Title: The phage integrase vector pIPI03 allows recA-independent, site-specific labeling of Staphylococcus lugdunensis strains

Keywords: Staphylococcus, Vector, Bacteriophage, competitive index, Integration

Abstract: Abstract
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Suggested Reviewers: Tarok Bae PhD, Associate Professor, University of Indiana, tbae@iun.edu
Chia Lee PhD, Professor, University of Arkansas, clee2@uams.edu
Francois Vandenesch MD, Professor, Université de Lyon, denesch@univ-lyon1.fr

Corresponding Author: Dr. Tim Foster,
Corresponding Author's Institution: Trinity College Dublin
First Author: Simon Heilbronner, MSc
Order of Authors: Simon Heilbronner, MSc; Ian R Monk, PhD; Tim Foster
Soeren Gatermann  MD  
Professor  
soeren.gatermann@rub.de  
Has studied pathogenesis of several coagulase negative staphylococci including S.lugdunensis.  
Performed some of the first genetic manipulations in this species

Andreas Peschel PhD  
Professor, University of Tubingen  
andreas.peschel@uni-tuebingen.de  
Wide experience of molecular pathogenesis of staphylococcal infections.

Opposed Reviewers:
Staphylococcus lugdunensis is a commensal and occasional pathogen of humans. Until recently easy genetic manipulation of this organism had not been reported. We previously set up a genetic system involving electrotransformation of plasmids and site specific allelic exchange mutagenesis. In this paper we report the construction and validation of a temperature sensitive plasmid which integrates site-specifically into the chromosome of most strains of S. lugdunensis. The vector was modified to allow it to be used to mark strains for competitive growth experiments. This approach could be adapted to other species of staphylococcus.
Highlights

- Plasmid vector constructed and validate that integrates site-specifically into the chromosome of most strains of *Staphylococcus lugdunensis*.

- Integrates at a phage attachment site directed by a phage integrase

- Vector stably integrated and maintained at 37 °C. No evidence for excision

- Vector labelled with different inducible antibiotic resistance markers

- Used for testing relative fitness of constructs in vitro
The phage integrase vector pIPI03 allows recA-independent, site-specific labeling of *Staphylococcus lugdunensis* strains

Simon Heilbronner\(^1\), Ian Monk\(^1\) and Timothy Foster\(^1\)

\(^1\) Microbiology Department, Trinity College, Dublin 2, Ireland.

Running title. The *Staphylococcus lugdunensis* phage-integrase vector pIPI03

Corresponding author. tfoster@tcd.ie
Abstract

*Staphylococcus lugdunensis* is a coagulase negative staphylococcus that is a commensal of man and an opportunistic pathogen. A site-specific integrative plasmid for the use in *S. lugdunensis* was constructed and validated. The integrase gene *ccrB* of bacteriophage φSL01 together with its attachment site was cloned into the thermosensitive plasmid pIMAY. The resulting plasmid pIPI03 integrated *recA*-independently, site-specifically and irreversibly into the *S. lugdunensis* chromosome. Two IPTG-inducible antibiotic resistance determinants were cloned into pIPI03 and the derivatives were used to construct strains suitable for competitive growth experiments in both *in vitro* and *in vivo*.

Keywords

*Staphylococcus lugdunensis*, plasmid vector, *recA* mutant, bacteriophage

1. Introduction

*S. lugdunensis* (Freney et al., 1988) is a coagulase negative staphylococcus (CoNS) that shows an unusual degree of virulence. The species is a commensal of human skin that is predominantly isolated of the moist areas such as perineum and the inguinal fold (Bieber and Kahlmeter, 2010). However it is particularly associated with cases of infective endocarditis (Anguera et al., 2005; Patil et al., 2011; Vandenbroucke et al., 1993) with a course of disease akin to *S. aureus* infections. Although *S. lugdunensis* is recognized to be an important pathogen, remarkably little is known about virulence factors and the molecular mechanisms of pathogenicity. Furthermore, genetic tools for the manipulation of the organism were limited until very recently when efficient transformation by electroporation was reported along with the use of the thermosensitive vector pIMAY for constructing site-specific mutations by allelic exchange (Heilbronner et al., 2013).
Bacteriophage integrases recognize attachment sites (attB) on the bacterial chromosome and mediate site-specific recombination with the phage attP site. This results in a single copy of the circular phage genome being inserted into the bacterial chromosome (Hirano et al., 2011). It has been demonstrated before that phage integrases can be used to promote site-specific integration into the bacterial chromosome of plasmids harboring an attP site. Such integrative plasmids have been used both in Gram-negative and Gram-positive bacterial species including Lactococcus lactis (Petersen et al., 2013) Staphylococcus aureus (Lei et al., 2012) and Listeria monocytogenes (Monk et al., 2008a; Monk et al., 2008b).

We examined the genome sequence of S. lugdunensis N920143 (Heilbronner et al., 2011) and identified a prophage φSL1 that is not present in strain HKU09-01 (Tse et al., 2010). The phage integrase of φSL1 was used to construct the integrative plasmid pIPI03 and its derivates pIPIery and pIPIkan which were tested in competitive growth experiments.

2. Materials and Methods

Unless stated otherwise, reagents and chemicals were obtained from Sigma Aldrich. Restriction endonucleases were obtained from Roche and Fermentas.

2.1 Bacterial strains and growth conditions

All strains are listed in Table 1. S. lugdunensis was grown in tryptic soy broth (TSB) or agar (TSA) (Difco). E. coli strains were grown in Luria-Bertani broth (LB) or agar (LA) (Difco). Unless stated otherwise, strains were grown at 37 °C.

2.2 Construction of pIPI03

To construct the S. lugdunensis phage integrase vector, the thermosensitive vector pIMAY (Monk et al., 2012) was modified using the SLIC cloning method reported earlier (Li and Elledge,
2007; Li and Elledge, 2012). Amplification of the φSL1 integrase (ccrB) together with the corresponding attachment site attP was only possible from the circular phage genome and not from the linearized, integrated genome of the lysogenic phage. Thus, *S. lugdunensis* N920143 was grown to exponential phase and induced for 1 h with 1 μg / ml mitomycin C to stimulate the excision of φSL1. Cells were harvested and DNA was extracted as described previously (Heilbronner et al., 2011). At their 5’ ends the primers ccrB-F / ccrB-R contain 27 bp sequence identity to the pIMAY sequence downstream of the cat gene to allow SLIC assembly of vector and insert. The 5.7 kb backbone of pIMAY was amplified (linearized after the cat stop codon) using primers pIMAY_SLICcat_F and pIMAY_SLICcat_R. All PCR reactions were carried out using Phusion polymerase (Finzymes). Primer sequences a summarized in Table 2.

The PCR products were purified and the vector product was digested with DpnI in order to remove the methylated template DNA. 1 μg of vector and insert DNA was treated with T4 DNA polymerase in a final volume of 40 μl (creation of single stranded 5’ ends). The reaction contained NEB Buffer 2 (New England Biolabs), molecular grade BSA, 5mM DTT, 200 mM Urea and 3 Units T4 DNA polymerase (New England Biolabs). The reaction was incubated for 20 min on 23 °C and stopped by the addition of 25 mM EDTA (pH 8) and subsequent incubation for 20 min at 75 °C. 5 μl of vector and 5 μl of insert were mixed and the single stranded overhangs (identical ends generated by the primers ccrB-F/ccrB-R) were allowed to anneal. For this, the reaction was placed in a PCR machine for 10 min at 65 °C followed by a decrease in temperature from 65 – 25 °C with 1 min hold for each degree. 2.5 μl of the reaction was subsequently used for the transformation of *E. coli*. Recovered clones were validated by DNA sequencing.

IPTG-inducible kanamycin and erythromycin resistance cassettes (Monk et al., 2008a) were excised from pIMCkan and pIMCery, respectively, using the restriction endonucleases KpnI / Sacl. The fragments were gel-purified and cloned into the MCS of pIPI03 treated with the same endonucleases. The resulting plasmids were named pIPI03kan and pIPI03ery.
2.3 Chromosomal integration of pIPI03

pIPI03 was used to transform *S. lugdunensis* HKU09-01 strains (Heilbronner et al., 2013; Zapotoczna et al., 2012). Due to the thermosensitive character of the plasmid transformants were selected at 28 °C on TSA containing 10 μg / ml chloramphenicol (TSA<sub>cm10</sub>). To select for plasmid integration, one colony from a plate grown at 28°C (with an autonomously replicating plasmid) was resuspended in TSB and dilutions were plated on TSA<sub>cm10</sub> and incubated at 37°C. Colonies were screened by PCR to confirm site-specific integration.

To determine the frequency of pIPI03 integration, a HKU09-01 (pIPI03) colony was resuspended in TSB. Dilutions were plated and incubated at 28 °C to determine the total number of CFUs and at 37 °C to determine the number of CFUs carrying integrated plasmid.

To test the stability of integration, HKU09-01:pIPI03 was grown for 4 consecutive subcultures (diluting 1 in 1000 each time) at 37 °C or 28 °C without antibiotic selection. Dilutions were plated on TSA and 50 colonies were screened for chloramphenicol resistance.

2.4 Isolation of *S. lugdunensis* HKU09-01 ΔrecA

The *recA* mutation in HKU09-01 was isolated by allelic replacement using the thermosensitive plasmid pIMAY (Monk et al., 2012). 500 bp fragments upstream and downstream of the *recA* gene were PCR-amplified and assembled using spliced overlap extension PCR. The resulting 1 kb deletion cassette was cloned into pIMAY and used to replace the chromosomal *recA* gene by allelic exchange (Heilbronner et al., 2013; Monk et al., 2012).

2.5 Growth curves

Growth kinetics of different strains was assessed using the SynergyH1 Hybrid reader (BioTeK) in 96 well plates (Nunc). Triplicate wells were filled with 200 μl TSB inoculated to an OD<sub>578</sub> = 0.05 with a fresh overnight culture. The optical density at 578 nm was measured every 45 min with
10 min of shaking prior to every read. Each growth curve was recorded three times using independent overnight starter cultures.

2.6 UV sensitivity

Colonies were picked from a fresh plate and streaked on a new TSA plate. Plates were exposed for indicated times to short wave UV light using the “Mineralight Lamp” Model CC10 (UVP) in a Chomato-VUE Cabinet. Plates were incubated for 24 h at 37 °C and growth was evaluated semi quantitatively.

2.7 Competitive growth experiments

Two strains (labelled with pIPI\textit{kan} or pIPI\textit{ery}) were grown in TSB$_{\text{cm10}}$ overnight. Cells were harvested (10 min at 9000 x g), washed with TSB and adjusted to an OD$_{578}$ = 3. 50 μl of each strain was inoculated into 10 ml TSB$_{\text{cm10}}$. Dilutions were plated on TSA$_{\text{IPTG/Kan}}$ (1 mM IPTG + 50 μg / ml kanamycin) and TSA$_{\text{IPTG/Ery}}$ (1 mM IPTG + 10 μg / ml erythromycin) to determine the ratio of cells at the start of the experiment (C0). The culture was incubated for 24 h with shaking at 200 rpm at 37 °C. The OD$_{578}$ of the overnight culture was measured and the approximate number of generations was calculated. Dilutions were plated on TSA$_{\text{IPTG/Kan}}$ and TSA$_{\text{IPTG/Ery}}$ to determine the ratio of the strains after 24 h growth (C1). A new TSB culture was inoculated to an OD$_{578}$ = 0.01 with the previous culture and incubated at 37 °C for another 24 h. The process was repeated four times and the ratio of strains in the consecutive cultures was determined.

3. Results

3.1 The phage-integrase vector pIPI03
S. lugdunensis N920143 carries the lysogenic phage φSL1 (Heilbronner et al., 2011). The phage is integrated into gene SLUG_08250 which encodes a putative phosphosugar transport protein. Strain HKU09-01 does not harbour the phage (Heilbronner et al., 2011; Tse et al., 2010).

To create a vector for the site-specific chromosomal integration, the integrase gene ccrB and the corresponding attachment site attP were amplified and cloned into the thermosensitive vector pIMAY (Monk et al., 2012) to form pIPI03 (Fig. 1A). The cat and ccrB genes form a transcriptional fusion and are constitutively expressed.

To be able to distinguish between strains with the same genetic background, two different variants of pIPI03 were constructed by cloning IPTG-inducible antibiotic resistance determinants (Monk et al., 2008a) into the multiple cloning site of the plasmid. pIPIery confers inducible erythromycin resistance and pIPIkan confers kanamycin resistance (Fig. 1B). The inducible cassettes contain a Phelp promoter followed by a LacO repressor binding site and the resistance determinant. The lacI (repressor) coding sequence is present and its expression is driven by a second Phelp promoter. Thus the antibiotic resistances are repressed unless induced by IPTG.

3.2 Integration of pIPI03

S. lugdunensis HKU09-01 was transformed with pIPI03 with selection on Cm agar at 28 °C, the permissive temperature for plasmid replication. Subsequent plating at 37 °C allowed the selection of clones with an integrated plasmid. Determination of the total number of CFUs grown at 28 °C and 37 °C revealed that 0.09 % to 0.13 % of cells present within a colony carried the plasmid integrated into the chromosome.

PCR experiments using primer combinations recognizing sequences in the chromosome and the pIPI03 backbone (Fig. 2) confirmed that the plasmid had integrated at the same site as phage φSL01 in N920143. The PCR1 experiment using the chromosomal primers scr.F and scr.R yielded a 600 bp fragment with DNA isolated from HKU09-01 (pIPI03) grown on 28 °C, showing that the phage attachment site is not occupied in this strain (Fig. 2B). No product was formed with DNA from
HKU09-01::pIPI03 grown on 37 °C, indicating that the site is occupied by the integrated plasmid. The PCR2 experiment used primers ccrB. F and attP. R and confirmed the presence of replicating plasmid in HKU09-01 (pIPI03). PCR2 failed to detect autonomous plasmid in HKU09-01::pIPI03 (Fig. 2B). The primers used in PCR 3 (scr.R and attP.R) bound to sites in the chromosome and the plasmid, respectively, and yielded a 400 bp fragment with DNA from HKU09-01::pIPI03. This confirmed the site-specific integration of the plasmid. Interestingly, this PCR produced a faint product with HKU9-01 (pIPI03) as well (Fig. 2B), showing that at growth on 28 C° a small subpopulation of cells carried the plasmid integrated in the chromosome.

The stability of the integrated form of pIPI03 was investigated. No loss of Cm resistance (<2%) was detected when strains with integrated plasmid were grown for four consecutive subcultures (ca. 40 generations) in TSB without antibiotic pressure at 28 °C and at 37 °C. To make sure that the integrated plasmid was maintained, 10 representative colonies (after growth on 28 °C and 37 °C ) were screened by PCR using the primers described above. All clones showed integrated plasmid and the PCR tests failed to detect autonomous plasmid (data not shown). These results suggest that the phage integrase of φSL01 is only responsible for the insertion of the plasmid into the chromosome and not for its excision.

Recently MLST typing was established for S. lugdunensis (Chassaîn et al., 2012) and revealed five clonal complexes (CCs). PCR1 experiments (using primers scr.F and scr.R) showed that none of the 10 strains in our collection representing isolates of all CCs carried a lysogenic phage at the chromosomal attB site of φSL1 (data not shown). This prompted us to investigate whether pIPI03 is able to integrate into the attB site of strains from different CCs. Since HKU09-01 represents a member of CC1 we chose five isolates representing other CCs (SL71 (CC2), SL27 (CC3), SL37 (CC3), SL62 (CC4) and SL81(CC5)). All strains could be transformed with pIPI03 and the plasmid integrated specifically into the attB site as confirmed by PCR3 using the primer combination scr.R and attP.R (data not shown).
3.3 Effects of pIPI03 integration on bacterial fitness

The ccrB gene in pIPI03 is expressed constitutively by the Phelp promoter. We performed growth curve analysis to exclude the possibility that this influences bacterial fitness. At 37 °C the HKU09-01 wild-type and HKU09-01::pIPI03 strains showed identical lag phases (ca. 2.5h) and similar exponential growth rates (generation times of 98 min and 93 min, respectively) (Fig. 3A). This shows that ccrB expression did not have any toxic effects. However, when the strains were grown at a temperature that allowed integrated plasmid to begin replication (28 °C), the strain had a significantly slower rate of growth (Fig. 3B). At 28 °C both strains showed a lag phase of ca. 4 h 20 min. However, the generation time of the wild-type strain was 181 min, while it was 246 min for the strain with the integrated plasmid. This is most likely due to the thermosensitive replication machinery of pIPI03 attempting to replicate the integrated plasmid which subsequently interferes with the chromosomal replication.

3.4 Integration of pIPI03 is recA independent

A recA mutant of HKU09-01 was tested to determine if integration of pIPI03 is independent of RecA-promoted recombination. RecA is known to be an important mediator of the bacterial SOS response and recA mutants have a reduced ability to repair damaged DNA using recombination by RecA-dependent recombination. As expected, the ΔrecA mutant displayed strongly increased sensitivity to UV-light. The wild-type strain could tolerate UV-light exposure for up to 20 seconds without visible effects on growth, while the ΔrecA mutant showed reduced viability even after only 5 seconds of exposure (Fig. 4A). In a growth curve experiment the ΔrecA mutant displayed a longer lag phase (3 h 10 min) and a reduced growth rate (163 min per generation) compared to the wild-type strain (Fig. 4B).

The integration of pIPI03 occurred independently of the recA genotype because pIPI03 integrants occurred at the same frequency in the recA mutant as in the wild-type (data not shown).
This supports the idea that CcrB does not need RecA-mediated processes to mediate integration into the chromosome.

3.5 pIPI03 in competitive growth experiments

Plasmids pIPI03ery and pIPIkan were integrated into the chromosome of different strains to facilitate competitive growth experiments and to discriminate between strains growing in the same culture. Both plasmids were integrated into the HKU09-01 wild-type strain, creating HK(WT):Ery and HK(WT):Kan. TSB was inoculated with the two strains at a ratio of 1:1, grown to saturation, diluted in fresh broth, grown again to saturation. The process was continued for four consecutive cultures. The ratio of erythromycin to kanamycin resistant cells was determined at the beginning and after each of the subcultures. The results are shown in Fig. 5 (red line). The ratio of erythromycin : kanamycin resistant bacteria remained constant over the course of the experiment. The only difference between the strains was the nature of the resistance determinant and it can be concluded that this did not influence the growth of the bacteria in the competition experiment.

The ∆recA mutant showed a growth defect in the growth curve analysis (Fig. 4B). We integrated both plasmids into the recA strain, creating HK(R):Ery and HK(R):Kan and compared the strains in competitive growth experiments. No differences were observed in the outcome when HK(R):Ery vs. HK(WT):Kan was compared to HK(R):Kan vs. HK(WT):Ery. Therefore the experiments were combined to one data set HK(R) vs HK(WT) (blue line in Fig. 5).

In contrast the data demonstrated a strong growth advantage for the wild-type compared to the recA mutant. The two strains started the experiment at a ratio of 1:1. However the recA mutant was rapidly outcompeted and was present at a $10^5$ fold lower number at the end of the experiment.

4. Discussion
Site-specific integrative plasmids are important tools for modern molecular microbiology since they allow the efficient insertion of DNA in a specific genomic region. Phage-integrase vectors represent a universal class of such vectors and can be constructed for every species. The ccrB gene together with the corresponding attP site of any phage can be used for the construction of integrative plasmids for any species as long as the strain to be tested is not lysogenic and the CcrB protein does not promote excision of the phage. The phage-integrase vector will integrate stably into the bacterial chromosome.

We have constructed a site-specific integration vector for *S. lugdunensis* using the coding sequence of the CcrB integrase and the corresponding attP site of phage φSl1 (Heilbronner et al., 2011). The plasmid was found to integrate specifically, irreversibly and recA-independently into the attB site of HKU09-01. The constitutive expression of ccrB in this vector did not have a negative effect on bacterial fitness. However, the integrated plasmid led to a reduction in growth rate at 28 °C. This is most likely due to the thermosensitive nature of the plasmid replication system, which allows replication to be attempted at 28 °C which could interfere with the chromosomal replication. This should be borne in mind when pIPI03 is employed to complement mutants, for example. Despite this no plasmid loss was detected after 40 generations of growth at 28°C. Apart from N920143, none of the *S. lugdunensis* strains from CC1-CC5 harbored a lysogenic phage in the attB site. Consequently we were able to integrate pIPI03 into the chromosome of each strain. This demonstrates the broad range of *S. lugdunensis* strains that can conveniently be labeled using pIPI03.

There are many possible applications of site-specific integrative plasmids such as pIPI03. The extensive multiple cloning site of pIPI03 facilitates insertion of DNA fragments into the plasmid. We cloned two IPTG-inducible antibiotic resistance cassettes into pIPI03 and demonstrated their use for competitive growth experiments. Competitive growth is a powerful technique to investigate the effects of the controlled expression of a single gene on the cell physiology under identical growth conditions and can be used both *in vitro* and *in vivo* (Monk et al., 2008a). pIPIery and pIPIkan allowed
easy labeling of *S. lugdunensis* strains with clearly distinguishable phenotypes and hence the accurate determination of the ratio of the two strains in competitive growth experiments. It is possible to detect subtle differences by prolonged competitive growth when differences in growth rate/yield in a conventional growth curve experiment would not be seen. Furthermore, the growth of different strains in the same culture allows the accurate analysis of environmental stress factors, such as antibacterial compounds or nutrient limitation and allows investigation of their effects on strains under direct competition.

Another application of integration vectors is complementation of mutants. This would be particularly attractive for experiments involving growth in experimental animals where selection for an autonomously replicating plasmid is difficult and where plasmid loss is magnified by stressful conditions of growth *in vivo*. Furthermore complementation may be distorted by the gene-dosage effect of expressing the wild-type gene from a multicopy plasmid. The integrated vector is very stable and provides expression from a single copy of the wild-type gene.

The *S. lugdunensis* integrative plasmid pIP03 is ideally suited for these applications and will facilitate future experiments to allow a deeper understanding of the virulence potential of this neglected coagulase negative organism.

### 5. Nucleotide sequence accession number

The nucleotide sequence of pIP03 was deposited in GenBank under accession number XXXXX.

### 6. Acknowledgements

We acknowledge the support of the Irish Research Council for Science, Engineering and Technology for an Embark scholarship (RS2000192) and Science Foundation Ireland for a Programme Investigator grant (08/IN.1/B1854) (to TJF). We thank Martine Pestel-Caron for supplying *S. lugdunensis* strains from different clonal complexes.
7. References


Lei, M. G., et al., 2012. A single copy integration vector that integrates at an engineered site on the *Staphylococcus aureus* chromosome. BMC Res Notes. 5, 5.


Figures and tables:

Fig. 1.
(A) Schematic diagram of pIPI03
The coding sequences of the thermosensitive plasmid pI MAY are shown in white. Coding sequences
of the φSL1 integrase (ccrB) and the corresponding attachment site (attP) are shown in black.
Unique restriction sites are indicated. For sequencing purposes, T3 and T7 primer binding sites are
present before the KpnI and after the SacI restriction sites, respectively.

(B) Schematic diagram of the IPTG-inducible antibiotic resistance cassettes.
These were cloned into the MCS of pIPI03 using SacI and KpnI. ermAM – erythromycin resistance;
apHA3 – kanamycin resistance.

Fig. 2.
(A) Schematic diagram of the integration of pIPI03.
The plasmid-encoded genes are shown in white and the the attP site on the plasmid is shown in
blue. The gene SLUG08250 harboring the attB site (red) in the HKU09-01 chromosome is shown in
tan. Arrows indicate the location of primers for the detection of integration by PCR. Integration of
pIPI03 disrupts the attachment sites resulting in ½ attB and ½ attP on either side of the integrated
plasmid.

(B) PCR to detect the integration of pIPI03.
Primer combination 1 (scr. F / scr. R) was used to determine if the chromosomal attB site was
occupied or not. Primer combination 2 (ccrB. F / attP. R) was used to detect autonomous plasmid.
Primer combination 3 (attP. R / scr. R) determined if pIPI03 was integrated into the attB site in the
chromosome.

Fig. 3.
(A) Growth curves of HKU09-01 and HKU09-01::pIPI03 (integrated plasmid) on 37 °C. The mean and standard deviation of three independent growth curves is shown.

(B) Growth curves of HKU09-01 and HKU09-01::pIPI03 (integrated plasmid) at 28 °C. The mean and standard deviation of three independent growth curves is shown.

Fig 4.

(A) Semi quantitative determination of UV sensitivity displayed by HKU09-01 and the isogenic ΔrecA mutant. Exposure to short wave UV light is given in seconds. Growth was assessed after 24 hours of incubation. +++ – normal growth; ++ – weakly impaired growth; + – strongly impaired growth; - – no growth

(B) Growth curves of HKU09-01 and the isogenic ΔrecA mutant at 37 °C. The mean and standard deviation of three independent growth curves is shown.

Fig. 5.

Competitive growth experiment

TSA cultures were inoculated with two strains (labeled with pIPIkan and pIPIery, respectively) and grown through four consecutive subcultures. CFUs were determined at stationary phase at each stage and the ratio of erythromycin : kanamycin resistant cells was calculated. The mean and standard deviation of three independent experiments is shown. The red line represents competitive growth of HKU09-01(WT):Ery and HK(WT):Kan. The number of erythromycin resistant CFUs was set to 1 and the number of kanamycin resistant CFUs was expressed in relation to this value. The blue line represents competitive growth of HK(WT) and the isogenic ΔrecA mutant HK(R). The number of HK(R) CFUs was set to 1 and the number of HK(WT) CFUs was expressed in relation to this value.
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<th>Bacterial strain or plasmid</th>
<th>Description</th>
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<tr>
<td>plM(\text{C}^\text{ery})</td>
<td>Site-specific integration vector for (L.) <em>monocytogenes</em></td>
<td>(Monk et al., 2008a)</td>
</tr>
<tr>
<td>plM(\text{C}^\text{kan})</td>
<td>Site-specific integration vector for (L.) <em>monocytogenes</em></td>
<td>(Monk et al., 2008a)</td>
</tr>
</tbody>
</table>
Table 2 Oligonucleotides used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>5’-3’ Sequence</th>
<th>Restriction site / SLIC homology</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔrecA-A</td>
<td>attttGagC'Tcaacgaataataataagtttacc</td>
<td>SacI</td>
</tr>
<tr>
<td>ΔrecA-B</td>
<td>Cctaaagcattatctagttcactgtccttttctttgtgtaagttc</td>
<td></td>
</tr>
<tr>
<td>ΔrecA-C</td>
<td>ctagtagataatgcttagcttaaaagaaatccagaagtaagag</td>
<td></td>
</tr>
<tr>
<td>ΔrecA-D</td>
<td>ACAAAACqATC'TACTTTTTTAGTTGTG</td>
<td>EcoRI</td>
</tr>
<tr>
<td>ΔrecA-Sc. F</td>
<td>gttttgtatgatatcttataaagaagc</td>
<td></td>
</tr>
<tr>
<td>ΔrecA-Sc. R</td>
<td>atcattaatttctcttttgtaagaagt</td>
<td></td>
</tr>
<tr>
<td>ccrB F</td>
<td>GATAGGCCTAATGACTGGCTTTTATATAAGGAGTGAAACAATGAAAGTAG</td>
<td>pIMAY overlap (behind cat)</td>
</tr>
<tr>
<td>attP R</td>
<td>AAAAAGTACAGTCGGCATTATCTCATAGCATGATATAATT</td>
<td>pIMAY overlap (behind cat)</td>
</tr>
<tr>
<td>pIMAY_SLICcat_F</td>
<td>TTATAAAAGCCAGTGCTATTAGGCCTATCTGAC</td>
<td></td>
</tr>
<tr>
<td>pIMAY_SLICcat_R</td>
<td>TATGAGATAATGCCGACTGTACTTTTTACAG</td>
<td></td>
</tr>
<tr>
<td>attB Sc.-F</td>
<td>CCCTTTATCTGCAATATTATTTTATATGTC</td>
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</tr>
<tr>
<td>attB Sc.-R</td>
<td>GACCAAAAAGCAAAAACGGGTACC</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2.tif
Click here to download high resolution image

A

HKU09-01 chromosome

SLUG08250 attB

attP

repA(ts)

ccrB

cat

MCS Phelp

attP, R

scr. F

scr. R

scr. F

ccrB, F

cat Phelp MCS

repA(ts)

⅓ attP ⅓ attB

⅓ attB ⅓ attP

B

pIPI03 28°C

pIPI03 37°C

1.5 kb

0.4 kb
<table>
<thead>
<tr>
<th>Time (s)</th>
<th>WT</th>
<th>recA^-</th>
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<tbody>
<tr>
<td>0</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>10</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>60</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**B**

37°C

![Graph showing OD578 over time for WT and ΔrecA strains.](image-url)
competitive growth

Strain ratio

Culture number

WT(ery) vs WT(kan)

WT vs ΔrecA