Exploitation of a β-lactamase reporter gene fusion in the carbapenem antibiotic production operon to study adaptive evolution in Erwinia carotovora

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INTRODUCTION

Erwinia carotovora subsp. carotovora (Ecc) strain ATCC 39048 is a Gram-negative phytopathogenic member of the Enterobacteriaceae and a causal agent of potato soft rot. In our earlier development of genetic analysis tools for this strain, Tn10 was introduced by λ::Tn10 mutagenesis into the genetically ‘virgin’ version of ATCC 39048, in the selection of a mutant defective in the endogenous DNA restriction system (McGowan et al., 1996). This restrictionless mutant strain was then subjected to fusaric acid eduction (Bochner et al., 1980) to yield the tetracycline (Tc)-sensitive derivative strain ATTN10, which was still restrictionless (McGowan et al., 1996). ATTN10 has subsequently been studied as a model genetically tractable phytopathogen with which to understand the regulation of bacterial virulence factors involved in plant disease.

ATTN10 is known to express the carABCDEFGH gene operon, which enables production of the β-lactam antibiotic 1-carbapen-2-em-3-carboxylic acid (carbapenem) by expressing the carABCDEFGH operon. Mutants exhibiting increased carbapenem gene transcription were positively selected using an engineered strain with a functional β-lactamase translational fusion in carH, the last gene of the operon. However, spontaneous ampicillin-resistant mutants were isolated even when transcription of carH::blaM was blocked by a strongly polar mutation in carE. The mechanism of resistance was shown to be due to cryptic IS10 elements transposing upstream of carH::blaM, thereby providing new promoters enabling carH::blaM transcription. Southern blots showed that IS10 was present in multicopy in ATTN10. In addition, a Tn10 genetic remnant was discovered. The results offer insights into the genetic archaeology of strain ATTN10 and highlight the powerful impacts of cryptic IS elements in bacterial adaptive evolution.

Abbreviations: Ap, ampicillin; Tc, tetracycline; Car, 1-carbapen-2-em-3-carboxylic acid; Ecc, Erwinia carotovora subsp. carotovora.

Erwinia carotovora subsp. carotovora strain ATTN10 produces the β-lactam antibiotic 1-carbapen-2-em-3-carboxylic acid (carbapenem) by expressing the carABCDEFGH operon. The presence of carFG does not confer cross-resistance to related β-lactam antibiotics such as imipenem (McGowan et al., 1997). CarF and CarG both contain predicted signal peptides that direct antibiotics such as imipenem (McGowan et al., 1997). It has been shown recently that the ATTN10 carABCDEFGH gene cluster contains two promoters (McGowan et al., 2005). The first promoter (P1) is located immediately upstream of carA. Transcription from P1 is increased by the presence of the quorum-sensing signal molecule, N-(3-oxohexanoyl)-l-homoserine lactone (OHHL), produced by the Car enzyme (McGowan et al., 1996). The OHHL signal molecule interacts with CarR with a dissociation constant of 1.8 μM (Welch et al., 2000). It is thought that the CarR:OHHL complex activates transcription of the carABCDEFGH operon, and that this leads to production of Car (McGowan et al., 1997). The second promoter (P2) is located within carD and is not regulated by quorum sensing (McGowan et al., 2005). P2 is a weak promoter that allows low levels of expression of a carG::lacZ transcriptional fusion (McGowan et al., 2005). P2 is presumed to maintain auto-resistance to leaky production of Car by an individual cell or by its neighbours.

The aim of this research was to create and exploit a strain with a β-lactamase translational fusion in the carH gene to enable the positive selection of mutants exhibiting increased transcription of the carABCDEFGH operon. We presumed that these spontaneous mutants would have point mutations.
within the P1 and P2 promoters that would lead to increased expression of the \textit{carABCDEFGH} genes. In addition, we predicted that we might isolate a class of mutants with extra-operonic mutations defining new genes involved in carbapenem operon regulation. However, our results were surprisingly unexpected, and led to the discovery that Tn10 had not been entirely lost from the ATTn10 genome, highlighting the powerful role of mobile genetic elements in the adaptive evolution of bacteria that are challenged by a potentially lethal chemical selection pressure.

\section*{METHODS}

\subsection*{Bacterial strains, plasmids and growth conditions.} All \textit{Ecc} and \textit{Escherichia coli} strains, bacteriophage and plasmids used in this study are listed in Table 1. Strains were routinely grown on Luria–Bertani broth agar (LBA) (Miller, 1972), at 30 \(^\circ\)C for \textit{Ecc} strains and 37 \(^\circ\)C for \textit{E. coli} strains, supplemented with antibiotics, as necessary. Liquid cultures of \textit{Ecc} were grown in sterile conical flasks containing one-tenth volume Luria–Bertani broth (LB) at 30 \(^\circ\)C at 300 r.p.m. in shaking water baths.

\subsection*{DNA manipulations and sequencing.} All molecular biological techniques, unless stated otherwise, were performed by standard procedures (Sambrook \textit{et al.}, 1989). Restriction enzymes and T4 DNA ligase were purchased from NEB and were used according to the manufacturer’s instructions. Oligonucleotide primers were obtained from Sigma Genosys and are listed in Table 2. DNA sequencing was performed by the DNA sequencing facility, University of Cambridge. Nucleotide sequence data were analysed using the GCG package (Genetics Computer Group, University of Wisconsin); sequences were compared to protein and nucleotide databases using the BLAST suite of programs (Altschul \textit{et al.}, 1990) at GenBank (http://www.ncbi.nlm.nih.gov/BLAST/).

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
Strain & Relevant genotype or description & Reference or source \\
\hline
\textit{E. coli} & \textit{araD139 \textit{(ara leu)7697 \DeltalacX74 phoA20 galE galK thi rpsE rpoB araEam recA1 (pir)}} & Herrero \textit{et al.} (1990) \\
CC118/\textit{pir} & & \\
HH26 (pNJ5000) & \textit{E. coli} helper strain used in marker exchange & Grinter (1983) \\
ES & \textit{\beta}-Lactam super-sensitive strain used in Car bioassays & Bainton \textit{et al.} (1992) \\
\textit{Ecc} & & \\
ATCC 39048 & Original ‘virgin’ Car\(^+\) strain & Laboratory stock \\
ATTn10 & Restrictionless Tc \(^+\) derivative of a Tn10-containing ATCC 39048 (made by fusaric acid curing) & McGowan \textit{et al.} (1996) \\
SBE1 & \textit{carH}:\textit{blaM} derivative of ATTn10 & This study \\
SM11 & \textit{Delta}c\textit{arB} derivative of ATTn10; Car\(^-\) & McGowan \textit{et al.} (1997) \\
SBE5 & \textit{carH}:\textit{blaM} derivative of SM11 & This study \\
SBE7 & \textit{Delta}c\textit{arB}, \textit{carE}:\textit{Omega}, \textit{carH}:\textit{blaM} derivative of SBE5 & This study \\
SBE9 & \textit{Delta}c\textit{arB}, \textit{carE}:\textit{Omega}, \textit{carH}:\textit{blaM} derivative of ATCC 39048 & This study \\
SBE7 5.1 & Spontaneous ampicillin-resistant mutant derived from SBE7 & This study \\
SBE7 5.2 & Spontaneous ampicillin-resistant mutant derived from SBE7 & This study \\
SBE7 5.3 & Spontaneous ampicillin-resistant mutant derived from SBE7 & This study \\
SBE7 5.4 & Spontaneous ampicillin-resistant mutant derived from SBE7 & This study \\
SBE7 5.5 & Spontaneous ampicillin-resistant mutant derived from SBE7 & This study \\
SBE7 5.6 & Spontaneous ampicillin-resistant mutant derived from SBE7 & This study \\
SBE7 5.7 & Spontaneous ampicillin-resistant mutant derived from SBE7 & This study \\
SBE7 5.8 & Spontaneous ampicillin-resistant mutant derived from SBE7 & This study \\
SBE7 5.9 & Spontaneous ampicillin-resistant mutant derived from SBE7 & This study \\
SBE7 5.10 & Spontaneous ampicillin-resistant mutant derived from SBE7 & This study \\
\textit{Plasmid} & & \\
pKNG101 & \textit{sacB}, \textit{oriR6K}, Sm\(^R\) & Kaniga \textit{et al.} (1991) \\
pSMG45HBlAMCAA + & pSMG45 plasmid with EZ TnBlAM \textit{carH} translational fusion (see Methods) & This study \\
pSMG45HBlAMTAAT + & pSMG45HBlAMCAA + with stop codon corrected to TAA (see Methods) & This study \\
pSMG45HBlAMTAAT - & SmaI/SnaBI-digested pSMG45HBlAMTAAT + 6-2 kb band self-ligated to remove \textit{oriR6K} & This study \\
pKNG101HBlAMTAAT - & BglII–SpeI 2-4 kb pSMG45HBlAMTAAT– band ligated into \textit{BanHI}–XbaI 6-5 kb pKNG101 band & This study \\
\textit{Bacteriophage} & & \\
\textit{phi}KP & Generalized \textit{Ecc} transducing phage & Toth \textit{et al.} (1993) \\
\hline
\end{tabular}
\caption{Strains, plasmids and phages used in this study.}
\end{table}
Engineering of carH::blaM translational fusion plasmids.

The plasmid pSMG45 was mutagenized in vitro using the EZ TnBlAM kit (Epicentre) as described in the manufacturer's manual. Briefly, 1 μl reaction mixture was used to transform electrocompetent CC118/pir cells, which were plated onto LBA supplemented with 5 μg ampicillin (Ap) ml⁻¹ and incubated overnight at 37°C. Resistant transformant colonies were screened by colony PCR using the primers CarF5' and BlaR5P1. One colony was identified as carrying an insertion in carH. Sequencing of the carH gene using primers CarHF1 and CarHB1 confirmed that the EZ TnBlAM transposon DNA was inserted into the carH gene at bp 7713 as defined by the carA–H gene cluster accession number U17224. This insertion was predicted to yield a functional translational fusion of the carH and blaM genes.

Unfortunately, sequencing revealed that bp 823 of the original EZ TnBlAM transposon DNA was a cytosine residue rather than the documented thymine residue listed in the manufacturer's manual. This single base pair alteration extended the ORF of the blaM gene by an additional 23 codons.

To correct the T-C base pair alteration, a PCR strategy was devised. Briefly, primers CarF5' and INBlAM3' were used to amplify frag. carH::blaM from pSMG45/HlMCAA+ using the Hi Fi PCR kit supplied by Roche. The PCR product was digested with SpeI and KpnI and cloned into the 5.0 kb pSMG45/HlMCAA+ SpeI/KpnI band to generate the plasmid pSMG45/HlMlMTA+. CC118/pir (pSMG45/HlMlMTA+) grown on LBA had a MIC >500 μg Ap ml⁻¹, whilst CC118/pir (pSMG45/HlMlMCAA+) had an MIC of <50 μg Ap ml⁻¹, confirming that the altered stop codon was dramatically influencing CarH/BlAM β-lactamase activity in E. coli (data not shown).

Marker exchange. Engineering of carE::Ω and carH::blaM was carried out using the pKNG101-derived plasmids pSMG112 and pKNG101/HlMlMlMTA+, respectively, as described previously (Kaniga et al., 1991). To avoid selecting for unwanted mutations during the marker exchange process that could lead to enhanced β-lactamase activity from the carH::blaM mutation, Ap was not used to select for colonies containing the carH::blaM marker. Instead, sucrose-resistant, streptomycin (Sm)-sensitive colonies were screened by patching onto LBA and LBA plus 5 μg Ap ml⁻¹. Any colonies that were able to grow on the LBA plus 5 μg Ap ml⁻¹ plates were then picked from the LBA-only plate, and the presence of the carH::blaM marker was confirmed by PCR analysis.

Carbapenem plate assays. Carbapenem production assays on overnight cultures of Ecc samples were carried out as described previously (McGowan et al., 1996), using the β-lactam sensor strain ESS. The only alteration was that, in some experiments, freshly prepared stock solutions of clavulanate were added to the LBA overlay to the desired final concentration to inhibit carH::blaM β-lactamase activity (Reading & Cole, 1977).

Nitrocefin-based β-lactamase assays. Expression of carH::blaM throughout growth was assayed using the chromogenic substrate nitrocefin (GlaxoSmithKline). Aliquots (1 ml) were extracted from liquid cultures of Ecc and resuspended in phosphate buffer, pH 7.0. The samples were then sonicated on ice for a total of 3 min and 45 s, composed of eight 15 s periods of sonication with 15 s gaps between them. Cell debris was pelleted using a benchtop centrifuge at 13,000 r.p.m. for 5 min. To 800 μl of the sample incubated at 30°C, 20 μl of 4 mg nitrocefin ml⁻¹ stock solution was added, and the change in OD560 was recorded in a spectrophotometer over time and recorded relative to the OD560 of the culture at each time-point.

Southern blotting using DIG-labelled probes. Southern blotting experiments were carried out using standard procedures (Sambrook et al., 1989). DIG-labelled probes hybridizing to IS10 and jensC were created by PCR using the DIG-labelled dNTP mix supplied by Roche. The IS10 probe was PCR-amplified using primers IS10'5 and IS103'. DIG-labelled probes were detected using Fab fragments supplied by Roche. Peroxidase activity was detected using the CDP-star kit supplied by Roche.

RESULTS AND DISCUSSION

CarH::BlAM is functionally expressed in Ecc throughout growth

A carH::blaM translational fusion was constructed in the ATTn10 carA–H operon by marker exchange to generate

![Diagram](http://mic.sgmjournals.org)
CarH::BlaM expression was detected throughout growth by measuring β-lactamase activity (Fig. 3). The level of β-lactamase activity in SBE1 increased dramatically during mid-exponential growth, as predicted, due to carA–H transcription via quorum sensing. The level of β-lactamase activity from SBE1 began to decline again once the cells were in stationary phase, suggesting that the CarH::BlaM hybrid was turned over in vivo, presumably because of proteolysis. Previous studies have shown that β-galactosidase also declines during stationary phase (McGowan et al. 2005). It seems likely that both CarH::BlaM and β-galactosidase are inactivated by proteases that are induced, or are most active, during the stationary phase of growth.

A third strain (SBE7, Fig. 1c) was constructed, in which transcription of carH::blaM was disrupted. SBE7 carH::blaM transcription from both of the known carA–H promoters was blocked by the presence of the strongly polar omega interposon sequence (Prentki & Krisch, 1984) in carE (Figs 1c and 3). Consistent with our previous data on transcription of the operon (McGowan et al. 2005), transcription of carH::blaM was switched off in strain SBE7.

The carH::blaM fusion strain can give rise to ampicillin-resistant mutants, despite the interposon insertion

The survival frequency of ATTn10 and SBE1 in response to increasing concentrations of Ap is recorded in Fig. 4. As a negative control we also included Ecc strain SBE7, which cannot transcribe the carH::blaM gene because of the carE::Ω mutation, as described above. Surprisingly, SBE7 had an e.o.p. on LBA supplemented with 5 μg Ap ml⁻¹ of 1 x 10⁻⁵, which was significant, because ATTn10 could not grow on this medium. Ecc growth on LBA supplemented with 25 μg Ap ml⁻¹ was undetectable after 24 h at 30 °C, with the exception of SBE1, which had an e.o.p. of 1 x 10⁻⁶ relative to that in the absence of Ap.

The e.o.p. values of SBE1 and SBE7 when grown on LBA supplemented with 5 μg Ap ml⁻¹ were 1 x 10⁻⁴ and 1 x 10⁻⁵, respectively. Because transcription of carH::blaM in SBE7 cannot occur from either of the carA–H promoters because of the polar carE::Ω mutation, SBE7
ApR colonies could only arise if the carH::blaM fusion was transcribed from a new promoter.

**Expression of CarHoBlaM can occur spontaneously in SBE7 because of the action of mobile DNA providing functional promoters**

To determine the nature of the SBE7 ApR mutants, 10 of the colonies (SBE7 5.1–5.10) that grew on 5 μg Ap ml⁻¹ were selected at random for further investigation. The most likely explanation for ApR in SBE7 5.1–5.10 was that a genetic alteration (presumably insertions or deletions) had occurred immediately upstream of carH::blaM. Therefore, the region between carH::blaM and carE::Ω in these mutants was amplified by PCR analysis using primers Sp2 and BlaRP1 (Fig. 5a). It was clear that, in the ApR SBE7 mutants, the size of the 1871 bp region (Fig. 5a, lane F) between carH::blaM and carE::Ω had increased. In most cases, the increase in size was approximately 1·3 kb. In the case of SBE7 5.5, the increase in size was approximately 1·8 kb (Fig. 5a, lane E). Sequencing of four of the PCR products from SBE7 5.1 to 5.4 revealed that IS10R from the transposon Tn10 had inserted in the region between carH::blaM and carE::Ω (Fig. 5b). The site of the IS10 insertion in strains SBE7 5.1, 5.3 and 5.4 was identical in each case, suggesting that they could be siblings. In all cases, the IS10 R Pout promoter was orientated in the correct direction to enable transcription of the downstream carH::blaM gene (Fig. 5c). Previous data has shown that both IS10 elements possess a fairly strong Pout promoter (Simons et al., 1983) that is capable of allowing expression of adjacent ORFs (Ciampi et al., 1982). It seems that, due to these random insertions, the Pout promoter from IS10 has enabled SBE7 5.1–5.10 to drive transcription of the otherwise cryptic carH::blaM fusion.

Our results suggest that ATTN10 provides a useful model to study the adaptive evolution processes seen in natural isolates that possess IS10R. As an example, earlier research by other groups has shown that IS elements can induce antibiotic resistance in E. coli (Jellen-Ritter & Kern, 2001; Kobayashi et al., 2001) and Salmonella enterica (Olliver et al., 2005). In all three of these studies, IS elements were shown to provide mechanisms of broad-spectrum antibiotic resistance by providing promoters that are capable of expressing the multidrug resistance pump AcrEF. Such experiments show that IS elements can play a crucial role in the evolution of antibiotic resistance in clinical isolates.

**Transposon Tn10 has been extensively degraded in the ATTN10 genome**

Mutant SBE7 5.5 was particularly interesting, because the PCR product from this mutant was enlarged by approximately 1·8 kb instead of 1·3 kb (Fig. 5a, lane E). Sequencing of the enlarged PCR product showed that this mutant contained genetic remnants of Tn10 orientated such that the IS10L end was closest to carH::blaM. In SBE7 5.5, transcription of carH::blaM appears to be from the Pout promoter of IS10L, as this promoter is in the correct orientation. The Tn10 sequence in this IS element was severely degraded, however, by the presence of two large deletions in its sequence (Fig. 6). Both deletions are defined by the Tn10 sequence accession number AF162223 (Chalmers et al., 2000), and are as follows: deletion 1 removes bp 340–4426, which includes most of IS10L and ends in the 5’ end of jemC, removing jemAB in the process; deletion 2 removes bp 4698–7818. Deletion 2 removes the 3’ end of the jemC gene and the entire tetRACD gene cluster, and ends precisely at the point at which the IS10R inverted repeat sequence begins. This 1939 bp Tn10 genetic remnant will from now on be referred to as Tn10Δ.

The process leading to the generation of deletion 1 is not clear, but may involve strand slippage mechanisms (Farabaugh et al., 1978; Albertini et al., 1982; Singer & Westlye, 1988; Marvo et al., 1983; Uematsu et al., 1999) during DNA replication or repair, because it is flanked at the...
termini by an imperfect 7 bp repeat sequence G(C/A)T(G/C)TAT. Alternatively, processes other than strand slippage (Balbinder, 2001) may be responsible for deletion 1.

Deletion 2 terminates with a 5 bp imperfect direct repeat, ATGC(T/A), and therefore may have formed by a strand misalignment mechanism (Farabaugh et al., 1978; Albertini et al., 1982; Singer & Westlye, 1988; Marvo et al., 1983; Uematsu et al., 1999). However, the direct transition of the IS10R inverted repeat sequence that defines the boundaries of the mobile genetic element to the site of a deletion is characteristic of an IS10 adjacent deletion, as described elsewhere (Roberts et al., 1991; Chalmers & Kleckner, 1996).

It seems very likely that deletion 2 has been generated by an IS10-mediated adjacent deletion. Two lines of evidence support this hypothesis: i) deletion 2 is immediately flanked at the 3’ end by the IS10 inside end, and ii) deletion 2 is immediately flanked at the 5’ end by a 5’-GGTTATGCT-3’ sequence which closely matches the 5’-NGCTNAGCN-3’ IS10 transposition consensus sequence (Halling & Kleckner, 1982).

The TeC phenotype of the ATTn10 strain can be explained by the presence of deletion 2. This is because deletion 2 has led to the complete loss of the tetRACD gene cluster from Tn10.

Furthermore, a Southern blot to probe for jemAB in ATTn10 genomic DNA cut with BsiEI, which cuts either end of Tn10, generated only one hybridizing band that corresponded to the size expected for Tn10, confirming that the tetRACD and jemAB genes are absent in the ATTn10 genome (data not shown). Deletion 2 would offer a selective advantage when Tn10-containing strains were exposed to fusaric acid because it removes the tetA gene that encodes the TetA efflux pump which confers fusaric acid sensitivity. An example of how fusaric acid treatment can lead to the generation of hybrid IS elements in strains containing Tn10 has been documented in E. coli (Bogosian et al., 1993). The IS10R fusion in the strain under study generated a 1329 bp element.
ATn10 contains multiple copies of IS10

Genomic DNA from SBE7, SBE9 and SBE7 5.1–5.10 was analysed by Southern blot experiments using DIG-labelled probes to the transposase gene of IS10 (Fig. 7). No hybridizing bands were detected in SBE9 DNA. SBE9 is not derived from ATn10, and therefore has never been exposed to Tn10. However, it was clear from the SBE7 sample, which does derive from ATn10, that there were at least 15 independent hybridizing bands. A band approximately 6·8 kb in size, corresponding to an IS10 insertion in carFG, was present in all the ApR SBE7 mutants except SBE7 5.5 (Fig. 7), supporting the PCR and DNA sequencing data (Fig. 5). A band at the predicted 7·4 kb was present in the SBE7 5.5 genomic DNA sample, but this co-migrated with at least one other band that was present in SBE7 and all the derived mutant strains. The 7·4 kb band was more distinguishable in SBE7 5.5 than in the other strains when probing for the presence of jemC (data not shown).

There are other examples in the literature of strains that contain multiple copies of IS10: a strain of Enterobacter cloacae has been found to contain at least 15 copies of IS10 (Matsutani, 1991), and a strain of Salmonella enterica has also been shown to possess at least 15 chromosomal copies (Olliver et al., 2005). IS10 copy number would normally be kept low by several mechanisms. Firstly the transcription and translation levels of the IS10R promoter are fairly weak (Simons et al., 1983). Secondly, transcription of the transposase gene is limited to a small fraction of the cell cycle because of methylation of the two dam sites located in IS10R (Roberts et al., 1985). Thirdly, IS10R has been shown to possess mechanisms to limit its copy number in the cell by a process known as ‘multicopy inhibition’ (Simons & Kleckner, 1983). This effect is believed to be due to RNA production by transcription from the Pout promoter inhibiting translation of the transposase gene by hybridizing to the first 36 bp of the mRNA message and occluding ribosome binding. This effect has been shown to function in trans, so that as the number of IS10 copies in the cell increases, multicopy inhibition becomes more effective at preventing IS10 transposition. It may be that multicopy inhibition is less effective in some genetic backgrounds, perhaps because the Pout RNA transcript is turned over rapidly in some strains.

It is possible to differentiate SBE7 5.1–5.10 from each other by the number and size of their IS10R hybridizing bands, which in many cases were different from each other. As an example, SBE7 5.10 contains an easily distinguishable hybridizing band at around 2·2 kb that is absent in SBE7 and the rest of the ApR SBE7 mutants. Whether these IS10 hybridizing bands were generated before or after selection on Ap is not known. It has been shown that growth in the presence of β-lactams can induce the SOS response (Miller et al., 2004). The SOS response in turn has been shown to...
induce IS10 transposition (Eichenbaum & Livneh, 1998). Therefore, any colonies that are initially able to grow in the presence of Ap may undergo increased rates of IS10R transposition that may explain the high level of heterogeneity among SBE7 5.1–5.10.

One observation from Fig. 7 is that none of the IS10 hybridizing bands present in the SBE7 parental strain is absent in SBE7 5.1–5.10. As IS10R has been shown previously to transpose by a non-replicative mechanism (Bender & Kleckner, 1986; Kleckner et al., 1995), it seems clear that during transposition, IS10R is maintained in our strains at the donor molecule site. Mechanisms for how this can occur have been described elsewhere (Bender et al., 1991), and our results agree with a model in which intramolecular rejoining to lose IS10R from the donor molecule site is an uncommon event.

Final summary

This research has generated a functionally expressed β-lactamase translational fusion in carH, and shown that in the absence of a functional promoter, this construct can serve as an effective mobile promoter trap. This has led to the identification of cryptic IS10 elements in the ATTn10 chromosome. This finding was initially surprising, as it was assumed that IS10 might have been lost from the chromosome during fusaric acid treatment to cure Tn10-dependent TcR in the progenitor strain. This promoter trap offered insights into the molecular events that may have led to ATTn10 Tc3R with the discovery of the Tn10 genetic remnant Tn10A in the SBE7 ApR mutant SBE7 5.5. Southern blot experiments subsequently demonstrated that ATTn10 contains at least 15 copies of IS10. These data show that ATTn10 may be a useful model strain in which to study adaptive evolution, and our recent observations that ATTn10 may be hypermutable are consistent with this notion (S. D. Bowden and G. P. C. Salmon, unpublished data).

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REFERENCES


Adaptive evolution in Erwinia carotovora


