and characterization of the *dif* site from *Bacillus subtilis*. *J. Bacteriol.* **183**: 1058-1068.

Sciochetti, S.A., Piggot, P.J., Sherratt, D.J. and Blakely, G. (1999) The *ripX* locus of *Bacillus subtilis* encodes a site-specific recombinase involved in proper chromosome partitioning. *J. Bacteriol.* **181**: 6053-6062.

Sherratt, D.J., Arciszewska, L.K., Blakely, G., Colloms, S., Grant, K., Leslie, N. (1995). Site-specific recombination and circular chromosome segregation. *Phil. Trans R. Soc. Lond. B. Biol. Sci.* **347**:37-42.

Sherratt, D. J., and Wigley, D. B. (1998). Conserved themes but novel activities in recombinases and topoisomerases. *Cell* **93:** 149–152.

Sirois, S. and Szatmari, G. (1995). Detection of XerC and XerD recombinases in Gram-negative bacteria of the family *Enterobacteriaceae*. *J. Bacteriol.* **177**: 4183-4186.

Slack, F.J., Serror, P., Joyce, E. and Sonenshein, A.L. (1995). A gene required for nutritional repression of the *Bacillus subtilis* dipeptide permease operon. *Mol. Microbiol.* **15**: 689-702.

Staats, J.J., Feder, I., Okwumabua, O., Chengappa, M.M. (1997). *Streptococcus suis*: past and present. *Vet. Res. Commun.* **21**: 381–407.

Steiner, W., Liu, G., Donachie, W.D. and Kuempel, P. (1999). The cytoplasmic domain of FtsK protein is required for resolution of chromosome dimers. *Mol. Microbiol.* **31**: 579-583.

Stirling, C.J., Szatmari, G., Stewart, G., Smith, M.C., and Sherratt, D.J. (1988). The arginine repressor is essential for plasmid-stabilizing site-specific recombination at the ColE1 cer locus. *EMBO J.* **7**: 4389-4395

Stirling, C.J., Colloms, S.D., Collins, J.F., Szatmari, G., and Sherratt, D.J. (1989). XerB, an *Escherichia coli* gene required for plasmid ColE1 site-specific recombination, is identical to *pepA*, encoding aminopeptidase A, a protein with substantial similarity to bovine lens leucine aminopeptidase. *EMBO J.* **8**: 1623-1627.

Subramanya, H.S., Arciszewska, L.K., Baker, R.A., Bird, L.E., Sherratt, D.J., Wigley, D.B. (1997). Crystal structure of the site-specific recombinase, XerD. *EMBO J.* **16**: 5178-5187.

Villion, M. and Szatmari, G. (2003). The XerC recombinase of *Proteus mirabilis*: characterization and interaction with other tyrosine recombinases. *FEMS Microbiol Lett.* **226**: 65-71.

Chapter III

Discussion

1. The *xerC* Gene of *Streptococcus suis*

Analysis of the available bacterial genome sequences reveals that XerC and XerD homologues are present in many bacteria that have circular chromosomes (e.g. see Nunes-Düby *et al.*, 1998). By homology searching, the *xerC* gene of *S. suis* was found, cloned and overexpressed as a maltose binding protein fusion. The molecular weight of the fusion protein was 82 kDa (including 43 kDa MBP fusion protein), in good accordance with its predicted molecular weight of 39 kDa (Figure 14). The deduced amino acid sequence of the *S. suis* XerC protein contains all the conserved triad residues RHR and the catalytic tyrosine residue (Y) of the tyrosine recombinase family (Appendix 1). Analysis of the sequence displays a very high degree of similarity with the Xer proteins in other streptococcal bacteria. It shows 79% identity and 88% similarity with the XerC protein of *S. pneumoniae*. However, it only displays 27% identity and 45% similarity with CodV of *B. subtilis* (chapter 2, Figure 1). When compared to XerC and XerD of *E. coli,* a similar low identity was also observed. This may be explained by the great evolutionary divergence of the two organisms from which the proteins were derived. According to the phylogenetic tree in Reichmann and Hakenbeck, 2002, it was proposed that two distinct groups exist in the XerD family, one of which is represented by the streptococcal protein. These proteins show lower identity when they were compared with the XerD proteins of *E. coli* or *B. subtilis*.

2. DNA Binding Activity of XerCSs-MBP

The *dif* site from *B. subtilis* (*difBs*) was initially used in our DNA binding assays as it represents the best characterized *dif* site from gram-positive bacteria (Sciochetti *et al.*, 2001). To ascertain if *S. suis* XerCSs-MBP protein could specifically bind to *difBs* site, we used gel retardation analysis of DIG-labeled *difBs* with purified protein. XerCSs-MBP, at concentration of 1μM and above, displayed specific binding to *difBs* site, by the formation of two protein-DNA complexes that migrated with reduced mobility (Figure 3 in chapter 2). This result differs from what has been observed with CodV binding to the *difBs* site which only gives rise to a single protein-DNA complex (Sciochetti *et al.*, 2001). It is possible that XerCSs-MBP might bind to both halves of *difBs*, which would require further investigation in the future.

Since the XerC protein of *S. suis* bound specifically to *difBs*, we speculated that the *S. suis* chromosome must possess a specific DNA sequence similar to *difBs*. One potential *dif* site from the *S. suis* chromosome was found by homology searching, which demonstrated high similarity with *difBs*. The *dif* sites from *E. coli, H. influenzae* and *B. subtilis* have characterized by the presence of two 11-bp half-sites that contain partial dyad symmetry separated by 6-bp central region (5'-ATAA N6 TTAT or 5'AGAA N6 TTAT) that delineate the positions of strand cleavage and exchange (Blakely *et al.*, 1997; Neilson *et al.*, 1999; Sciochetti *et al.* 2001). The right half-site of the potential *difSs* shows a high degree of homology conservation when compared to the gram-negative bacterial sites *difEc* and *difHin* and the Gram-positive bacterial site *difBs* (former 9 of 11-bp match, latter totally 11-bp match) considering their great evolutionary divergence (Figure 3). The left half-site of *difSs* is much more divergent compared to other XerC binding sites. It is important to note mutation at positions –9

and –13 of *difEc* abolished XerC binding (Hayes and Sherratt, 1997). We also noted that the central region sequence of *difSs* was very similar to *difHin* site (5 of 6 matches) and suggests that maintenance of this sequence may be of major functional importance.

XerC was overexpressed and purified as a maltose binding protein fusion. Specific binding to *difSs* site was detected at XerCSs-MBP concentrations of 67 nM and above, in the presence of poly dIdC competitor (Figure 2A). This binding was of a higher affinity than that observed for *difBs* (67 nM vs 1 uM). In the Xer recombination site, different nucleotides at the left half-site DNA sequence determine the binding specificity of XerC (Hayes and Sherratt, 1997). Additional retarded bands were observed with increasing concentrations of protein. Multiple protein-DNA complexes were also observed with XerCSs binding to *difBs* (Figure 2). It had been previously shown that *B. subtilis* RipX (XerD) could bind both half-sites of *difBs* (Sciochetti *et al.* 2001), but CodV (XerC) only bind to one half-site (Sciochetti *et al.*, 2001). It would require further investigations to determine if the formation of more than one complex was caused by XerCSs-MBP binding to both half-sites of *difSs.* This could be done by creating half-sites of *difSs* and using these as substrates in gel shift assays.

Because of the strong similarity between *difEc* and *difSs*, XerC and XerD of *E. coli* were tested for binding to *difSs*. Our results showed XerDEc (1.5μM) could bind to *difSs* but no binding of XerCEc was observed (Figure 2B). XerDEc binding to *difSs* was not surprising, given the high degree of conservation of the right-half sites of *difSs* and *difEc*. The failure to detect XerCEc binding to *difSs* is similar to what has been observed with its binding behaviour to *difBs*. Even the cooperative interaction between XerC and XerD of *E.coli* are not sufficiently strong to overcome the low affinity of XerC for the *difBs* (Sciochetti *et al.*, 2001). It might be the similar case for *difSs* site and

might be related to the divergence of the left-half site between two recombination core sites.

Since the *S. suis* XerC protein possesses all conserved residues of site-specific recombinases and displays some similarity with XerC protein from *E. coli*, the *dif* site of *E. coli* (*difEc*) was initially used to analyze its binding activity by gel retardation assay. However, no specific binding to *difEc* was detected in our assays. We speculated several possibilities of the failure to detect binding. Firstly, the failure to detect binding to *difEc* might be due to differences between the XerC proteins of *E. coli* and *S. suis* in the residues implicated in base and phosphate contacts that determine binding specificity (Subramanya *et al.*, 1997). Secondly, The XerC binding site of *dif* sites are much more divergent than XerD binding sites. There are some important nucleotides implicated in binding specificity, such as –9 and –13, mutations at which *difEc* binding was abolished (Hayes and Sherratt, 1997), which might be one possible explanation why we were unable to detect specific binding to *difEc*. Finally, the binding might be of such low affinity that our assays are unable to detect it.

3. Cooperative Binding studies

In *E. coli*, XerC and XerD bind cooperatively (Blakely *et al.*, 1993); and it is possible that *S. suis* XerC might require its potential partner such as XerD for binding to *difEc*, but the addition of XerDEc to the binding reaction did not affect the binding of XerCSs to *difEc* in our assay. It suggests that XerDEc could not cooperatively stimulate XerC *S. suis* binding to *difEc*. It is possible that the high degree of divergence between these proteins may not allow for cooperative binding at *difEc* or at *difSs*. We also attempted to find the *xerD* gene of *S. suis*. An open reading frame was identified that

displayed a strong similarity with *S. aureus* XerD, but this protein was truncated by about 100 aa at its amino terminus. With the completion of *S.suis* genomic sequencing, the putative *xerD* gene might be explored and the two proteins cooperatively binding might be examined in the future study. Alternatively, it is possible that this truncated XerD protein may still be able to interact with XerCSs for binding cooperativity and to catalyze site-specific recombination. Future studies will look at this possibility.

However, it was interesting to note that stronger retardated bands were observed for XerDEc binding to *difEc* in the presence of XerCSs-MBP (Figure 3 in article). This suggests that *S. suis* XerC protein stimulated XerDEc binding to *difEc*. XerCEc and XerDEc have been shown to bind cooperatively *dif* site (Blakely *et al.*, 1993). Our observation suggests that these two proteins may cooperate in binding to *difEc*, but do not form additional complexes as observed in Blakely *et al.*, 1993.

4. Phenotypic Assay of *xerC* Mutant

The Xer site-specific recombination system is important to ensure circular chromosomes are monomeric before their segregation to daughter cells at cell division. *xer* mutants in *S. pneumoniae, S. aureus* and *P. fluorescens* also affect the pathogenicity of these bacteria (Chalker *et al*, 2000; Reichmann and Hakenbeck, 2002; Dekkers *et al*, 1998). It suggests that Xer recombinases may function in processes other than chromosome dimer resolution, or that defects in chromosome dimer resolution may affect other cellular processes. The thermosensitive vector pBEA756 was used to inactive the *xerC* gene of *S. suis* to explore the potential role of *xer* gene in the growth and viability of *S. suis.*

The *xerC* gene was inactivated by the homologous recombination of the suicide vector pBEA756XerCint into the *S. suis* chromosome. *xerC* mutants were viable but grew with a considerably longer generation time compared with wild-type *S.suis* (Figure 5). Mutants also had a tendency to grow in longer chains of cells (Figure 5). Similar phenomena were observed with an *xerD* mutant of *S. pneumoniae* and an *xerC* mutant of *S. aureus* (Chalker *et al.*, 2000; Reichmann and Hakenbeck, 2002). It will be required to further elucidate whether the longer chains are due to the *xerC* mutation are in a downstream gene, this could be demonstrated by complementing the *xerC* mutant strain with a cloned *xerC* gene in a plasmid vector. We speculated that the slower growth rate might be related to a defect in chromosome segregation. Nucleoid morphology was investigated by DAPI-staining wild-type and mutant cells, but no significant morphological changes were observed by this method. A failure to detect defects in nucleoid morphology was also found with the *xerC* mutant of *S. aureus* (Chalker *et al.*, 2000). In coccus bacteria, dimensional changes resulting from perturbation of chromosome segregation may be rather subtle as they have the potential to occur in more than one plane, and this may explain why fluoresence microscopy was insufficiently sensitive to detect the morphological changes. It might require other efficient methods to detect the possible change of *xerC* mutant.

5. *In vitro* Recombination Reaction

E. coli recombinases form Holliday junctions on a plasmid containing two *cer* sites in the presence of accessory proteins and PepA (Colloms *et al.*, 1996). After digestion of the plasmid DNA having undergone the action of recombinase, this junction can be distinguished from the linear form of the plasmid in an agarose gel; because of its

particular structure, it migrates more slowly than the linear form. This Holliday junction was obtained during *in vitro* reactions with plasmid pCS201 (which contains two *cer* sites flanking the lacZ gene)(Stirling *et al.*, 1989; Manuela and Szatmari, 2003) . We have characterized XerC recombinase and it showed that it bound specifically to the *difSs*. *In vitro* recombination reactions were performed to examine its catalytic activity and to determine if XerC alone could perform the recombination reaction at *difSs*. We constructed a plasmid containing two directly repeated *difSs* sites and *in vitro* recombination reactions were performed (described in Colloms *et al.*, 1996) to detect the possible recombination products or intermediate products. However, we did not detect any recombination product by using XerCSs-MBP, under any of the conditions used (data not shown). Because of the similarity between *difSs* and *difEc*, we assumed that XerC and XerD from *E. coli* might act at the two cloned *difSs* sites vector. *In vitro* recombination was also carried out with these two proteins on the two *difSs* vector but no recombination products were detected (data not shown).

The failure to detect recombination products might be due to difference between *in vivo* and *in vitro* reaction conditions. Additional factors may be required for the recombination reaction, for example, XerD and FtsKc. Aussel *et al.*, (2002) proposed two alternative pathways (FtsK-dependent/independent pathway) of Xer recombination at *dif*, one initiated by XerC and the other by XerD. The role of FtsK in promoting chromosome dimer resolution is to switch the activity of the XerCD recombinases in the synaptic complex, so that Xer recombination follows one pathway in which XerD mediates the first pair of strand exchanges to form HJ intermediates that are resolved to recombination products by XerC (FtsK-dependent pathway). Another possibility is that the small amount products might be undetectable in out assay. Holliday junctions

formed at *dif in vitro* are the result of catalysis by XerC (Barre *et al.*, 2000). The HJ intermediates are rapidly converted back to substrates in cycles of XerC-mediated strand exchanges in the absence of FtsK (FtsK-independent pathway) and make them undetectable.

6. *In vivo* Recombination

It has been proposed that the failure to observe recombination between *dif* sites *in vitro* reflects the requirement for an *in vivo* control that activates Xer recombination in cells containing chromosome dimers (Neilson *et al.*, 1999). Therefore, we tried to study the catalytic activity of XerCSs *in vivo*.

Firstly, we introduced a plasmid containing two directly repeated *difSs* site into different *E. coli* strains to detect possible recombination reaction *in vivo*. It was interesting that no recombination products were detected and that plasmids were eventully lost from *E. coli xerC*$^+$ strains. The 2-*dif* plasmid was stable in *E.coli* strain with *xerC or xerD* mutations or *xerC/xerD* double mutants. However, it was unstable in *xerC*$^+$ and *xerD*$^+$ *E. coli* strains. It is quite likely that the plasmid integrated into the chromosome of *E. coli*; further studies like southern blotting, are currently being undertaken to explore this possibility. Because of the short region of homology between *difSs* and *difEc* in the chromosome, the possibility of homologous recombination between these two sequences is very low. Furthermore, the putative integration phenomenon was observed in *recA*$^-$ strains (DH5 α). We also observed stable *2-dif* plasmids in *E. coli* strains harboring a deletion of *dif* site, further suggesting that the

instability phenomena we observed was due to Xer-mediated recombination between the *S. suis* and *E. coli dif* sites.

Since Xer proteins from *E. coli* have showed effects at two *difSs* site. It would be interesting to examine this effect in *S. suis*, factual attempts to examine this phenomenon were not successful due to the instability of one *E. coli-S. suis* shuttle vector in Xer^+ *E. coli* strains. This problem can easily be overcome in the future by constructing xer^- of the *E. coli* strain which expresses the RepA protein, which is required for the replication of one shuttle vector. Once this plasmid is constructed, it would be interesting to test the effects of this vector in *xerC* wild-type and mutant strains of *S. suis* to see if this instability is connected to Xer-related recombination.

7. Perspectives

Our report is the first functional characterization of an XerC protein in streptococcal bacteria, and its site of action, *difSs*. The XerC recombinase showed DNA binding activities and an *xerC* mutant demonstrated significant growth and morphological changes. In the future, further *in vitro* investigations of XerC's catalytic activity and its interaction between protein and DNA would be interesting. Animal infection studies should also be performed to analyze the pathogenicity of *xerC* mutant. The potential *difSs* sequence would require further characterization, especially to investigate whether XerC could bind to both half sites of *difSs*. Since an open reading frame was identified that displayed a strong similarity with *S. aureus* XerD, but this protein was truncated by about 100 aa at its amino terminus. In the future, the cloning and expression of the truncated XerD would help to give further insight in the mechanisms of Xer recombination in this species of bacteria.

REFERENCES

Abremski, K.E., and Hoess, R.H. (1992). Evidence for a second conserved arginine residue in the integrase family of recombination proteins. Protein Eng.5, 87-91.

Alén, C., Sherratt, D.J., and Colloms, S.D. (1997). Direct interaction of aminopeptidase A with recombination site DNA in Xer site-specific recombination. EMBO J.16, 5188-5197.

Arciszewska, L.K., Baker, R.A., Hallet, B., and Sherratt, D.J. (2000). Coordinated control of XerC and XerD catalytic activities during Holliday junction resolution. J.Mol. Biol. 299, 391–403.

Arciszewska, L.K., and Sherratt, D.J. (1995). Xer site-specific recombination *in vitro*. EMBO J. 14, 2112–2120.

Arciszewska, L.K., Grainge, I., and Sherratt, D.J. (1997). Action of site-specific recombinases XerC and XerD on tethered Holliday junctions. EMBO J.16, 3731–3743.

Arends, J.P., and Zanen, H.C. (1988). Meningitis caused by *Streptococcus suis* in humans. Rev. Infect. Dis.10,131–137.

Argos, P., Landy, A., Abremski, K., Egan, J.B., Haggard-Ljungquist, E., Hoess, R.H., Kahn, M.L., Kalionis, B., Narayana, S., Pierson, L.S., Sternberg, N., and Leong, J.M. (1986). The integrase family of site-specific recombinases: regional similarities and global diversity. EMBO J. 5, 433-440.

Aussel, L., Barre, F.X., Aroyo, M., Stasiak, A., Stasiak, A.Z., and Sherratt, D.J. (2002). FtsK is a DNA motor protein that activates chromosome dimer resolution by switching the catalytic state of the XerC and XerD recombinases. Cell 108, 195–205.

Austin, S., Ziese, M., and Sternberg, N. (1981). A novel role for site-specific recombination in maintenance of bacterial replicons. Cell 25, 729-736.

Azaro, M.A., and Landy, A. (1997). The isomeric preference of Holliday junctions influences resolution bias by lambda integrase. EMBO J.16, 3744–3755.

Barre, F.X., Aroyo, M., Colloms, S.D., Helfrich, A., Cornet, F., and Sherratt, D.J. (2000). FtsK functions in the processing of a Holliday junction intermediate during bacterial chromosome segregation. Genes Dev 14, 2976–2988.

Barre, F.X., Soballe, B., Michel, B., Aroyo, M., Robertson, M., and Sherratt, D.J. (2001). Circles: The replication-recombination-chromosome segregationconnection. Proc. Natl. Acad. Sci. USA 98, 8189-8195.

Barre, F.X., and Sherratt, D.J. (2002). Xer site-specific recombination: promoting chromosome segregation. In Mobile DNA II, Craig N.L., Craigie R., Gellert M., Lambowitz (eds) Vol.1, pp149-161. Washington, DC: ASM Press.

Bartling, D., and Weiler, E.W. (1992). Leucine aminopeptidase from *Arabidopsis thaliana*. Eur. J. Biochem. 205, 425–443.

Becker, J., and Brendel, M. (1996). Molecular characterization of the *xerC* gene of *Lactobacillus leichmannii* encoding a site-specific recombinase and two adjavent heat shock genes. Curr Microbiol 32, 232-236.

Bernardi, A., and Bernardi, F. (1984). The complete sequence of the pCS101 plasmid. Nucleic Acid Res. 12, 9415-9426.

Bigot, S., Corre, J., Louarn, J.M., cornet, F., and Barre, F.X. (2004). FtsK activities in Xer recombination, DNA mobilization and cell division involve overlapping and separate domains of the protein. Mol Microbiol #vol. 1365-2958.

Bigot, S., Saleh, O.A., Lesterlin, C., Pages, C., Karoui, M. E., Dennis, C., Grigoriev, M., Allemandm, J.F., Barre, J.F., and Cornet, F. (2005). KOPS: DNA motifs that control *E. coli* chromosome segregation by orienting the FtsK thanslocase. EMBO J. 24, 3370-3780.

Blakely, G., Colloms, S., May. G., Burke, M., and Sherratt, D., (1991). *Escherichia coli* XerC recombines is required for chromosomal segregation at cell division. New Biol. 3789-798.

Blakely, G.W., May, G., McCulloch, R., Arciszewska, L., Burke, M., Lovett, S., and Sherratt, D.J. (1993). Two related recombinases are required for site-specific recombination at *dif* and *cer* in *Escherichia coli* K12. Cell, 75, 351–361.

Blakely, G.W., and Sherratt, D.J. (1996). Cis and trans in site-specific recombination. Mol. Microbiol. 20, 233-238.

Blakely, J.A.R., Ganguly, N., and Sherratt, D.J. (1997). DNA sequence of recombinase binding sites can determine Xer site-specific recombination outcome. Mol.Microbiol. 23,387–398.

Blattner, F. R., Plunkett, G., 3rd, Bloch, C.A., Perna, N.T., Burland, V., Riley, M., *et al.* (1997). The complete genome sequence of *Escherichia coli* K-12. Science 277, 1453–1474.

Burke, M., Merican, A.F., and Sherratt, D.J. (1994). Mutant *Eschericia coli* arginine repressor proteins that fail to bind L-arginine, yet retain the ability to bind their normal DNA-binding sites. Mol. Microbiol. 13, 609–618.

Burley, S.K., David, P.R., Sweet, R.M., Taylor, A., and Lipscomb, W.N. (1992). Structure determination and refinement of bovine lens leucine aminopeptidase and its complex with bestatin. J. Mol. Biol., 224, 113–140.

Capiaux, H., Cornet, F., Corre J., Guijo, M.I., Pérals, K., Rebollo, J.E., and Louarn, J.-M. (2001). Polarization of the Escherichia coli chromosome. A view from the terminus. Biochimie 83, 161–170.

Capiaux, H., Lesterlin, C., Pérals, K., Louarn, J.M., and Cornet, F. (2002). A dual role for the FtsK protein in *Escherichia coli* chromosome segregation. EMBO Rep. 3, 532–536.

Chalker, A.F., Lupas, A., Ingraham, K.A., So, C.Y., Lunsford, R.D., Li, T., Bryant, A., Holmes, D.J., Marra, A., Pearson, S.C., Ray, J., Burnham, M.K.R. Palmer, L.M., Biswas, S., and Zalacain, M. (2000). Genetic characterization of gram-positive homologs of the XerCD site-specific recombinases. Biotechnol. 2, 225-233.

Chanter, N., Jones, P.W., and Alexander, T.J.L. (1993). Meningitis in pigs caused by *Streptococcus suis*– a speculative review. Vet Microbiol 36, 39–55.

Charland, N., Harel J., Kobisch M., Lacasse, S., and Gottschalk, M.. (1998). *Streptococcus suis* serotype 2 mutants deficient in capsular expression. Microbiology 144, 325–332.

Charland, N., Kobisch, M., Martineau-Doize B., Jacques, M., and Gottschalk, M. (1996). Role of capsular sialic acid in virulence and resistance to phagocytosis of *Streptococcus suis* capsular type 2. FEMS Immunol. Med. Microbiol. 14, 195–203.

Charlier, D., Hassanzadeh, Gh., G., Kholti, A., Gigot, D., Pierard, A., and Glansdorff, N. (1995). *carP*, involved in pyrimidine regulation of the *Escherichia coli* carbamoylphosphate synthetase operon encodes a sequence-specific DNA-binding protein identical to XerB and PepA, also required for resolution of ColE1 multimers. J. Mol. Biol. 250, 392–406.

Chen, J.W., Evans, B.R., Yang, S.H., Teplow, D.B., and Jayaram, M. (1991). Domain of a yeast site-specific recombinase (Flp) that recognizes its target site. Proc. Natl Acad. Sci. USA 88, 5944–5948.

Chen, J.W., Lee, J., and Jayaram, M. (1992). DNA cleavage in trans by the active site tyrosine during FLP recombinase: switching protein partners before exchanging strands. Cell 69, 647-658.

Chin-a-Woeng, T.F.C., Bloemberg, G.V., Mulders, I.H.M., Dekkers, L.C., and Lugtenberg, B.J.J. (2000). Root colonization by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato foot and root rot. Mol Plant–Microbe Interact 13, 1340–1345.

Colloms, S.D., Sykora, P., Szatmari, G., and Sherratt, D.J. (1990). Recombination at ColE1 requires the *Escherichia coli* xerC gene product, a member of the lambda integrase family of site-specific recombinases. J. Bacteriol. 172, 6973-6980.

Colloms, S.D., Alen, C., and Sherratt, D.J. (1998). The ArcA/ArcB two-component regulatory system of *Escherichia coli* is essential for Xer site-specific recombination at *psi*. Mol. Microbiol, 28, 521-530.

Colloms, S.D., McCulloch, R., Grant, K., Neilson, L., and Sherratt, D.J (1996). Xer-mediated site-specific recombination *in vitro*. EMBO J., 15, 1172–1181.

Colloms, S.D., Bath, J., and Sherratt, D.J. (1997). Topology of Xer mediated site-specific recombination at *psi* and *cer*. Cell. 88, 855–864.

Cornet, F., Mortier, I., Patte, J., and Louarn, J.M. (1994). Plasmid psc101 harbors a recombination site, *psi*, which is able to resolve plasmid multimers and to substitute for the analogous chromosomal *Escherchia coli* site *dif*. J. Bacteriol. 176, 6973-6980.

Cornet, F., Louarn, J., Patte, J., and Louarn, J.M. (1996). Restriction of the activity of the recombination site *dif* to a small zone of the *Escherichia coli* chromosome. Genes Dev 10, 1152–1161.

Corre, J., and Louarn, J.M. (2002). Evidence from terminal recombination gradients that FtsK uses replichore polarity to control chromosome terminus positioning at division in *Escherichia coli*. J. Bacteriol 184, 3801–3807.

Corre, J., Patte, J., and Louarn, J.M. (2000). Prophage lambda induces terminal recombination in *Escherichia coli* by inhibiting chromosome dimer resolution. An orientation dependent *cis*-effect lending support to bipolarization of the terminus. Genetics 154, 39–48.

Cox, M.M., GoodmanM, F., Kreuzer, K.N., Sherratt, D.J., Sandler, S.J., and Marians, K.J. (2000). The importance of repairing stalled replication fork. Nature 404, 37-41.

Craig, N.L. (1988). The mechanism of conservative site-specific recombination. Annu Rev Genet, 22, 77-105.

Cromie, G.A., and Leach, D.R. (2000). Control of crossing over. Mol Cell 6, 815-826.

Cunin, R., Glansdorff, N., Pierard, A., and Stalon, V. (1986). Biosynthesis and metabolism of arginine in bacteria. Microbiol Rev 50, 314-352.

Cuypers, H.T., van Loon-Klaassen, L.A.H., Vree Egberts, W.T.M., de Jong, W.W., and Bloemendal, H. (1982). The primary structure of leucine aminopeptidase from bovine eye lens. J. Biol. Chem., 257, 7077–7085.

de Massy, B., Béjar S., Louarn, J., Louarn, J.M., and Bouché, J.P., (1987). Inhibition of replication forks exiting the terminus region of the *E.coli* chromosome occurs at two loci separated by 5 min. Proc. Natl. Acad. Sci. USA 84, 1758-1763.

Dekkers, L.C., Phoelich, C.C., Van Der Fits, L., and Lugtenberg, B.J.J. (1998). A site-specific recombinase is required for competitive root colonization by *Pseudomonas fluorescens* WCS365. Proc. Natl. Acad. Sci. USA 95, 7051–7056.

Dekkers, L.C., Mulders, I.H.M., Phoelich, C.C., Chin-a-Woeng, T.F.C., Wijfjes, A.H.M., and Lugtenberg, B.J.J. (2000). The *sss* colonization gene of the tomato–*Fusarium oxysporum f. sp. radicislycopersici* biocontrol strain *Pseudomonas fluorescens* WCS365 can improve root colonization of other wild-type *Pseudomonas spp.* bacteria. Mol Plant–Microbe Interact 13, 1177–1183.

Draper, G.C., McLennan, N., Begg K., Masters, M., and Donachie, W.D. (1998). Only the N-terminal domain of FtsK functions in cell division. J. Bacteriol 180, 4621–4627.

Erickson, M.J., and Meyer, R.J. (1993). The origin of greater-than unit-length plasmids generated during bacterial conjugation. Mol. Microbiol. 7, 289-298.

Esposito, D., and Scocca, J.J. (1997). The integrase family of tyrosine recombinases: evolution of a conserved active site domain. Nucleic Acid Res. 25, 3605-3614.

Evans, B.R., Chen, J.W., Parsons, R.L., Bauer, T.K., Teplow, D.B., and Jayaram, M. (1990). Identification of the active-site tyrosine of FLP recombinase. J. Biol. Chem., 265, 18504–18510.

Gopaul, D.N., Guo, F., and Van Duyne, G.D. (1998). Structure of the Holliday junction intermediate in Cre-loxP site-specific recombination. EMBO J., 17, 4175–4187.

Gottschalk, M., Higgins, R., and Quessy, S. (1999) Dilemma of the virulence of *Streptococcus suis* strains. J. Clin. Microbiol. 37: 4202–4203.

Gottschalk, M., and Segura, M. (2000). The pathogenesis of the meningitis caused by *Streptococcus suis*: the unresolved questions. Vet Microbiol 76, 259–272.

Grandori, R., Lavoie, T.A., Pflumm, M., Tian, G., Niersbach, H., and Mass, W.K. (1995). The DNA-binding domain of the hexameric arginine repressor. J. Mol. Biol. 254, 150-162.

Grindley, N.D.F. (1994). Resolvase-mediated site-specific recombination. In: Nucleic Acids and Molecular Biology (Eckstein, F. and Lilley, D.M.J., Eds.), pp. 236-267. Springer-Verlag, Berlin.

Guhathakurta, A., and Summers, D. (1995). Involvement of ArgR and PepA in the pairing of ColE1 dimer resolution sites. Microbiology, 141, 1163–1171.

Guhathakurta, A., Viney, I., and Summers, D. (1996). Accessory proteins impose site selectivity during ColE1 dimer resolution. Mol. Microbiol. 20, 613–620.

Guo, F., Gopaul, D.N., and Van Duyne, G. (1997). Structure of Cre recombinase complexed with DNA in a site-specific recombination synapse. Nature 389, 40–47.

Guo, F., Gopaul, D.N., and Van Duyne, G. (1999). Asymmetric DNA bending in the Cre-*loxP* site-specific recombination synapse. Proc. Natl. Acad. Sci. USA 96, 7143-7148.

Haataja, S., Tikkanen, K., Liukkonen, J., Francois-Gerard, C., and Finne, J. (1993). Characterisation of a novel bacterial adhesion specificity of *Streptococcus suis* recognizing blood group P receptor oligosaccharides. J. Biol. Chem. 268: 4311–4317.

Hallet, B., and Sherratt D.J. (1997). Transposition and site-specific recombination: adapting DNA cut-and-paste mechanisms to a variety of genetic rearrangements. FEMS Microbiol. Rev. 21, 157-178.

Hall, R.M., and Vockler C. (1987). The region of the IncN plasmid R46 coding for resistance to beta-lactam antibiotics, streptomycin/spectinomycin and sulphonamides is closely related to antibiotic resistant segments found in IncW plasmids and Tn21-like transposons. Nucleic Acids Res. 15, 7491-7501.

Hallet, B., Arciszewska, L.K., and Sherratt, D.J. (1999). Reciprocal control of catalysis by the tyrosine recombinases XerC and XerD: an enzymatic switch in site-specific recombination. Mol. Cell 4, 949–959.

Hatfull, G. F., and Grindley, N.D.F. (1988). Resolvases and DNA-invertases: a family of enzymes active in site-specific recombination. In: Genetic Recombination (Kucherlapati, R. and Smith, G., Eds.), pp. 357-396. American Society for Microbiology, Washington, DC.

Hayes, F., and Sherratt, D.J. (1997). Recombinase binding specificity at the chromosome dimer resolution site *dif* of *Escherichia coli*. J. Mol. Biol., 266, 525-537.

Hickman, A.B., Waninger, W., Scocca, J., and Dyda, F. (1997). Molecular organization in site-specific recombination: the catalytic domain of bacteriophage HP1 integrase at 2.7 Å resolution. Cell, 89, 227-237.

Higgins, R., Gottschalk, M., Boudreau, M., Lebrun, A., and Henricson, J. (1995). Description of six new capsular types (28 though 34) of *Streptococcus suis*. J. Vet. Diagn. Invest. 7, 405–406.

Hodgman, T.C., Griffiths, H., and Summers, D.K. (1998). Nucleoprotein architecture and ColE1 dimer resolution: hypothesis. Mol. Microbiol, 29, 545-558.

Hofte, M., Dong, Q.G., Kourambas, S., Krishnapillai, V., Sherratt, D., and Mergeay, M. (1994). The *sss* gene product, which affects pyoverdin production in *Pseudomonas aeruginosa* 7NSK2, is a site-specific recombinase. Mol Microbiol, 14, 1011–1020.

Huber, K.E., and Waldor, M.K. (2002). Filamentous phage integration requires the host recombinases XerC and XerD. Nature 417, 656–659.

Hoess, R.H., and Abremski, K. (1985). Mechanism of strand cleavage and exchange in the Cre-lox site-specific recombination system. J. Mol. Biol, 181, 351-362.

Jacobs, A.A., Loeffen, P., van der Berg, A., and Storm, P. (1994). Identification, purification and characterisation of a thiol-activated hemolysin (suilysin) of *Streptococcus suis*. Infect. Immun. 62, 1742–1748.

Jayaram, M. (1994). Phosphoryl transfer in Flp recombination: a template for strand transfer mechanisms. Trends Biochem. Sci. 19, 78-82.

Jouan, L., and Szatmari, G. (2003). Interactions of the *Caulobacter crescentus* XerC and XerD recombinases with the *E. coli dif*. FEMS Microbiol Lett 222, 257-262.

Johnson, R.C. (1991). Mechanism of site-specific DNA inversion in bacteria. Curr. Opin. Genet. Dev. 1, 404-411.

Klippel, A., Mertens, G., Patschinsky, T., and Kahmann, R. (1988). The DNA invertase Gin of phage Mu: formation of a covalent complex with DNA via a phosphoserine at amino acid position 9. EMBO J. 7, 1229-1237.

Kohara, Y, Akiyama, K., and Isono, K (1987). The physical map of the whole *Escherichia coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell 50, 1389-1393.

Kopic, J., Paradzik, M.T., and Pandak, N. (2002). *Streptococcus suis* infection as a cause of severe illness: 2 case from Croatia. Scan J Infect Dis 34, 683–684.

Kuempel, P., Hogaard, A., Nielsen, M., Nagappan, O., and Tecklenburg, M. (1996). Use of a transposon (Tn*dif*) to obtain suppressing and nonsuppressing insertions of the *dif* resolvase site of *Escherichia coli*. Genes Dev 10, 1162–1171.

Kuempel, P.L., Henson J.M., Dircks L., Tecklenburg M., and Lim. D.F. (1991). *dif*, a RecA-independent recombination site in the terminus region of the chromosome of *Escherichia coli*. New Biol. 3, 799-811.

Kunst, F., Ogasawara, N., Moszer, I., Albertini, A.M., Alloni, G., Azevedo, V., and Bertero, M.G. (1997). The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. Nature 390, 249–256.

Kwon, H.J., Tirumalai, R., Landy, A., and Ellenberger, T. (1997). Flexibility in DNA recombination: structure of the lambda integrase catalytic core. Science, 276, 126–131.

Landy, A (1989). Dynamatic, structural, and regulatory aspects of lambda site-specific recombination. Annu Rev Biochem, 58, 913-949.

Landy, A. (1993). Mechanistic and structural complexity in the site-specific recombination pathways of Int and FLP. Curr. Opin. Genet. Dev. 3, 699-707.

Lee, J., Jayaram, M., and Grainge, I. (1999). Wild-type Flp recombinase cleaves DNA in trans. EMBO J. 18, 784–791.

Lee, L., and Sadowski, P.D. (2001). Directional resolution of synthetic Holliday structures by the Cre recombinase. J. Biol. Chem., 276, 31092–31098.

Lee, J., Serre, M.C., Yang, S.H., Wang, I., Araki, H., Oshima, Y., and Jayaram, M. (1992). Functional analysis of boxII mutations in yeast site-specific recombinases FLP and R. J. Mol. Biol. 228, 1091-1103.

Leschziner, A. E., Boocock, M. R., and Grindley, N. D. (1995). The tyrosine-6 hydroxyl of gamma delta resolvase is not required for the DNA cleavage and rejoining reactions. Mol. Microbiol. 15, 865-870.

Leslie, N.R., and Sherratt, D.J. (1995) Site-specific recombination in the replication terminus region of *Escherichia coli*: functional replacement of *dif*. EMBO J. 14, 1561-1570.

Lesterlin, C., Barre, F.X., and Cornet, F. (2004). Genetic recombination and the cell cycle: what we have learned from chromosome dimmers. Mol. Microbiol. 54, 1151-1160.

Levy, O., Ptach, J., Pease, P., Gore, J., Eisen, M.B., and Bustamante, C. (2005). Identification of oligonucleotide sequences that direct the movement of the *Escherichia coli* FtsK translocase. Proc. Natl. Acad. Sci. USA 2005, Vol.102, 17618-17623.

Lim, D.B., Oppenheim, J. D., Eckhardt, T., and Maas, W. K. (1987). Nucleotide sequence of the argR gene of *Escherichia coli* K-12 and isolation of its product, the arginine repressor. Proc. Natl Acad. Sci. USA, 84(19), 6697–6701.

Lim, D. J., Oppenheim, J., Eckhardt, T., and Maas, W. K. (1988). The unitary hypothesis for the repression mechanism of arginine biosynthesis in *E. coli* B and *E. coli* K12-revisited after 18 years. In Gene Expression and Regulation: the Legacy of Luigi Gorini (M. Bissel *et al.*, ed.), pp. 55–63, Elsevier Science Publishers BV, Amsterdam.

Liu, G, Draper, G.C. ,and Donachie, W.D. (1998) FtsK is a bifunctional protein involved in cell division and chromosome localization in *Escherichia coli*. Mol Microbiol 29, 893–903.

Lobry, J.R., and Louarn, J.M. (2003) Polarisation of prokaryotic chromosomes. Curr Opin Microbiol 6, 101–108.

Lovett, S.T., and Kolodner, R.D. (1991). Nucleotide sequence of the *Escherichia coli recJ* chromosomal region and construction of RecJ-overexpression plasmids. J. Bacteriol. 173, 363-364.

Lu, C.D., Houghton, J.E., and Abdelal, A.T. (1992). Characterization of the arginine repressor from *Salmonella typhimurium* and its interactions with the carAB operator. J. Mol. Biol. 225(1), 11–24.

Manen, D., and L. Caro. (1991). The replication of plasmid pCS101. Mol. Microbiol. 5, 233-237.

Martinez-Granero, F., Capdevila, S., Sanchez-Contreras, M., Martin, M., and Rivilla, R. (2005). Two site-specific recombinases are implicated in phenotypic variation and

competitive rhizosphere colonization in *Pseudomonas fluorescens*. Microbiology, 151, 975–983.

Massey, T.H., Aussel, L., Barre, F.X., and Sherratt, D.J. (2004). Asymmetric activation of Xer site-specific recombination by FtsK. EMBO Rep. 4, 399–404.

McCulloch, R., Burke, M.E., and Sherratt, D. J. (1994). Peptidase activity of *Escherichia coli* aminopeptidase A is not required for its role in Xer site-specific recombination. Mol. Microbiol. 12, 241–251.

McGlynn, P., and Lloyd, R.G. (2000). Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. Cell 101, 35-45.

Messer, W., and Weigler, C., (1996). Initiation of chromosome replication, in: Neidhardt F.C., et al. (Eds), *Escherichia coli* and *Salmonella* cellular and molecular biology, 2nd ed., pp. 1579-1601, Ameican Society for Microbiology, Washington DC.

Michel, B., Recchia, G.D., Penel-Colin, M., Ehrlich, S.D., and Sherratt, D.J. (2000). Resolution of Holliday junctions by RuvABC prevents dimer formation in *rep* mutants and UV irradiated cells. Mol Microbiol 37, 181–191.

Miller, C.M., Baumberg, S., and Stockley, P.G. (1997). operator interactions by the *Bacillus subtilis* arginine repressor/activator, AhrC: novel positioning and DNA-mediated assembly of a transcriptional activator at catabolic sites. Mol Microbiol 26, 37-48.

Moitoso de Vargas, L., Pargellis, C.A., Hasan, N.M., Bushman, E.W., and Landy, A. (1988). Autonomous DNA binding domains of λ. integrase recognize two different sequence families. Cell, 54, 923–929.

Stockhofe-Zurwieden, N., and Smits, M.A. (1999). Identification and characterization of the *cps* locus of *Streptococcus suis* serotype 2: the capsule protects against

phagocytosis and is an important virulence factor. Infect. Associated Immun. 67, 1750–1756.

Nash, H.A. (1996). Site-specific recombination: integration, excision, resolution, and inversion of defined DNA segments. In: Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhart, F.C. *et al.*, Eds.), 2nd edn, Vol. 2, pp. 2363-2376. American Society for Microbiology, Washington, DC.

Ni, J., Sakanyan, V., Charlier, D., Glansdorff, N., and van Duyne, G.D. (1999). Structure of the arginine repressor from *Bacillus stearothermophilus*. Nature Struct Biol, 6, 427–432.

North, A.K., Smith, M.C., and Baumberg, S. (1989). Nucleotide sequence of a *Bacillus subtilis* arginine regulatory gene and homology of its product to the *Escherichia coli* arginine repressor. Gene, 80(1), 29–38.

Nunes-Düby, S., Tirumalai, R.S., Dorgai, L., Yagil, E., Weisberg, R.A., and Landy, A. (1994). Integrase cleaves DNA in cis. EMBO J. 13, 4421–4430.

Nunes-Düby S., Azaro M.A., and Landy A. (1995). Swapping DNA strands and sensing homology without branch migration in lambda site-specific recombination. Curr Biol, 5,139-148.

Nunes-Düby, S.E., Kwon, H.J., Tirumalai, R.S., Ellenberger, T., and Landy, A. (1998). Similarity and differences among 105 members of Int family site-specific recombinases

Pan, G. and Sadowski, P.D. (1993). Identification of the functional domains of the FLP recombinase. J. Biol. Chem., 268, 22546–22551.

Pargellis, C.A., Nunes-Düby, S.E., Devargas, L.M., and Landy, A. (1988). Suicide recombination substrates yield covalent lambda integrase-DNA complexs and lead to identification of the active-site tyrosine. J.Biol.Chem.263, 7678-7685.

Pease, P.J., Levy, O., Cost, G.J., Gore, J., Ptacin, J.L., Sherratt D., Bustamante, C., and Cozzarelli, N.R. Sequence-directed DNA translocation by purified FtsK. Science Jan 28; 307(5709), 586-90.

Pérals, K., Capiaux, H., Vincourt, J.B., Louarn, J.M., Sherratt, D.J., and Cornet, F. (2001). Interplay between recombination, cell division and chromosome structure during chromosome dimer resolution in *Escherichia coli*. Mol Microbiol 39, 904–913.

Pérals, K., Cornet, F., Merlet, Y., Delon, I., and Louarn, J.M. (2000). Functional polarization of the *Escherichia coli* chromosome terminus: the *dif* site acts in chromosome dimer resolution only when located between long stretches of opposite polarity. Mol Microbiol 36, 33–43.

Prasad, P.V., young, L.J., and Jayaram, M. (1987). Mutation in the 2μm circle site-specific recombinase that abolish recombination without affecting substrate recognition. Proc. Natl. Acad.Sci. USA 84, 2189-2193.

Recchia, G..D., Aroyo, M., Wolf, D., Blakely, G., and Sherratt, D.J. (1999). FtsK-dependent and –independent pathways of Xer site-specific recombination. EMBO J. 18, 5724-5734.

Reams, R.Y., Glickman, L.T., Harrington, D.D., Thacker, H.L., and Bowersock, T.L. (1994) *Streptococcus suis* infection in swine: a retrospective study of 256 cases. Part II. Clinical sings, gross and microscopic lesions, and coexisting microorganisms. J Vet Diagn Invest 6, 326–334.

Reed, R.R., and Moser, C.D. (1984). Resolvase-mediated recombination intermediates contain a serine residue covalently linked to the DNA. Cold Spring Harbor Symp. Quant. Biol. 49, 245-249.

Reichmann, P., and Hakenbeck, R. (2002). XerD recombinase with unusual active site motifs in streptococcus pneumoniae. J. Mol. Microbiol. Biotechnol. 4(1), 101-110.

Reijns, M., Lu, Y., Leach, S., and Colloms, S.D. (2005). Mutagenesis of PepA suggests a new model for the Xer/cer synaptic complex. Mol Microbiol. 57, 927-41.

Richaud, C., Higgins, W., Mengin-Lecreux, D., and Stragier, P. (1987). Molecular cloning, characterization and chromosomal localization of *dapF*, the *Escherichia coli* gene for diaminopimelate epimerase. J Bacterial 169, 1454-1459.

Richaud, C., and Printz, C. (1988). Nucleotide sequence of the *dapF* gene and flanking regions from *Escherichia coli* K12. Nucleic Acids Res 16, 10367.

Sadowski, P.D. (1986). Site-specific recombinase: changing partners and doing the twist. J.Bacteriol. 165, 341-347.

Sadowski, P.D. (1995). The Flp recombinase of the 2μm plasmid of *Saccharomyces cerevisiae.* Prog Nucleic Acid Res. Mol Biol. 51, 53–91.

Saleh, O.A., Perals, C., Barre, F.X., and Allemand, J.F. (2004). Fast, DNA-sequence independent translocation by FtsK in a single-molecule experiment. EMBO J 23, 2430-2439.

Salzberg, S.L., Salzberg, A.J., Kerlavage, A.R., and Tomb, J.F. (1998). Skewed oligomers and origins of replication. Gene 217, 57–67.

Sciochetti, S.A., Piggot, P.J., and Blakely, G.W. (2001). Identification and characterization of the *dif* site from *Bacillus subtilis*. J. Bacteriol, 183, 1058-1068.

Sherratt D.J., Arciszewska L.K., Blakely G., Colloms S., Grant K., Leslie N., and McCulloch R. (1995). Site-specific recombination and circular chromosome segregation. Philos Trans R Soc Lond B Biol Sci, 347, 37-42.

Sherratt, D.J., and Wigley, D.B. (1998). Conserved themes but novel activities in recombinases and topoisomerases. Cell 93, 149–152.

Sherratt, D. J., Arciszewska, L. K., Blakely, G., Colloms, S., Grant, K., and Leslie, N. (1995). Site-specific recombination and circular chromosome segregation. Phil. Trans. R. Soc. Lond. B. Bio.l Sci. 347, 37-42.

Smithe, M.C., Czaplewski, L., North A.K., Baumberg, S., and Stockley, P.G. (1989). Sequence required for regulation of arginine biosynthesis promoters are conserved between *Bacillus subtilis* and *Escherichia coli*. Mol. Microbiol, 3(1), 23-8.

Smith, H.E., Damman, M., van der Velde, J., Wagennar, F., Wisselink, H., Stockhofe-Zurvieden, N., and Smith, M.A. (1999). Identification and characterisation of the *cps* locus of *Streptococcus suis* serotype 2: the capsule protects against phagocytosis and is an important virulence factor.Infect. Immun. 67: 1750–1756.

Stark, W.M., and Boocock, M.R. (1995). Topological selectivity in site-specific recombination. In: Mobile Genetic Elements (Sherratt, D.J., Ed.), pp. 101-129. IRL, Oxford.

Stark, W.M., Boocock, M.R. and Sherratt, D.J. (1992). Catalysis by site-specific recombinases. Trends Genet. 8, 432-439.

Sträter N., Sherratt D.J., and Colloms S.D. (1999). X-ray structure of aminopeptidase A from *Escherichia coli* and a model for the nucleoprotein complex in Xer site-specific recombination. EMBO J. 18, 4513-22.

Steiner, W., Liu, G., Donachie, W. D., and Kuempel, P. (1999). The cytoplasmic domain of FtsK protein is required for resolution of chromosome dimers. Mol. Microbiol. 31, 579–583.

Steiner, W.W, and Kuempel, P.L, (1998a). Cell division is required for resolution of dimer chromosomes at dif locus of *Escherichia coli*. Mol. Microbiol 27, 257-268.

Steiner, W.W, and Kuempel, P.L, (1998b). Sister chromatid exchange frequencies in *Escherichia.coli* analyzed by recombination at the *dif* resolvase site J.Bacteriol. 180, 6269-6275.

Stirling, C.J., Colloms, S., Collins, J.F., Szatmari, G., and Sherratt. D.J. (1989). *XerB*, an *Escherichia coli* gene required for plasmid ColE1 site-specific recombination, is identical to *pepA*, encoding aminopeptidase A, a protein with substantial similarity to bovine lens leucine aminopeptidase. EMBO J. 8, 1623-1627.

Stirling, C. J., Szatmari, G., Stewart, G., Smith, M.C.M., and Sherratt, D.J. (1988). The arginine repressor is essential for plasmid-stabilizing site-specific recombination at the ColE1 *cer* locus. EMBO J. 7, 4389-4395.

Subramanya, H.S., Arciszewska, L.K., Baker, R.A., Bird, L.E., Sherratt, D.J., and Wigley, D.B. (1997). Crystal structure of the site-specific recombinase, XerD. EMBO J. 16, 5178-5187.

Summers, D., Yaish, S., Archer, J., and Sherratt. D. (1985). Multimer resolution systems of ColE1 and ColK: localization of the crossover site. Mol. Gen. Genet. 201, 334-338.

Summers, D.K. (1989). Derivatives of ColE1 *cer* show altered topological specificity in site-specific recombination. EMBO J. 8, 309-315.

Summers, D. K., and Sherratt, D. J. (1984). Multimerization of high copy number plasmids causes instability: ColE1 encodes a determinant essential for plsmid monomerization and stability. Cell 36, 1097-1103.

Summers, D.K., and Sherratt, D.J. (1988). Resolution of ColE1 dimers require a DNA sequence implicated in the three-dimentional organization of the *cer* site. EMBO J. 7, 851-858.

Summers, D.K., Beton, C.W.H., and Wthers, H.L. (1993). Multicopy plasmid stability: the dimer catastrophe hypothesis. Mol. Microbiol. 8,1031-1038.

Takamatsu, D., Osaki, M., and Sekizaki, T. (2001). Thermosensitive Suicide Vectors for Gene Replacement in *Streptococcus suis.* Plasmid 46, 140-148,

Tecklenburg, M., Naumer, A., Nagappan, O., and Kuempel, P. (1995). The *dif* resolvase locus of the *Escherichia coli* chromosome can be replaced by a 33-bp sequence, but function depends on location. Proc. Natl. Acad. Sci. USA 92, 1352–1356.

Tian, G., and Maas, W.K. (1994). Mutational analysis of the arginine repressor of *Eschericia coli.* Mol. Microbiol. 13, 599–608.

Tian, G., Lim, D., Carey, J., and Maas, W.K. (1992). Binding of the arginine repressor of *Escherichia coli* K12 to its operator sites. J. Mol. Biol. 226, 387-397.

Tikkanen, K., Haataja, S., and Finne, J. (1996). The galactosyl-(alfa1–4)-galactose-binding adhesin of *Streptococcus suis*: occurence in strains of different haemagglutination activities and induction of opsonic antibodies. Infect. Immun. 64: 3659–3665.

van de Putte, P., and Goosen, N. (1992). DNA inversions in phages and bacteria. Trends Genet. 8, 457-462.

van Duyne G.D., Ghosh G., Maas W.K., and Sigler P.B. (1996). Structure of the oligomerization and L-arginine binding domain of the arginine repressor of *Escherichia coli*. J Mol Biol, 256, 377–391.

Van Gool, A.J., Hajibagheri, N.M., Stasiak, A., and West, S.C. (1999). Assembly of the *Escherichia coli* RuvABC resolvasome directs the orientation of Holliday junction resolution. Genes Dev 13, 1861–1870.

Vogt, V.M. (1970). Purification and properties of an aminopeptidase from *Escherichia coli*. J. Biol. Chem., 245, 4760–4769.

Warren, G.J., and A.J. Clark. (1980). Sequence-specific recombination of plasmid ColE1. Proc. Natl. Acad. Sci. 77, 6724-6728.

Winterhoff, N., Goethe, R., Gruening, P., Rohde, M., Kalisz, H., Smith, H.E., and Valentin-Weigand, P. (2002). Identification and characterisation of two temperature-induced surface associated proteins of *Streptococcus suis* with high homologies to members of the arginine deiminase system of *Streptococcus pyogenes*. J. Bacteriol. 184: 6768–6776.

Wisselink, H.J., Smith, H.E., Stockhofe-Zurwieden, N., Peperkamp, K., and Vecht, U. (2000). Distribution of capsular types and production of muramidase-released protein (MRP) and extracellular factor (EF) of *Streptococcus suis* strains isolated from diseased pigs in seven European countries. Vet. Microbiol. 74: 237–248.

Wood, D., Solomon, M., and Speed, R. (1993). Characterisation of the *Rickettsia prowazekii pepA* gene encoding leucine aminopeptidase. J. Bacteriol. 175, 159–165.

Wu, L.J., Lewis, P.J., Allmansberger, R., Hauser, P.M., and Errington, J. (1995). A conjugation-like mechanism for prespare chromosome partitioning during sporulation in *Bacillus subtilis*. Genes & Dev. 9, 1316–1326.

Yates, J., Aroyo, M., Sherratt, D.J., and Barre, F.X. (2003). Species specificity in the activation of Xer recombination at *dif* by FtsK. Mol Microbiol 49, 241–249.

Yu, X.C., Weihe, E.K., and Margolin, W. (1998). Role of the C terminus of FtsK in *Escherichia coli* chromosome segregation. J. Bacteriol. 180, 6424–6428.

Vecht, U., Wisselink, H., Jellema, M., and Smith, H.E. (1991). Identification of two proteins associated with virulence of *Streptococcus suis* type 2. Infect. Immun. 59: 3156–3162.

APPENDIX I

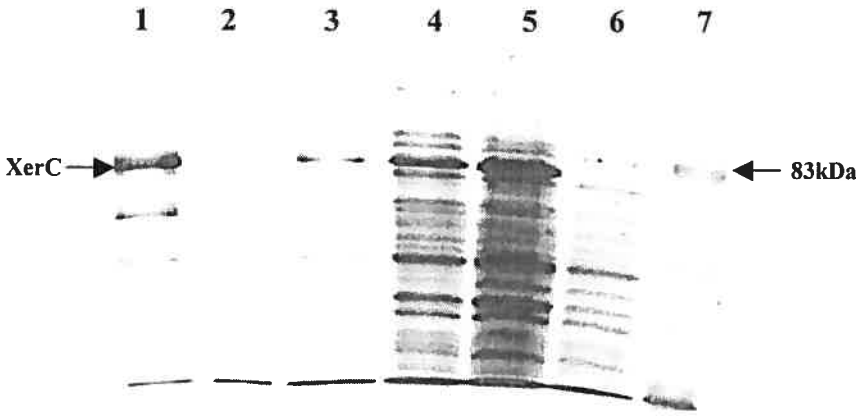


Figure 14. Overexpression and purification of *S. suis* XerC. Lane 1, partial purified XerC; lane 2, 3 washing fraction; lane 4, flow-through; lane 5, induced crude extract; lane 6, no induced crude extract; lane 7, protein molecular weight standard.

APPENDIX II

XerC gene of *S. suis*

atgagacgcgagttattattggaaaaaattgatcaactaaaagaaattat

gccttggtttgttctggaatattatcagtcaaaattggctgtgccttaca

gttttactaccttatatgaatacttgaaagaataccgtcgttttttgaa

tggttacaggattcggatttggtggctgttgaacggattgctgacattcc

gctggatgttctggaacatctgacaaaaaaagatatggaagctttcattc

tttatctgcgggagcgtcccttgctgaacgccaataccacgcagaatggt

gtgtcgcagaccaccattaaccgtaccctctcggccctttctagtctctt

caagtatttaaccgaagaagtggagaatgagcagggcgagccctacttct

accgcaatgtcatgaaaaaggtatctactaagaagaagaaggaaaccttg

gcagcgcgggcggagaacatcaagcaaaagctctttttgggcgatgaaac

gatggagttcttggactatgtggacaaggaataccaagtcaatctctcta

aacgtgccctctcctccttccagaaaaataaggagcgggatttggcgatt

ctggcgcttctcttggcttctggcgtccgtctgtcagaagcggtgaattt

ggatcttcgagatgtaaacctcaatatgatgattatcgaagtaactcgta

agggtggtaaacgggactcggtcaatgtggctgggtttgctaagctctac

ttagaagcctacatgggcatccgtcagcaacgctacaaggctgaaaaaac

ggatacagccttcttcctgtccgaataccgtggtctgcctaaccgtatcg

atgcttcttctattgaaaaaatggttgccaagtactctgcggacttcaag

atacgcgtaacccccccacaaactccgtcacacattggcaactcgtctcta

cgacgccaccaagtcgcaagttctagtcagtcatcagctgggccatgcca

atacccaggtcaccgatctctacacccatatcgtcaacgatgaacagaaa

aatgctctggatcagttgtga

1071 nucleotides

Amino Acid Sequence of XerC *S. suis*

MRRELLLEKI DQLKEIMPWF VLEYYQSKLA VPYSFTTLYE YLKEYRRFFE
WLQDSDLVAV ERIADIPLDV LEHLTKKDME AFILYLRERP LLNANTTQNG
VSQTTINRTL SALSSLFKYL TEEVENEQGE PYFYRNVMKK VSTKKKKETL
AARAENIKQK LFLGDETMEF LDYVDKEYQV NLSKRALSSF QKNKERDLAI
LALLLASGVR LSEAVNLDLR DVNLNMMIIE VTRKGGKRDS VNVAGFAKLY
LEAYMGIRQQ RYKAEKTDTA FFLSEYRGLP NRIDASSIEK MVAKYSADFK
IRVTPHKLRH TLATRLYDAT KSQVLVSHQL GHANTQVTDL YTHIVNDEQK
NALDQL

356 amino acids

ACKNOWLEDGEMNETS

I would like to take this opportunity to appreciate my research director, Dr. George Szatmari, for his encouragement, patience, consideration, and constructive criticism. I especially appreciate that he provided me a very freely atmosphere to learn new knowledge and new techniques. I am also appreciated for his instructive suggestion and judgement on my work.

I would also like to express my appreciation to Dr Josée Harel of Faculté de Médecine Véterinaire for providing *S. suis p1/7* and *31533* genomic DNA, *S. suis S735* strain and plasmid pBEA756; Dr Newman Laboratory of Concordia University for providing DAPI-staining protocol; Monic Vasseur for technical assistance. I also wish to thank Dr. Marc Drolet for his kind supporting and permission to use the instruments in his lab. Dr Xiaojian Yao for his good suggestion;

I would also like to thank all members in our lab, Manuela villion and Hélène Sénéchal for their very kindly help on experimental manipulation and good suggestions on my project; Vincent Deslandes, Jérémy Desleques, for their cooperation, help, suggestions, supports and consideration.

I am also grateful to my friends, Yong Xiao, Imad Baaklini, Patrick Sanscartier, Zhujun Ao, Tan Wang, for their encouragement, good suggestion, support to my project.