Molecular cloning and genetic characterization of the rfb region from *Yersinia pseudotuberculosis* serogroup IIA, which determines the formation of the 3,6-dideoxyhexose abequose

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(Received 20 May 1991; revised 1 August 1991; accepted 28 August 1991)

The *rfb* region of *Yersinia pseudotuberculosis* serogroup IIA has been cloned and expression of O antigen in *Escherichia coli* K12 was demonstrated. Transposon mutagenesis analysis confined the DNA region required for O antigen expression to a 19.3 kb fragment, and the O antigen expressed was visualized by SDS-PAGE and silver staining. Southern hybridization analysis demonstrated significant levels of similarity between the *Yersinia rfb* region and the 3,6-dideoxyhexose pathway genes *rfbF* and *rfbG*, previously isolated from *Salmonella enterica* LT2, but no similarity to the abequose synthase gene *rfbJ* of the same strain or the paratose synthase gene *rfbS* isolated from *S. enterica* Ty2. The evolutionary relationship between the abequose biosynthetic genes of the two species of *Salmonella* and *Yersinia* is discussed.

Introduction

Lipopolysaccharides (LPS) are important antigens of the cell surface in Gram-negative bacteria, responsible for the serological O specificity of many clinically relevant genera such as *Salmonella, Escherichia, Shigella* and *Yersinia*. The immunogenic properties of a given O antigen are entirely due to the sugar composition and structure of the O-specific polysaccharide moiety, which forms, together with an oligosaccharide core and lipid A, the complete LPS molecule embedded in the outer membrane (Lüderitz *et al.*, 1971). Of a considerable number of monosaccharides identified as components of O-specific polysaccharides, derivatives of the 3,6-dideoxyhexose group have attracted particular attention for their highly immunogenic character. Within Gram-negative bacteria, only a limited number of species, all in the family *Enterobacteriaceae*, is able to form these unusual sugars. Colitose can be found in the LPS of some *Escherichia coli* serotypes, while O antigens of some *Citrobacter* strains contain abequose. In these cases, the repertoire seems to be limited to the synthesis of one particular derivative only in each genus. On the other hand, within *Salmonella* and *Yersinia*, a whole variety of 3,6-dideoxyhexoses can be found throughout a spectrum of serotypes. Of the five dideoxyhexoses known to occur naturally, four have been found in various strains of *Salmonella enterica* (abequose, tyvelose, paratose and colitose), while among serotypes of *Yersinia pseudotuberculosis*, all five including ascorylase are present (Lüderitz *et al.*, 1971). Considering the evolutionary distance between *Salmonella* and *Yersinia* (Brenner, 1978), the simultaneous occurrence of this otherwise unknown polymorphism is somewhat surprising. The genetic principles underlying LPS formation have been most extensively studied in *S. enterica* LT2 (serovar *typhimurium*, group B) as a model system (Mäkelä & Stocker, 1984).

In *Salmonella*, the *rfb* cluster includes all the genes necessary for the synthesis of the O unit sugar components and their subsequent assembly. The *rfb* region of *S. enterica* LT2 has been cloned, sequenced and analysed (Brahmbatt *et al.*, 1988; Jiang *et al.*, 1991; Verma *et al.*, 1988; Wyk & Reeves, 1989; Liu *et al.*, 1991): most of the genes necessary for formation of the O unit backbone including three genes from the abequose biosynthetic pathway (*rfbF* and *rfbG* coding for the two enzymes initiating abequose formation, and *rfbJ* coding for abequose synthase) have been allocated to specific open reading frames, while other genes such as *rfbH* and *rfbI* of the abequose pathway have only been located approximately (Fig. 1). Sequence analysis has revealed an unusually low G+C content for the entire LT2 *rfb* region, suggesting a relatively recent transfer of the gene cluster to *S. enterica* from a non-enterobacterial donor with low G+C content; the data indicate that the *rfb*
gene cluster itself may have been formed by combination of DNA segments derived from various sources, before being transferred to S. enterica (Jiang et al., 1991). Other S. enterica serovars such as typhi (group D) and paratyphi A (group A) possess an O antigen backbone structure similar to that of strain LT2 (containing mannos, rhamnose and galactose), but feature different immunodominant 3,6-dideoxyhexose side chains, such as tyvelose and paratose, respectively (Liideritz et al., 1987; Liu et al., 1991). The boundary between the groups of genes involved in the four sugar pathways is indicated above, and the location of the DNA fragments used for hybridization (pPR1127 and rfbJ) are indicated by brackets below. Below: the rfb gene cluster of strain Ty2 (group D). Regions of near identity are shown as a simple line. Stippled areas indicate low homology (Verma et al., 1988, 1989). The rfbE gene is not present in group B (dark area = no homology). The strain IMVS1316 (group A) rfb region (allowing synthesis of the 3,6-dideoxyhexose paratose) differs from the group D rfb region (determining tyvelose synthesis) essentially by a single point mutation in the rfbE gene, resulting in the loss of the CDP-tyvelose-2-epimerase function (Verma et al., 1989). The position of the DNA region used for hybridization experiments (rfbS) is indicated by the bracket below.

![Fig. 1. Polymorphism of the rfb gene cluster in S. enterica serovar typhi and paratyphi A (serogroups B, D, and A, respectively). Above: The rfb gene cluster of S. enterica SL1654 (serogroup B). Open reading frames which have been identified are given their rfb designation (A, B, etc.). The approximate positions of rfbC, rfbD, rfbH and rfbI are also given (Jiang et al., 1991). The region involved in the synthesis of the 3,6-dideoxyhexose side chains, such as tyvelose or paratose, respectively (Liideritz et al., 1987; Liu et al., 1991). The boundary between the groups of genes involved in the four sugar pathways is indicated above, and the location of the DNA fragments used for hybridization (pPR1127 and rfbJ) are indicated by brackets below. Below: the rfb gene cluster of strain Ty2 (group D). Regions of near identity are shown as a simple line. Stippled areas indicate low homology (Verma et al., 1988, 1989). The rfbE gene is not present in group B (dark area = no homology). The strain IMVS1316 (group A) rfb region (allowing synthesis of the 3,6-dideoxyhexose paratose) differs from the group D rfb region (determining tyvelose synthesis) essentially by a single point mutation in the rfbE gene, resulting in the loss of the CDP-tyvelose-2-epimerase function (Verma et al., 1989). The position of the DNA region used for hybridization experiments (rfbS) is indicated by the bracket below.

Methods

Bacterial strains, cloning vectors and transposons. E. coli K12, S. enterica and Y. pseudotuberculosis strains used are listed in Table 1; all strains were cultivated in LB broth as described by Maniatis et al. (1982). The low copy number cosmids pPR691 (Jiang et al., 1987) was used as a cloning vector for the construction of the Y. pseudotuberculosis M85 gene bank, to avoid possible lethal effects of a high copy number of the rfb gene cluster; kanamycin (50 μg ml⁻¹) was used in this case for plasmid selection. The plasmid pUC18 was selected for with ampicillin (50 μg ml⁻¹). E. coli strain S17-1 served as a donor for the transposon Tn5-B50 (Samuelsson et al., 1974).

Enzymes, chemicals and antisera. Restriction enzymes, DNA polymersase I, RNAase A, proteinase K, pronase, bacterial alkaline phosphatase and T4 DNA ligase were purchased from Boehringer; Taq polymerase was purchased from Pharmacia; DNAase I and chemicals were obtained from Sigma and Ajax Chemicals. S. enterica O4-specific antisera was purchased from Wellcome Diagnostics. The 'Packgene' kit from Promega was used for in vitro packaging of recombinant DNA. Nitrocellulose for colony blotting was obtained from Schleicher and Schuell.

DNA techniques. DNA extraction, general DNA manipulation methods and transformation were carried out as described by Maniatis et al. (1982). Southern hybridization was carried out overnight at 37 °C. For low stringency conditions, the hybridization solution contained 30% (v/v) formamide, and washing was carried out at room temperature in 2 x SSC, 0-1% SDS; for high stringency conditions, 50%
TTBS to remove unbound antibodies. Goat anti-rabbit IgG-horse-
vivo TTBS (TBS as outlined by Leinonen (1985). For screening of large numbers of
bound to the filter were then lysed
were blocked by incubation with
(diluted 1:1000 in TTBS) for 1 h was followed by three washes with
TTBS to remove unbound antibodies. Goat anti-rabbit IgG–horse-
radish peroxidase conjugate was diluted 1:5000 in TTBS, added to the
filters, and left to incubate for 1 h. Subsequent extensive washing (four
times with TTBs, twice with TBS) was followed by addition of staining
substrate (4-chloro-1-naphthol and hydrogen peroxide) in TBS.

**Table 1. Bacterial strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Our stock no.</th>
<th>Characteristics</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> K12</td>
<td>P3851</td>
<td>lacY1 glnV44 galK2 galT22 metB1 ΔthyA57</td>
<td>Jacobs et al. (1986)</td>
</tr>
<tr>
<td>y2819</td>
<td></td>
<td>gudR2 recA55</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Yersinia pseudotuberculosis</em></td>
<td></td>
<td>Strain LT2, ΔhisD-rgb-388</td>
<td>Nikaido et al. (1967)</td>
</tr>
<tr>
<td>M85</td>
<td></td>
<td>Wild-type, serogroup IIA</td>
<td>Germanier &amp; Furer (1975)</td>
</tr>
<tr>
<td><em>Ty21a</em></td>
<td>M18</td>
<td>Serovar typhi, galE mutant of Ty2</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
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</table>

(v/v) formamide was used, and a further wash of 1 h at 56 °C in
1 × SSC, 0·1% SDS was added (Howley et al., 1979). A genomic DNA
library from strain M85 was constructed as described by Bates & Swift
(1983), using the low copy number vector pPR91 (Jiang et al., 1987).
The recombinant DNA was packaged in vitro, and E. coli y2819 was
transfected with the lysate.

The *S. enterica* LT2 gene rjbJ and the *S. enterica* Ty2 gene rjbS were
amplified in vitro using PCR as described by Saiki et al. (1988).
Transposon mutagenesis was carried out using a modification of the
protocol outlined by Simon et al. (1989): overnight cultures of the donor
strain S17-1 (carrying the transposon Tn5-B50 on a mobilizable
plasmid) and recipient strain P4309 (carrying the M85 rjb region on
pPR981) were mixed and incubated for 3 h at 30 °C to allow
conjugation. Cells were spread onto plates containing kanamycin and
tetracycline, thereby selecting for the presence of both the cosmid
and the transposon. Isolated colonies were grown to an OD530 of 0·3, and in
vitro packaging of the cosmid DNA was induced by heat shock (Jacobs
et al., 1986). Bacterial cells were then lysed by treatment with
chloroform, and cellular debris was removed by centrifugation. After
filter sterilization, the phage lysate obtained was used to infect E. coli
y2819, which was then plated onto media containing kanamycin and
tetracycline. Only in cases where the transposon had inserted into
the cosmid was a simultaneous transfer of both resistance markers to
the host strain possible, thereby allowing specific selection for the desired
recombinant clones.

**Immuchemical procedures.** Slide agglutination tests were carried out
as outlined by Leinonen (1985). For screening of large numbers of colonies,
a modification of the protocol described by Hawkes et al. (1982) was used.
Agar plates with colonies to be screened were overlaid with
nitrocellulose discs, and incubated at 37 °C for 1 h. The cells
bound to the filter were then lysed in situ by placing the discs for 30 min
onto Whatman 3MM paper wetted with 0·5 M-HCl. Cell debris was
removed by washing with saline, and non-specific protein binding sites
were blocked by incubation with 5% (w/v) skimmed milk powder in
TTBS (TBS + 0·05% Tween 20, TBS = 20 mM-Tris/HCl, 200 mM-
NaCl, pH 7·4). Incubation of the filters with O4-specific antibody
(diluted 1:1000 in TTBS) for 1 h was followed by three washes with
TTBS to remove unbound antibodies. Goat anti-rabbit IgG–horse-
radish peroxidase conjugate was diluted 1:5000 in TTBS, added to the
filters, and left to incubate for 1 h. Subsequent extensive washing (four
times with TTBs, twice with TBS) was followed by addition of staining
substrate (4-chloro-1-naphthol and hydrogen peroxide) in TBS.

**Extraction of LPS, SDS-PAGE and silver staining.** Cells from a 10 ml
overnight culture were washed with saline, then resuspended in 2 ml
acetone before drying in a vacuum oven at 50 °C. Crude LPS was
extracted with 90% (v/v) phenol as outlined by Johnson & Perry (1975),
then further purified by treatment with DNAase I and RNAase A
(0·1 mg ml−1 final concentration of each) for 4 h at 37 °C, followed by
an overnight incubation with pronase (0·1 mg ml−1). After overnight
dialysis of the sample against water, its volume was reduced to about
50 µl in a vacuum oven at 50 °C. To this 50 µl 2× loading buffer
(Lugtenberg et al., 1975) was added; 1–5 µl of the sample was boiled for
5 min in a total volume of 20 µl 1× loading buffer, then 1 µl proteinase
K (25 µg ml−1) was added. SDS-PAGE was carried out as described by
Lugtenberg et al. (1975), using an 8% stacking gel and an 18% running
gel containing 0·2% SDS. For silver staining of LPS, the protocol
developed by Hitchcock & Brown (1983) was used.

**Results**

**Cloning of the M85 rjb region**

A gene library of 800 colonies containing chromosomal
DNA of *Y. pseudotuberculosis* M85 (serogroup IIA) was
established by transfection of *E. coli* y2819. As abequose
is the common immunodominant O antigen sugar in *S.
enterica* LT2 and *Y. pseudotuberculosis* M85, abequose-
specific O4 antiserum raised against strain LT2 was a
suitable agent to screen the gene library obtained. One
clone, P4309, containing the recombinant cosmid
pPR981, was detected by colony blotting and used for
further analysis. The cosmid pPR981 was transferred
into *E. coli* Sm874, which carries a chromosomal deletion covering the whole *rfb* region. Agglutination of the transformants with O4 antiserum suggested the presence of the complete M85 *rfb* region on the clone, as no complementation by the host *rfb* functions was needed for O antigen expression.

**Restriction enzyme mapping**

After making a preliminary restriction map, three plasmids, pPR1212, pPR1213 and pPR1214, together covering the full length of the cosmid pPR981, were isolated by digestion of pPR981 with SalI, ligation into pUC18 and transformation into *E. coli* JM109. The clones were subsequently analysed in detail using suitable restriction enzymes to give the complete map presented in Fig. 3.

**Localization of the rfb region by transposon mutagenesis**

Tn5-B50, a derivative of the transposon Tn5, was used for insertion mutagenesis of the cosmid clone pPR981, as described in Methods. All colonies showing both transposon-borne tetracycline resistance and cosmid-borne kanamycin resistance were isolated. The isolates were tested for production of O antigen by colony blotting and slide agglutination, and the location of each transposon insertion was mapped using *HindIII* and *XhoI*. Most insertions located between map positions 18.1 and 37.4 inhibited expression of the O antigen, while insertions outside this area generally had no effect on O antigen production (Fig. 3). Thus, the position of the M85 *rfb* gene cluster could be narrowed down to a DNA region 19.3 kb in length, from map position 18.1 to 37.4 in pPR981. Expression of O antigen in occasional clones carrying transposons within this 19.3 kb region is thought to be due to its insertion into a non-essential region or perhaps initiation of transcription by the npt promoter present on the transposon Tn5-B50.

One clone carrying a transposon insertion well outside the region for O antigen expression, but nevertheless inhibiting O antigen production, was isolated. The question whether the actual transposon insertion or a second, as yet unidentified mutational event within the *rfb* region caused this effect, was not investigated.

**Construction of a functional subclone**

Results from the transposon mutagenesis experiment indicated that the location of the *rfb* region was within the terminal half of the cosmid, between map positions 18.1 and 37.4 (Fig. 3). A plasmid, pPR1197, was constructed by digesting the original cosmid pPR981 with BamHI and religating the fragment carrying the vector, thereby removing most of the areas outside of the region thought to contain the *rfb* locus. This construct was shown to determine the formation of O4-cross-reacting antigen in both the original cloning host *E. coli* strain, JM109, and the *rfb*-deleted strain, *E. coli* Sm874.

**SDS-PAGE of O antigen**

The O antigens produced by the *E. coli* strains P4309, P4494 and P4554 were visualized by silver staining of whole LPS phenol extracts after SDS-PAGE (Fig. 4). Both plasmids pPR981 and pPR1197 allowed formation of long chain LPS structures in *E. coli* K12 transformants with the K12 *rfb* region present (y2819 or JM109). LPS chains determined by pPR1197 (lane 4) showed a
Yersinia pseudotuberculosis serogroup IIA rfb region

Fig. 4. SDS-PAGE of O antigen produced by strains containing the rfb region cloned from Y. pseudotuberculosis M85. Lanes: 1, M85; 2, Sφ874; 3, P4309 (α2819 containing pPR981); 4, P4554 (JM109 containing pPR1197); 5, Sφ874 containing pPR981; 6, Sφ874 containing pPR1197. O antigen was visualized by silver staining.

different length distribution to chains determined by pPR981 (lane 3).

In the rfb-deleted strain Sφ874, only pPR981 determined long chain LPS similar in appearance to the structures formed in other E. coli host strains (lane 5). The subclone pPR1197 in this strain only allowed the formation of short LPS chains with up to about five O antigen units, slightly larger in size than the LPS core (lane 6).

Southern hybridization

Southern hybridization experiments were carried out using several 3,6-dideoxyhexose pathway genes from S. enterica to probe Y. pseudotuberculosis M85 chromosomal DNA and the cosmid pPR981 (positions of the probes within the respective S. enterica rfb clusters are indicated in Fig. 1). The plasmid probe pPR1127 (containing the S. enterica LT2 genes rfbF and rfbG) hybridized to pPR981 under both low and high stringency conditions. The hybridizing fragments of M85 chromosomal DNA corresponded in size to those obtained with pPR981, confirming that no rearrangements had occurred during cloning (Fig. 5). However, the 8.4-kb cosmid DNA fragment hybridizing to pPR1127 also contained a large segment of the cloning vector, and was therefore represented by a fragment of different size (4.5 kb) in the M85 chromosomal DNA. Stronger signals obtained with pPR981 cosmid DNA as compared to M85 chromosomal DNA are the consequence of a much higher copy number of the probed DNA fragment in the former case. The signals obtained with M85 chromosomal DNA were much weaker in comparison to those obtained with S. enterica chromosomal DNA controls, which indicates significant DNA sequence dissimilarities between the radioactive probes and the hybridizing M85 chromosomal fragments.

PCR-amplified DNA fragments containing the abequose synthase gene rfbJ from S. enterica LT2 or the paratose synthase gene rfbS from S. enterica Ty2 failed to hybridize to either pPR981, or M85 chromosomal DNA, even under low stringency conditions (data not shown).

Fig. 5. Homology between rfb regions of Salmonella and Yersinia. Probing of Y. pseudotuberculosis M85 chromosomal DNA with pPR1127, which contains the 3,6-dideoxyhexose biosynthesis pathway genes rfbF and rfbG of S. enterica SL1654 (group B). All DNAs were digested with EcoRI; probing was carried out under high stringency conditions. Lanes: 1–3 chromosomal DNA: 1, S. enterica P9029; 2, S. enterica SL1654; 3, Y. pseudotuberculosis M85. Lane 4, pPR981 cosmid DNA cloned from Y. pseudotuberculosis M85. Sizes (in kb) of the fragments hybridizing are indicated; the hybridizing fragment slightly larger than 2.3 kb in the lane containing pPR981 is due to incomplete digestion of the cosmid DNA.

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Discussion

*Y. pseudotuberculosis* and *S. enterica* are the only species in nature known to contain a variety of 3,6-dideoxyhexose derivatives in their O antigen. The cloning of the *Y. pseudotuberculosis* serogroup IIA rfb region, which includes the abequose biosynthesis genes, is a first step in a closer investigation of this unusual polymorphism in *Yersinia*.

Cloning and subcloning of the rfb region

We have cloned a 39 kb DNA fragment from *Y. pseudotuberculosis* serogroup IIA which determines the synthesis of an O antigen cross-reacting with *S. enterica* O4 antiseraum. As O antigen synthesis was also conferred upon an rfb-deleted *E. coli* strain, the cosmid pPR981 must contain all the genes required for the synthesis and assembly of this structural component. The region responsible for O antigen expression covered 19.3 kb, as shown by transposon mutagenesis, and this region was subsequently subcloned to give the plasmid pPR1197. This plasmid also conferred an O4-cross-reacting epitope upon the rfb-deleted *E. coli* strain, showing that it still contained all the required genes.

The O antigen of original clone and subclone

*E. coli* K12 transformants containing the original cosmid pPR981 or the subclone pPR1197 are believed to form a typical *Y. pseudotuberculosis* M85 O antigen unit: spacing of the O antigen ladder banding pattern after SDS-PAGE of membrane preparations was found to be similar to the wild-type pattern, suggesting structural identity of the O units formed. The LPS patterns produced by strains containing plasmids pPR981 and pPR1197, however, differed significantly in the distribution of the O antigen chain length: pPR981 allowed synthesis of long-chain O antigen in all *E. coli* hosts tested irrespective of their genotype, while in the case of pPR1197, the formation of long-chain O antigen was dependent on the presence of the host rfb region. Recently, during the analysis of rfb regions from *S. enterica* serogroup C2 and *E. coli* serogroup O111, similar observations led us to postulate an additional factor located within or near the rfb region of *E. coli* K12, which is probably responsible for the determination of the chain length of a given LPS (Bastin *et al.*, 1991; Brown *et al.*, 1991). DNA regions encoding such a factor were apparently present on pPR981, allowing the synthesis of O antigen with a similar LPS chain length distribution in all *E. coli* host strains tested. Subcloning probably removed this region in pPR1197, with complementation of the missing determinant by the *E. coli* host factor giving the long-chain LPS phenotype in strain P4554. The predominance of shorter LPS chains in this strain may be a consequence of a different chain length specificity of the complementing host determinant. The rfb-deleted *E. coli* host strain Sph874 on the other hand was unable to supply the missing chain length determinant, and therefore produced only short-chain O antigen when carrying pPR1197. The presence of polymerized O antigen in all K12 transformants carrying pPR981 or pPR1197 indicates the participation of an O antigen polymerase (coded for by rfc) in the LPS formation process (Mäkelä & Stocker, 1984). This function may either be provided by a *Y. pseudotuberculosis* gene present on the recombinant plasmids, or be complemented by the O antigen polymerase of *E. coli* K12. The latter seems unlikely as rfc function appears to be very specific (Mäkelä & Stocker, 1984).

Relationship of *S. enterica* abequose genes to the *Y. pseudotuberculosis* IIA rfb region

In order to compare the genes responsible for the biosynthesis of 3,6-dideoxyhexoses in *Salmonella* and *Yersinia*, Southern hybridization experiments using *S. enterica* 3,6-dideoxyhexose genes to probe the cloned *Y. pseudotuberculosis* M85 rfb DNA region were carried out. The *S. enterica* LT2 genes rbfF and rbfG showed a significant level of similarity to the M85 rfb region, whilst the abequose synthase gene rbfJ of the same *Salmonella* strain has no apparent homologous equivalent in *Y. pseudotuberculosis* M85. Similarly, the paratose synthase gene rbfS from *S. enterica* Ty2 also failed to hybridize to the cloned DNA region. RbfS has previously been shown to be distantly related to rbfJ with 46% sequence similarity (Verma & Reeves, 1989). Thus it appears that different genes of the abequose biosynthetic pathway in *Y. pseudotuberculosis* M85 have different ancestral histories. Whilst rbfF and rbfG in *Salmonella* and *Yersinia* have been derived from a common source, the genes for abequose synthase (rfbJ) in both organisms seem to have originated from independent sources or alternatively, if they have derived from the same source, they have undergone significant sequence divergence since their separation, as detected by Southern hybridization.

Although a common origin for at least some of the 3,6-dideoxyhexose biosynthetic genes of *Y. pseudotuberculosis* M85 and *S. enterica* seems likely, their common ancestor is unlikely to be the source of the rfb genes: the *Salmonella* rfb region is thought to have originated from an unrelated non-enterobacterial donor (Wyk & Reeves, 1989). The rfb gene transfer into *S. enterica* is believed to have taken place in a relatively recent event, after its
divergence from *E. coli* (Jiang et al., 1991; Verma & Reeves, 1989; Wyk & Reeves, 1989). The separation of *Yersinia* and *Salmonella* is thought to be much more ancient than that of *Salmonella* and *E. coli* (Brenner, 1978), but the time of entry of the *rfb* genes into *Yersinia* is unknown at present. The considerable differences in DNA sequence of *Salmonella* and *Yersinia* abequose genes, as shown by Southern hybridization experiments, suggest that the two gene sets have been separated for quite some time, which may indicate a much earlier time for the *rfb* gene transfer into *Yersinia* than for *Salmonella*.

Sequence analysis of the cloned *rfb* region will allow more precise statements about relationship, homology and history of the DNA regions determining 3,6-dideoxyhexose synthesis in both *Yersinia* and *Salmonella*, and will lead to a better understanding of the evolution of *O* antigen variation.

This work was supported by a grant from the Australian Research Council.

**References**


