Detection of Pathogenic *Yersinia enterocolitica* Serotype O:3 by Biochemical, Serological, and PCR Methods

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**Abstract**


In this study, the pathogenic *Y. enterocolitica* of serotype O:3 was monitored. The serotype is widely spread in Europe and has been linked to human yersiniosis. For the detection of pathogenic strains were used biochemical and serological methods as well as PCR methods based on the identification of virulence genes (*ail*, *rfbC*, *ystA*, *yadA*, *virF*). The occurrence of *Y. enterocolitica* O:3 strains was monitored in slaughter animals from a number of farms in the Czech Republic. A total of 3748 samples were collected coming from pigs (1388), cattle (633), poultry (902), and slaughter facilities (825). Fifty-two *Y. enterocolitica* O:3 isolates were identified by biochemical and serologic methods, and 53 *Y. enterocolitica* O:3 isolates were identified by PCR methods (46 isolates from pigs, 2 isolates from poultry, 3 isolates from cattle, and 2 isolates from a poultry slaughtering facility). All isolates of *Y. enterocolitica* O:3 carried genes *ail* and *rfbC*, 83% isolates carried gene *ystA*, 79% isolates carried gene *yadA* and 49% isolates carried gene *virF*. The use of PCR methods based on the identification of *ail* and *rfbC* genes provides for a sufficiently specific identification of pathogenic *Y. enterocolitica* O:3 strains with optimum time consumption compared to biochemical and serological methods. It is not recommendable to use other PCR methods (detection of the *ystA*, *yadA*, and *virF* genes) for the detection of pathogenic *Y. enterocolitica* strains because those methods are not very specific for the determination of pathogenicity.

**Keywords**: *Yersinia enterocolitica*; serotype O:3; PCR; virulence; genes; biochemical methods; cultivation; DNA

The list of bacterial agents causing food-borne diseases includes, besides the most frequently encountered *Salmonella* and *Campylobacter*, also *Yersinia enterocolitica*. *Y. enterocolitica* is a Gram-negative bacterium belonging to the genus *Yersinia*, family *Enterobacteriaceae*. Of the 12 species that comprise the genus *Yersinia*, three are important from the human pathogenicity point of view, namely *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*. *Y. pestis* is the causative agent of the bubonic plague, and *Y. pseudotuberculosis* and *Y. enterocolitica* are intestinal pathogens (BOER 1992; SPRAGUE & NEUBAUER 2005; TENNANT et al. 2005).

*Y. enterocolitica* is divided into about 60 serotypes according to the variability of the O-antigen present in the outer membrane of bacteria (SKURNIK et al. 1999). Of the 11 serotypes important from

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the human infections point of view, the most frequently occurring are serotypes O:3; O:9; O:5.27, and O:8. Pathogenic strains of O:4, O:8, O:13a/b, O:18, O:20, and O:21 serotypes are prevalent in the USA. In Europe and Japan, only O:3 and O:9 serotypes occur (WANNET et al. 2001).

Y. enterocolitica are widely distributed throughout the environment and have been isolated from raw milk, sewage-contaminated water, soil, sea-food, humans, and many warm-blooded animals such as poultry and, most importantly, pigs (SCHMIDT & RODRICK 2003). Therefore, pork meat is thought to be an important source of the infection (LAMBERTZ & DANIELSSON-THAM 2005). Pathogenic strains of Y. enterocolitica are transmitted to man mainly from contaminated water or food (meat, milk or vegetables), more specifically raw or undercooked pork, and they may cause various infectious diseases (enteritis, enterocolitis, mesenteric lymphadenitis) (SCHMIDT & RODRICK 2003).

Until recently, pathogenic strains of Y. enterocolitica used to be identified on the basis of the detection of the virulence plasmid pYV (70 kb). Localised on the plasmid are virulent genes yadA and virF (CORNELIS 1998; THOERNER et al. 2003; PLATT-SAMORAJ et al. 2006). The yadA gene encodes the production of the YadA membrane protein, which functions as a protection of Y. enterocolitica cells against the activity of polymorphonuclear leukocytes (RUCKDESCHEL et al. 1996). The virF gene activates the transcription of yop genes that encode the secreted proteins (Yops) (MICIELS et al. 1990). Proteins (Yops) protect bacterial cells against the activity of macrophages (CORNELIS 1998). The plasmid pYV might be lost relatively easily when the bacteria are stored for a longer period of time, subjected to numerous passages or grown at temperatures above 37°C (PLATT-SAMORAJ et al. 2006).

To determine pathogenicity, it is therefore better to use chromosomal genes (ail, rfbC, and yst) (WEYNANTS et al. 1996; WANNET et al. 2001; THOERNER et al. 2003). The ail gene encodes Ail protein, it occurs only in the pathogenic strains of Y. enterocolitica. This protein participates in bacterial adhesion to mammalian cells, facilitates their invasion into eukaryotic cells, and intensifies their resistance to the bacteriocidal effects of complement (PLATT-SAMORAJ et al. 2006). The rfbC gene is localised on the chromosome only in pathogenic strains of Y. enterocolitica serotype O:3 (WEYNANTS et al. 1996). Also important for the pathogenicity reasons is the Yst thermostable enterotoxin, which is a protein with the molecular weight of 7494 Da. The enterotoxin is encoded by the yst gene, and facilitates the invasion of the microorganism into tissues by damaging the intestinal epithelium (PLATT-SAMORAJ et al. 2006). Pathogenic strains of Y. enterocolitica produce the thermostable enterotoxin YstA (ystA gene), which, sporadically, may also be produced by non-pathogenic strains of Y. intermedia and Y. enterocolitica of the biotype 1A (THOERNER et al. 2003; SINGH & VIRDI 2004; PLATT-SAMORAJ et al. 2006).

The countries with the highest prevalence of yersiniosis include Germany, Scandinavian countries, and Belgium. In the Czech Republic (No. of cases/during 2000–2004: 231/2000, 301/2001, 403/2002, 372/2003, 498/2004), the number of the reported cases of yersiniosis is not as high as in some other European countries, such as Germany (No. of cases/during 2000–2004: 4778/2000, 7186/2001, 7515/2002, 6571/2003, 6182/2004) (EFSA, Trends and sources of Zoonoses, Zoonotic Agents and Antimicrobial resistance in the European Union in 2004). In view of an increase in the incidence of yersiniosis in the Czech Republic (EPIDAT, CR), the risks presented by pathogenic strains of Y. enterocolitica and the need for an additional specific identification, the authors of this study focused on the monitoring of the pathogenic Y. enterocolitica of serotype O:3, which is widely spread across Europe and mentioned in connection with human yersiniosis (GÜRTLER et al. 2005).

MATERIAL AND METHODS

In 2005, a total of 3748 samples were collected in slaughterhouses (Czech Republic), of which 1388 samples were collected from pigs (tongues, tonsils, carcass halves, surface), 633 samples from cattle (tongues, carcass halves, skin), 902 from poultry (surface, intestines) and 825 samples from slaughter facilities.

Sterile cotton swabs were used to collect smear samples from the rectum, tongues, tonsils, and carcasses surfaces of the slaughtered pigs, cattle, and poultry, and from various surfaces of the slaughterhouse premises. As to pigs, samples were taken from the skin surface before bleeding, the surface of the tongue and tonsils after evisceration and, after the carcasses had been halved, from the carcass surfaces in the rectum region. With cattle, samples were taken from the skin surface before
bleeding, the surface of the tongue after eviscerat-
on and, after the carcasses had been halved, from
the carcass surfaces in the rectum region. As to
poultry, samples were collected from the carcass
surfaces after washing (i.e. after scald and defeath-
ering), from surfaces after evisceration, and from
the intestinal (caecum) content. Samples from the
slaughtering premises were taken at pig slaughter
facilities for pigs, poultry, and cattle, and they were
collected from the instruments (chopping knives,
saws, knives), work surfaces, trays, etc. The smear
samples collected were placed in the transport
Amies Agar Gel Medium (Copan Italia S.p.A) and
taken to the laboratory for immediate processing.
The bacterial strains were recovered after selec-
tive enrichment in ITC Broth Base (HiMedia,
Čaderský-Envitek, spol. s r. o., CR) for 48 h at
24°C and incubation on Selective Agar base (CIN)
(HiMedia, Čaderský-Envitek, spol. s r. o., CR) for
24 h at 30°C (ČSN EN ISO 10273).

Pathogenic Y. enterocolitica O:3 strains were
detected by biochemical and serological methods,
and by PCR methods.

Culture methods, biochemical tests and sero-
typing. The isolation of Y. enterocolitica strains,
their biochemical identification and pathogenic-
ity determination were performed in accordance
with ČSN EN ISO 10273 and Bottone (1997)
(Table 1).

Suspected colonies were taken out of the pure
culture obtained and used for preliminary tests
(oxidase, urease production, glucose and lactose
fermentation, hydrogen sulphide production, and
gas formation from glucose). Colonies with positive
urease and glucose, negative oxidase and lactose
fermentation, and negative hydrogen sulphide pro-
duction and gas formation from glucose were then
selected. In these selected colonies, biochemical
confirmation tests were performed (lysine decar-
boxylase and ornithine decarboxylase, sucrose,
rhamnose, xylose and trehalose fermentation,
and citrate). Colonies with typical characteristics
(positive sucrose fermentation and ornithine
decarboxylase, negative lysine decarboxylase and
citrate and trehalose, rhamnose and xylose positive
or negative) were tested for pathogenicity (esculin
hydrolysis, pyrazinamidase activity, and salicin).

Biochemical identification of the strains was
supplemented with biochemical reactions for Y. en-
terocolitica identification (indole, hydrogen sul-
phide, lysine, ornithine, urease, arginine, simmons
citrate, malonate, phenylalanine, β-galactosidase,
inositol, adonitol, cellobiose, sucrose, trehalose,
mannitol, acetoin, esculin, sorbitol, rhamnose,
melibiose, raffinose, dulcitol, glucose), which are
part of the commercially available Enterotest24
(Pliva-Lachema, CR).

In the isolated pathogenic strains, the O:3 se-
rotype was identified by slide agglutination using
commercially available antiserum (O:3) (Itest plus,
s. r. o., Hradec Králové, CR).

DNA extraction. Bacterial DNA was prepared
by the phenol-chloroform method as described
by Sambrook et al. (1989). Cultivated colonies of
bacterial cells identified as Y. enterocolitica were
suspected in Tris-HCl-EDTA buffer, pH 8.0, frozen,

<table>
<thead>
<tr>
<th>Test</th>
<th>Reaction</th>
<th>Test</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species identification</strong></td>
<td></td>
<td><strong>Species identification</strong></td>
<td></td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>Trehalose</td>
<td>+/–</td>
</tr>
<tr>
<td>Indole</td>
<td>–/+</td>
<td>Rhamnose</td>
<td>–/+</td>
</tr>
<tr>
<td>Glucose</td>
<td>–/+</td>
<td>Xylose</td>
<td>–/+</td>
</tr>
<tr>
<td>Gas formation from glucose</td>
<td>–</td>
<td>Simmons citrate</td>
<td>–</td>
</tr>
<tr>
<td>Lactose</td>
<td>–</td>
<td>Simmons citrate</td>
<td>–</td>
</tr>
<tr>
<td>Hydrogen sulphide</td>
<td>–</td>
<td>Simmons citrate</td>
<td>–</td>
</tr>
<tr>
<td>Oxidase</td>
<td>–</td>
<td>Simmons citrate</td>
<td>–</td>
</tr>
<tr>
<td>Lysine decarboxylase</td>
<td>–</td>
<td>Simmons citrate</td>
<td>–</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>+</td>
<td>Simmons citrate</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>Simmons citrate</td>
<td>–</td>
</tr>
</tbody>
</table>

**Pathogenicity determination**

<table>
<thead>
<tr>
<th>Test</th>
<th>Reaction</th>
<th>Test</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eesculin hydrolysis</td>
<td>–</td>
<td>Pyrazinamidase activity</td>
<td>–</td>
</tr>
<tr>
<td>Salicin</td>
<td>–</td>
<td></td>
<td>–</td>
</tr>
</tbody>
</table>

Table 1. Interpretation of biochemical and pathogenicity tests of Y. enterocolitica
thawed, and subsequently lysed by incubation with 
proteinase K at 55°C overnight. The released DNA 
was extracted twice with an equal volume of 
phenol:chloroform:isoamylalcohol (Serva, Germany) 
and once with chloroform. DNA concentrated by 
ethanol precipitation was dissolved in 40 μl TE 
buffer and stored at −20°C.

**PCR methods.** For the detection of (ail, yadA, 
ystA, rfbC, virF) genes, specific primers and PCR 
conditions as described by (Weynants et al. 1996; 
Lantz et al. 1998; Wannet et al. 2001; Thoerner 
et al. 2003) were used (Table 2). Specific DNA se-
quencies of genes (ail, yadA, ystA, rfbC, virF) were 
amplified in a thermocycler (Mastercycler Personal, 
Germany). Reference bacterial strains Yersinia en-
terocolitica CCM 5671, Yersinia rohdei CCM 4875T, 
and Campylobacter jejuni subsp. jejuni ATCC 
33560 (FCCM, CZ) were used in this study.

The reaction mixture (12.5 μl) contained 1 μl of 
DNA template, 4.2 μl H2O, 6.3 μl PCR-Mix (PP 
MASTER MIX, Top-Bio, s. r. o., CR), 0.5 μl of each 
primer (0.01 nM/μl).

PCR products were analysed on 3% agarose gel 
(Serva, Germany) run at 120 V in a Tris-H

3 buffer, pH 8.3. The DNA fragments were stained 
with ethidium bromide and photographed using 
a UV-transilluminator (UltraLum, Claremont) to 
visualise the bands. The GeneRuler™ 50 bp DNA 
Ladder (MBI Fermentas, Lithuania) was used as a 
molecular weight marker.

**RESULTS AND DISCUSSION**

In 2005, 53 isolates of Y. enterocolitica serotype 
O:3 were isolated from a total of 3748 samples 
collected from slaughter animals and slaughter 
facilities. Fifty-two Y. enterocolitica O:3 isolates 
were identified by biochemical and serological 
methods, and 53 Y. enterocolitica O:3 isolates 
were identified by PCR methods (Table 2) based 
on the identification of ail and rfbC genes. The 
list of isolates Y. enterocolitica O:3 strains from 
individual sources is given in Table 3.

The highest incidence of isolates of Y. enterocol-
itica O:3 was found in pigs (3.3%), while in cattle 
and poultry their incidence was low (0.5% and 
0.2%, respectively). Compared with the incidence 
among slaughter animals, the incidence rate of 
pathogenic strains in slaughter surroundings was 
negligible (Table 3). Pigs are generally reported 
as the main reservoir of pathogenic strains of 
Y. enterocolitica, which has been corroborated by 
a number of studies. For instance, studies from 
Germany report up to 64.4% incidence of patho-
genic strains of Y. enterocolitica O:3 (Gürtler 
et al. 2005) in pork samples. In Italy, pathogenic 
strains of Y. enterocolitica have been reported in 
14.7% of 150 samples collected from tonsils of 
pigs (Bonardi et al. 2003). In a Norwegian study 
investigating the prevalence of Y. enterocolitica 
pathogenic strain in 249 samples from five different 
slaughterhouses, 15.2% of the samples tested were 
positive for Y. enterocolitica pathogenic strains 
(Johannessen et al. 2000).

To detect the genes (ail, yadA, rfbC, ystA, virF) 
using the PCR methods, specific sequences of 
genes were amplified and individual amplified 
fragments were detected by agarose gel electro-
phoresis (Figure 1).

The occurrence of virulence genes (ail, yadA, 
rfbC, ystA, virF) in isolates of Y. enterocolitica O:3 
and reference strains is shown in Table 4.

According to literary data, the ail gene occurs 
only in pathogenic strains of Y. enterocolitica, and 
the rfbC gene in pathogenic strains of Y. enteroc-
litica, serotype O:3 (Weynants et al. 1996; Wan-
net et al. 2001; Lambertz & Danielsson-Tham 
2005). In the present study, the ail and rfbC genes 
were found in all isolates and pathogenic reference 
strain of Y. enterocolitica CCM 5671.

While the presence of the ystA gene is reported 
in the studies on pathogenic strains of Y. enterocoli-

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primers</th>
<th>Primers sequence (Reference)</th>
<th>Amplicon length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ail</td>
<td>A1, A2</td>
<td>Wannet et al. (2001)</td>
<td>425</td>
</tr>
<tr>
<td>yadA</td>
<td>P1, P2</td>
<td>Lantz et al. (1998)</td>
<td>600</td>
</tr>
<tr>
<td>rfbC</td>
<td>rfbC1, rfbC2</td>
<td>Weynants et al. (1996)</td>
<td>405</td>
</tr>
<tr>
<td>ystA</td>
<td>ystA1, ystA2</td>
<td>Thoerner et al. (2003)</td>
<td>79</td>
</tr>
<tr>
<td>virF</td>
<td>virF1, virF2</td>
<td>Thoerner et al. (2003)</td>
<td>561</td>
</tr>
</tbody>
</table>
tica, it is only rarely mentioned in connection with non-pathogenic Y. enterocolitica strains (biotype 1A) (Tennant et al. 2005). In contrast with the studies of pathogenic strains of Y. enterocolitica O:3 by Thoerner et al. (2003) and Platt-Samoraj et al. (2006) where the ystA gene was identified in 100% and 99%, respectively, in our study the ystA gene was found in only 83% of isolates. A study of reference strains showed the presence of the gene in pathogenic strain of Y. enterocolitica CCM 5671.

The yadA and virF genes were identified in 79% and 49% of isolates of Y. enterocolitica O:3, respectively, the yadA gene was found only in pathogenic reference strain of Y. enterocolitica CCM 5671, and the virF gene was not identified in any of the reference strains. Lantz et al. (1998) and Lambertz & Danielsson-Tham (2005) found the virF and yadA genes in 92% and 66% of isolates of Y. enterocolitica O:3, respectively, but failed to find them in any other pathogenic or non-pathogenic Yersinia spp. strains. Both genes (yadA and virF) were identified in the pathogenic strain of Y. enterocolitica CCM 5671.

Table 3. Evaluation appearance of Y. enterocolitica O:3

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of samples</th>
<th>No. of isolates Y. enterocolitica O:3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs</td>
<td>1388</td>
<td>46 (3.3)</td>
</tr>
<tr>
<td>tongue</td>
<td>296</td>
<td>15</td>
</tr>
<tr>
<td>tonsils</td>
<td>296</td>
<td>17</td>
</tr>
<tr>
<td>carcass halves</td>
<td>402</td>
<td>4</td>
</tr>
<tr>
<td>surface</td>
<td>394</td>
<td>10</td>
</tr>
<tr>
<td>Cattle</td>
<td>633</td>
<td>3 (0.5)</td>
</tr>
<tr>
<td>tongue</td>
<td>180</td>
<td>1</td>
</tr>
<tr>
<td>carcass halves</td>
<td>273</td>
<td>2</td>
</tr>
<tr>
<td>surface</td>
<td>180</td>
<td>0</td>
</tr>
<tr>
<td>Poultry</td>
<td>902</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>surface after wash</td>
<td>400</td>
<td>2</td>
</tr>
<tr>
<td>surface after full-dressed</td>
<td>266</td>
<td>0</td>
</tr>
<tr>
<td>intestines</td>
<td>236</td>
<td>0</td>
</tr>
<tr>
<td>Slaughter surroundings</td>
<td>825</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>pigs</td>
<td>358</td>
<td>1</td>
</tr>
<tr>
<td>cattle</td>
<td>245</td>
<td>0</td>
</tr>
<tr>
<td>poultry</td>
<td>222</td>
<td>1</td>
</tr>
<tr>
<td>Σ</td>
<td>3748</td>
<td>Σ 53 (1.4)</td>
</tr>
</tbody>
</table>

Table 4. Occurrence of virulence genes (ail, yadA, rfbC, ystA, virF) in Yersinia spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Serotype</th>
<th>No. positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ail</td>
</tr>
<tr>
<td><strong>Pathogenic Y. enterocolitica</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yersinia enterocolitica CCM 5671</td>
<td>O:3</td>
<td>1/1</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>O:3</td>
<td>53/53</td>
</tr>
<tr>
<td><strong>Nonpathogenic Yersinia spp.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yersinia rohdei CCM 4075T</td>
<td></td>
<td>0/1</td>
</tr>
<tr>
<td>Campylobacter jejuni subsp. jejuni ATCC 33560</td>
<td>23</td>
<td>0/1</td>
</tr>
</tbody>
</table>

Figure 1. Agarose gel electrophoresis of PCR products amplified with PCR methods for the ail (425 bp), yadA (600 bp), rfbC (405 bp), ystA (79 bp) and virF (561 bp) genes from Yersinia enterocolitica CCM 5671.
are contained on the virulent plasmid pYV, and the fact that the passaging of strains may lead to the loss of the plasmid may explain the less frequent detection of pathogenic strains of *Y. enterocolitica*. In their comparative study on the prevalence of the *ail* and *yadA* genes in *Y. enterocolitica* strains and non-pathogenic *Yersinia* spp. strains, Blais & Phillippe (1995) found the *ail* gene in 100% of pathogenic *Y. enterocolitica* strains, the *yadA* gene in only 86% of pathogenic *Y. enterocolitica* strains, but they found neither of the genes in non-pathogenic strains of *Yersinia* spp.

Biochemical and serological methods were able to identify 52 isolates of *Y. enterocolitica* O:3. In addition to biochemical identification, Enterotest24 was also performed, and on the basis of the results obtained, 52 isolates were identified as *Y. enterocolitica*. The list of the results of biochemical tests is presented in Table 5.

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of isolates</th>
<th><em>Y. enterocolitica</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species identification</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urease</td>
<td>+ (92%), – (8%)</td>
<td></td>
</tr>
<tr>
<td>Indole</td>
<td>+ (9%), – (91%)</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+ (100%), –</td>
<td></td>
</tr>
<tr>
<td>Gas formation from glucose</td>
<td>+ (15%) weak positive, – (85%)</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>+, – (100%)</td>
<td></td>
</tr>
<tr>
<td>Hydrogen sulphide</td>
<td>+ (6%) weak positive, – (94%)</td>
<td></td>
</tr>
<tr>
<td>Oxidase</td>
<td>+, – (100%)</td>
<td></td>
</tr>
<tr>
<td>Lysine decarboxylase</td>
<td>+, – (100%)</td>
<td></td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>+ (96%), – (4%)</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>+ (100%), –</td>
<td></td>
</tr>
<tr>
<td>Trehalose</td>
<td>+ (100%), –</td>
<td></td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+, – (100%)</td>
<td></td>
</tr>
<tr>
<td>Xylose</td>
<td>+ (60%), – (40%)</td>
<td></td>
</tr>
<tr>
<td>Simmons citrate</td>
<td>+ (2%), – (98%)</td>
<td></td>
</tr>
<tr>
<td><strong>Pathogenicity determination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eesculin hydrolysis</td>
<td>+ (8%), – (92%)</td>
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</tr>
<tr>
<td>Pyrazinamidase activity</td>
<td>+ (6%), – (94%)</td>
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</tr>
<tr>
<td>Salicin</td>
<td>+ (6%), – (94%)</td>
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</tbody>
</table>

PCR methods were able to identify 53 isolates of *Y. enterocolitica*. PCR methods based on the identification of *ail* and *rbfC* genes identified 100% of *Y. enterocolitica* O:3 isolates. PCR methods based on the identification of *ytsA*, *yadA*, and *virF* genes identified 83%, 79% and 49% of *Y. enterocolitica* isolates, respectively.

Differences in the rate of identification of *Y. enterocolitica* pathogenic strains using traditional cultivation and biochemical methods and PCR methods were also found by Johannessen et al. (2000), who reported even much greater differences in the detection of pathogenic strains between individual methods than those found in our study. When they used cultivation and biochemical methods, the authors were able to detect 6 pathogenic strains of *Y. enterocolitica*, but when they used PCR methods, they successfully identified up to 50 pathogenic strains.

The specificity of identification of pathogenic strains of *Y. enterocolitica* O:3 by PCR methods based on the identification of *ail* and *rbfC* genes is better than that of biochemical and serological methods. It is not recommendable to use other PCR methods (detection of *ytsA*, *yadA* or *virF* genes) for the detection of pathogenic strains because those methods are not very specific for *Y. enterocolitica* pathogenicity determinations. The use of PCR methods (detection of *ail* and *rbfC* genes) provides for a sufficiently specific identification of pathogenic *Y. enterocolitica* O:3 strains with optimum time consumption in comparison with biochemical and serological methods.

**References**


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