DIFFERENTIATION OF STAPHYLOCOCCUS EPIDERMIDIS ISOLATES FROM RAW MATERIAL, FOODSTUFFS AND FOOD CONTACT SURFACES

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INTRODUCTION

The capability of cell adhesion and biofilm formation is a significant characteristic of *Staphylococcus epidermidis* virulence, conditioned by the presence of chromosomal ica locus with icaA, icaB, icaC and icaD genes. The microorganism is particularly dangerous due to its capability of being able to colonize synthetic and metal surfaces of replacements in medicine. Biofilm also present a risk because of its higher resistance to antimicrobial agents than planktonic bacteria. However, biofilms are also found on technological surfaces in the food industry.

MATERIALS AND METHODS

From 2000 to 2005, 197 *S. epidermidis* isolates were obtained from swabs of contact surfaces on the equipment of food processing plants, raw materials and foodstuffs produced from meat and milk. Capability of this microorganism to form a biofilm was investigated by phenotype and multiplex PCR method (Table 1). Resistance of selected isolates to antimicrobial agents was investigated using the microdilution method.

- **phenotyping**
  *Staphylococcus epidermidis* isolates were incubated in brain heart infusion at 35°C / 72 hours in glass tubes. Then the tubes were rinsed with phosphate buffer and suspect biofilm was dried turning the tubes upside down and coloured by 0.1% safranin solution for 3 min. The intensity of red coloured biofilm structure was rated visually (Figure 1).

- **genotyping**
  Total genomic DNA was obtained from an individual bacterial strain, in a brain heart infusion for 24 hours. The bacterial suspension was centrifuged and the pellet washed in 500 µl of sterile distilled water. The suspension was then incubated at 80°C for 20 min and finally centrifuged at 1400 rpm / 1 min. The primers for the detection of ica genes (amplicon 546 bp), primers UNB applied as internal control (370 bp) and primers SE705 specific for *S. epidermidis* (174 bp) were used for multiplex PCR method. The amplification mode was 96°C / 10 sec, 66°C / 10 sec, 72°C / 40 sec for 45 cycles with an extension of 72°C / 2 min. Amplification products were separated by electrophoresis in 2% ethidium bromide-stained agarose gel using a 100-bp ladder as a molecular weight standard (Figure 2).

- **resistance**
  The susceptibility test to antimicrobial agents in selected isolates was carried out using the microdilution method (Tisos, Cz), according to the recommendations of CLSI, 2006. Isolates were screened for resistance to 12 antimicrobial agents: penicillin, oxacillin, ampicillin-sulbactam, vancomycin, teicoplanin, gentamicin, erythromycin, tetracycline, ciprofloxacin, clindamycin, trimethoprim-sulfamethoxazole, chloramphenicol.

RESULTS

A total of 197 *S. epidermidis* isolates were obtained from raw food materials, milk and meat products and from swabs of equipment used in meat and milk processing plants. The production of biofilm was detected in 12 isolates (6%). Most of them originated from raw milk and milk products (both 4 isolates) (Table 1). The ica operon was detected in 9 isolates and the phenotypical ability to produce biofilm was determined in 10 isolates. Different results between the phenotypical and genotypical method were detected in 5 isolates.

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Table 1: Incidence of phenotype and genotype biofilm-positive isolates of *S. epidermidis*

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Isolate weigh (n) (%)</th>
<th>Phenotype positive</th>
<th>Genotype positive</th>
<th>Total biofilm-positive isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>milk products</td>
<td>88 (47,2)</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>raw milk</td>
<td>41 (23,7)</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>equipment in milk processing plants</td>
<td>33 (19,1)</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>total milk isolates</td>
<td>179 (100)</td>
<td>9</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>meat products</td>
<td>77 (40,5)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>raw meat</td>
<td>31 (17,5)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>equipment in meat processing plants</td>
<td>41 (16,7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>total meat isolates</td>
<td>124 (100)</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Resistance to antimicrobial agents was observed in 41 isolates of *S. epidermidis*. The most common resistance was to penicillin. Multiresistant isolates (resistance to 3 and more agents) were detected in 5 cases, all of them were biofilm-positive, too. One of them was resistant to 8 antimicrobial agents (penicillin, oxacillin, ampicillin-sulbactam, tetracycline, trimethoprim-sulfamethoxazole, erythromycin, clindamycin, and gentamicin) (Graph 1).

Graph 1: Resistance to antimicrobial agents to biofilm-positive and biofilm-negative isolates (Intermediate ones not included)

Graph 2: Amplification products of expected sizes

- 1. *S. epidermidis* biofilm-positive
- 2. *S. epidermidis* biofilm-negative
- 3. *Staphylococcus non-epidermidis*
  M = 100 bp ladder

CONCLUSION

We found strains of *Staphylococcus epidermidis* with the ability to form biofilm in some food processing environments. It is possible for the microorganisms released from biofilm to carry resistance genes and could represent potential source of food contamination.

REFERENCES


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