Pathogenicity of sucrose-negative *Yersinia* strains found in pig tonsils

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**Abstract:** The aim of this work was to study the pathogenicity of sucrose-negative *Yersinia* strains isolated from pig tonsils with phenotypic and genotypic methods. All *Yersinia pseudotuberculosis* and 5 out of 8 of the sucrose-negative *Y. enterocolitica* strains were pathogenic. Three sucrose-negative *Yersinia enterocolitica* strains carried the *ail* gene and were pyrazinamidase negative showing that they were potentially pathogenic and may have lost the virulence plasmid during isolation and storage. Pyrazinamidase test was not reliable to predict the pathogenicity of *Y. pseudotuberculosis* strains. Using the PCR assays targeting the *inv*, *ail* and *yadA* genes sucrose-negative *Yersinia* strains could be differentiated from each other.

**Keywords:** *Yersinia*, pathogenicity, sucrose, pig tonsils

**Introduction:** The genus *Yersinia* presently consists of 11 species, three of which are sucrose-negative: *Yersinia pseudotuberculosis*, *Yersinia kristensenii* and *Yersinia aldovae* (Bercovier et al. 1980). Sucrose fermentation is one of the biochemical tests used to differentiate sucrose-positive *Yersinia enterocolitica* from sucrose-negative *Y. kristensenii*. Rare strains of sucrose-negative *Yersinia enterocolitica* have been isolated in humans and pigs (Fukushima et al., 1988; Guiyoule et al., 1998). The aim of this work was to study pathogenicity of sucrose-negative *Yersinia* strains isolated from pig tonsils with phenotypic and genotypic methods.

**Materials and Methods:** A total of 17 sucrose-negative *Yersinia* strains, including 8 *Y. pseudotuberculosis*, 8 *Y. enterocolitica*, and 1 *Y. kristensenii* strain, were studied. All strains were isolated from tonsils of clinically healthy fattening pigs in Finland. The sucrose-negative isolates were identified using the API 20E system.
Due to the temperature-dependent Voges-Proskauer test (Bercovier et al., 1980),
the API 20E system was incubated at 25°C instead of 37°C, which is the correct
temperature according to the manufacturer’s instructions. Three chromosomal-
encoded virulence markers; pyrazinamidase activity, presence of ail and inv genes,
and 4 plasmid-encoded virulence markers; Congo red uptake, calcium dependence,
virF and yadA genes were tested. Calssium dependence and Congo red absorption
were tested using Congo red-magnesium oxalate agar (CRMOX) (Riley and Toma,
1986). The virF, the ail, the inv and the yadA were detected using PCR (Nakajima
et al., 1992; Kapperud et al., 1993; Kaneko et al., 1995). The PCR assays targeting
ail and yadA genes are designed for detection of Y. enterocolitica and the PCR
assay targeting the inv gene for Y. pseudotuberculosis.

Results: All Y. pseudotuberculosis strains harboured the inv and virF genes, and
showed Congo red adsorption and calssium dependence. The Y. kristensenii was
negative in all pathogenicity tests used. 5 out of 8 Y. enterocolitica strains were
positive for all the plasmid-encoded virulence markers, and all 8 strains harboured
the ail gene and showed no pyrazinamidase activity.

Table 1. Different phenotypic and genotypic characters of sucrose-negative Yersinia

<table>
<thead>
<tr>
<th>Species</th>
<th>Nr. of strains</th>
<th>Phenotypic tests</th>
<th>Genotypic tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y. pseudotuberculosis</td>
<td>5</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Y. pseudotuberculosis</td>
<td>2</td>
<td>(+)</td>
<td>-</td>
</tr>
<tr>
<td>Y. pseudotuberculosis</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Y. kristensenii</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

a PYZ=pyrazinamidase, b CRMOX= Congo red uptake and calssium dependence, c weak positive

Discussion and conclusions: All Y. pseudotuberculosis and 5 out of 8 of the
sucrose-negative Y. enterocolitica strains were pathogenic. 3 sucrose-negative Y.
enterocolitica strains carried the ail gene and were pyrazinamidase negative
showing that they were potentially pathogenic and may have lost the virulence
plasmid during isolation and storage. Pyrazinamidase test was not reliable to
predict the pathogenicity of Y. pseudotuberculosis strains. Using the PCR assays
targeting the inv, ail and yadA genes sucrose-negative Yersinia strains could be differentiated from each other.

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References


