Different plasmids coding for heat stable enterotoxins in porcine *Escherichia coli* strains of O-group 149

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1. INTRODUCTION

Several reports have been published demonstrating linkage between the genes encoding production of heat-stable enterotoxin (ST) and colicin B (ColB) (Franklin et al., in prep.) or between ST and different colonisation factors [10,11]. The method generally used to assay ST was the infant mouse test [2,4]. In porcine *Escherichia coli* strains producing both heat stable and heat labile (LT) enterotoxins, these toxins on the basis of tests in ligated pig intestines, were considered to be encoded for by a single plasmid [5]. However, recently it has been shown, that the LT and ST enterotoxins were encoded by different plasmids in both human [11] and porcine LT\(^+\)ST\(^+\) strains (Franklin et al., in prep.). These conclusions were also based on the reliability of the infant mouse test for detection of ST.

Lately, Burgess and co-workers [1], Olsson and Söderlind [9] reported that porcine LT\(^+\) strains, which are negative regarding ST production in the infant mouse assay frequently are positive when investigated in pig intestinal loops. They concluded that the infant mouse assay could not be totally relied upon with regard to demonstration of ST production. Burgess and coworkers suggested that there are two kinds of ST, STA and STB. STA was active in the intestine of neonatal piglets and in infant mice, while STB was active only in the intestine of weaned pigs. Another view was put forward by Gyles [6], who suggested that one form of ST (ST 1) reacted both in infant mice and in the intestine of weaned pigs, while another form of ST (ST 2) was active in the intestine of weaned pigs only.

The aim of the present study was to investigate whether the different ST patterns, exhibited by enterotoxigenic porcine *E. coli* strains, might be explained by the presence of different genetic determinants coding for ST production. Therefore, the plasmids presumed to be coding for ST production were transferred into *E. coli* K-12 strains, and the phenotypical expression of the ST genes contained in *E. coli* K-12 was studied by means of the infant mouse test and the pig intestinal loop assay.

2. MATERIALS AND METHODS

2.1. Strains

Four *E. coli* strains of O-group 149 were used as donor strains, all of which produced LT. All strains were ST\(^+\) both in neonatal piglets and older pigs (STP\(^+\)), while two strains were ST\(^-\) in infant mice (STM\(^-\)) (Table I). The terms STM and STP only indicate that ST production has been assayed in infant mice and 6-weeks-old pigs, respectively.
Thus, the donor strains represented typical ST producing _E. coli_ strains isolated from pigs in Sweden [8,9,13,14]. The transfer of the STm and LT determinants from these strains to an _E. coli_ C strain has been described (Franklin et al., in prep.). As recipient strain in the present study an _E. coli_ K-12 recA met his Raf - nalR strain was used.

### 2.2. Enterotoxin testing

ST production was determined by the infant mouse method [2,4] and by the pig intestinal loop assay using heat inactivated culture supernatants prepared as described by Olsson and Söderlind [9]. The toxin preparations were tested in 6-week-old pigs for 6 h and some preparations were further tested in piglets only 1 week old. In the older pigs, strains producing a thin fluid accumulation in ml exceeding the length of the ligated intestinal loops in cm (V/L ratio) were regarded as ST +, while in the neonatal piglets obtaining of a V/L ratio exceeding 0.5 was regarded as evidence of ST production. LT production was assayed in the adrenal cell test [3,13].

#### 2.3. Transfer of the ST determinants

24-h crosses in nutrient broth between the donor and recipient strains were performed with subsequent selection for raffinose fermentation (Raf) on minimal salts medium [7]. Surveying Raf + transconjugant _E. coli_ C sublines for co-transfer of genes coding for ST production had earlier proved successful in order to obtain STm + transconjugants (Franklin et al., in prep.). In order to obtain STp + transconjugants the LT plasmids of the donor strains were transferred to _E. coli_ K-12, since plasmids coding for both STp and LT production in porcine _E. coli_ strains have been described [5]. Also the epidemiological correlation between LT and STp production suggested that genes encoding STp production might be transferred to _E. coli_ K-12 together with the LT plasmid [8,9]. LT + transconjugants were obtained at a high frequency (25%) by investigating Raf + transconjugant clones derived from LT + ColB - donor strains for cotransfer of the LT plasmid (Franklin et al., in prep.). Raf + LT + and Raf + LT - transconjugant clones were investigated for ST production in infant mice (STm) and subsequently in 6-weeks-old pigs (STp) and neonatal piglets.

### Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Raff</th>
<th>K88</th>
<th>ColB</th>
<th>LT</th>
<th>STm</th>
<th>STp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bd 91/75</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>B 549/75</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Bd 2450/75</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bd 1877/75</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* STp + strains were ST + both in neonatal and older pigs.

### Table 2

<table>
<thead>
<tr>
<th>Plasmid source</th>
<th>Enterotoxigenicity patterns of Raf + transconj. clones</th>
<th>STp + clones</th>
<th>Total numbers investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bd 91/75</td>
<td>STm - LT +</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>STm - LT -</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Bd 549/75</td>
<td>STm - LT +</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>STm - LT -</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Bd 2450/75</td>
<td>STm + LT +</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>STm - LT +</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>STm - LT -</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>ST - LT</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Bd 1877/75*a</td>
<td>STm - LT</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

* No transconjugant STm - clones were found.
Table 3
Phenotypical expression of STm and STp genes, transmitted to E. coli K-12, in piglets 1 week-old

<table>
<thead>
<tr>
<th>Plasmid source</th>
<th>Enterotoxigenicity patterns of Raf + transconj. clones</th>
<th>ST + clones</th>
<th>Total number tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bd 2450/75</td>
<td>STp+STm+LT -</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>STp+STm - LT +</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Bd 1877/75</td>
<td>STp- STm+LT -</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

3. RESULTS

All Raf +LT + transconjugant E. coli K-12 sublines, containing LT determinants derived from STm - donor strains, were STp +, while none of the LT - sublines was STp + (Table 2). The transconjugant clones containing LT determinants originating from the LT + STm + STp + donor strain Bd 2450 were all ST + both in neonatal and 6-week-old pigs, regardless of whether they were STm + or STm -. The LT - STm + clones tested were all ST - in the 6-week-old pigs but ST + in the neonatal piglets (Tables 2 and 3).

The mean V/L ratio of the ligated intestinal segments in the older pigs was 1.2 while in the neonatal piglets the corresponding figure was 0.9 as for the segments regarded as ST +.

4. DISCUSSION

The results of the conjugation experiments showed that there were at least two different genes encoding ST in E. coli strains of O-group 149, one coding for STp and one for STm. These genes behaved as if they were genetically independent entities also in strains which were phenotypically STp + STm +. The STp toxin was active in the intestines of neonatal and 6-week-old pigs, but ST - in infant mice. The STm toxin was active both in infant mice and in the intestines of neonatal piglets but not in older pigs.

The STp determinants seemed to be linked exclusively to the LT plasmid in E. coli strains of O-group 149, since the STp determinants only were transferred to E. coli K-12 together with the LT determinants. In contrast, the STm determinants behaved as transposable elements, although they were never found on the LT plasmid (Franklin et al., in prep.). These findings were in line with recent reports concerning comparisons of different ST assays, where no porcine LT + "only" E. coli strains were found which were not ST + when investigated in piglet intestinal loops [8,9].

The results are not consistent with the view that there is one ST (ST 1) active in both infant mice and in the intestines of weaned piglets [6], nor with the concept of an STb active only in the intestine of older but not of neonatal pigs [1]. There may be several explanations to these diverging results. The present study was limited to only one, in Sweden important O-group, and it is possible that other forms of ST occur in other O-groups or geographic areas. Probably a whole family of different forms of ST will be revealed in the near future (Falkow, personal communication).

The STp toxin originating from E. coli 0149 strains in Sweden was active in both older and neonatal pigs, in contrast to the STb toxin, which reacted only in weaned pigs. Nevertheless, STb and STp are probably identical or nearly identical as indicated by their stability [1], their occurrence in the same O-group of 149 [1,6] and their epidemiological connection with the LT plasmid [8].

The ST toxin produced by the STm + transconjugant clones might be identical with the ST mouse toxin, described by Olsson et al. [8] produced by E. coli strains producing the adhesin K99 or with STa. Besides, the transposable nature of the porcine STm genes (Franklin et al., in prep.) may explain the instability of ST production as assayed in infant mice, reported by others [2,6].

In conclusion, two distinct ST toxins have been identified in porcine E. coli strains of O-group 149.
These toxins were encoded for by two genetically independent determinants, residing on different plasmids. Furthermore, the present study indicates, that for screening of ST production in the neonatal piglet diarrhoea the pig loop test has to be combined with the infant mouse assay, or to be performed in neonatal piglets.

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REFERENCES