Bacterial presence in *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)

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**Abstract**

Bacteria from the digestive system of larvae, adults, and dead adults of *Prostephanus truncatus* (Horn), maize stored pest, from Mexico, Tanzania, and Togo were isolated and identified *Pseudomonas fluorescens*. Cellulolytic bacterium, was isolated from the guts of the three colonies of *P. truncatus* and the two insect stages studied. The presence of this bacterium suggests that could be involved in the *P. truncatus* digestive system, therefore this insect is able to survive in a great variety of environments and to be considered as a potential pest for any agricultural crop. *Streptococcus faecalis*, *Streptococcus dysgalactiae*, and *Burkholderia cepacia*, isolated from guts and dead insects, could be the cause of *P. truncatus* mortality during stress periods.

**Introduction**

The large grain borer, *Prostephanus truncatus* (Horn), has been recognized as a common but minor pest of farm-stored maize in Mexico and Central and South America. Tigar et al. (1994) reported its distribution and abundance in Mexico. There are also published records of its occurrence in the Americas (Rees et al., 1990). During the early 1980s, this insect was introduced into Africa and is now the most destructive pest to farm-stored maize (*Zea mays* L.) and dried cassava (*Manihot esculenta* Crantz) and wooden structures (Hodges, 1986; McFarlane, 1988).

Cellulose, the major component of most lignocellulases, is a linear homopolymer of β-1, 4-linked glucose. Enzymatic hydrolysis of cellulose to glucose occurs by the action of cellulase, a mixture of enzymes with different activities and specificities (Breznak and Brune, 1994).

**Materials and Methods**

**Insects**

Three colonies of *P. truncatus*, from Mexico, Tanzania, and Togo, were maintained as previously described (Vazquez-Arsta et al., 1997). Fifteen *P. truncatus* last instars (30d old) and 2-wk-old adults were dissected by using a cold drop of Ringer solution.

**Gut extract preparation**

Fat tissue was physically removed. Whole guts were thoroughly rinsed 3 times with cold, sterile distilled water and then homogenized by using a sterile Potter homogenizer in 1 ml of sterile, distilled water. Rinses and homogenization were done in a biological safety cabinet.

**Dead insects extract preparation**

Twenty five dead insect adults of each colony were used. Dead insects were rinsed 3 times with sterile distilled water, then were washed during 15 min with 10% chloride solution, and finally were thoroughly rinsed 3 times with...
sterile distilled water. Dead insects were homogenized by using a sterile Potter homogenizer in 1 ml of sterile distilled water. Dead insects extract preparation was done in a biological safety cabinet (Rady, et al., 1992).

Isolation of aerobic microorganisms

A loopful of guts and dead insects homogenates was inoculated in petri plates containing nutrient agar (23 g/liter) This procedure was repeated to obtain the separation of microbial colonies. Pure cultures were obtained from the isolated colonies by inoculation in nutritive agar (23 g/liter). These pure cultures were used for further assays. Microorganisms were conserved as stock cultures by maculation of a pure culture loopful in test tubes with maculated nutrient agar (23 g/liter). The streak-plate method was used for inoculation. Petri plates and test tubes were incubated at 29 ± 1°C under aerobic conditions (Brock et al., 1997a).

Gram staining and bacterial identification

The gram-staining and identification techniques were performed as previously described (Vazquez-Arist 1997).

Results

Isolation of aerobic microorganisms

Seventeen bacteria were isolated from the guts of P. truncatus Six were from the Mexican colony and were named M1 and M2 (from adult guts), M3 and M4 (from larval guts), and M5 and M6 (from dead adults) Six were from the Tanzanian colony, Z1 and Z2 (from adult guts), Z3 and Z4 (from larval guts), and Z5 and Z6 (from dead adults) The last 5 were from the Togo colony, G1 and G2 (from adult guts), G3 and G4 (from larval guts), and G5 (from dead adults).

Gram staining

Eight gram-negative (M2, M4, Z1, Z3, Z6, G1, G4, and G5) and 9 gram-positive (M1, M3, M5, M6, Z2, Z4, Z5, G2, and G3) bacteria were isolated.

Bacterial identification

Table 1 shows the identification of the bacteria isolated from the P. truncatus adults and larval guts, as well as dead insects.

Discussion

This research shows the presence of Pseudomonas fluorescens, cellulolytic bacteria; Streptococcus (Enterococcus) faecium; and Streptococcus dysgalactiae in the digestive system of P. truncatus from Mexico, Tanzania, and Togo; in addition to Burkholderia cepacia (formerly known as Pseudomonas cepacia) from dead adults.

<p>| Table 1. Bacteria isolated and identified from P. Truncatus |</p>
<table>
<thead>
<tr>
<th>Origin of P. truncatus</th>
<th>Source</th>
<th>Code</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mexico</td>
<td>adult guts</td>
<td>M1</td>
<td>Streptococcus faecium</td>
</tr>
<tr>
<td></td>
<td>adult guts</td>
<td>M2</td>
<td>Pseudomonas fluorescens</td>
</tr>
<tr>
<td></td>
<td>larval guts</td>
<td>M3</td>
<td>Streptococcus dysgalactiae</td>
</tr>
<tr>
<td></td>
<td>larval guts</td>
<td>M4</td>
<td>Pseudomonas fluorescens</td>
</tr>
<tr>
<td></td>
<td>dead insects</td>
<td>M5</td>
<td>Streptococcus faecium</td>
</tr>
<tr>
<td></td>
<td>dead insects</td>
<td>M6</td>
<td>Streptococcus faecium</td>
</tr>
<tr>
<td></td>
<td>adult guts</td>
<td>Z1</td>
<td>Pseudomonas fluorescens</td>
</tr>
<tr>
<td></td>
<td>adult guts</td>
<td>Z2</td>
<td>Streptococcus faecium</td>
</tr>
<tr>
<td>Tanzania</td>
<td>larval guts</td>
<td>Z3</td>
<td>Pseudomonas fluorescens</td>
</tr>
<tr>
<td></td>
<td>larval guts</td>
<td>Z4</td>
<td>Streptococcus faecium</td>
</tr>
<tr>
<td></td>
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<td>Z5</td>
<td>Streptococcus faecium</td>
</tr>
<tr>
<td></td>
<td>dead insects</td>
<td>Z6</td>
<td>Burkholderia cepacia</td>
</tr>
<tr>
<td>Togo</td>
<td>adult guts</td>
<td>G1</td>
<td>Pseudomonas fluorescens</td>
</tr>
<tr>
<td></td>
<td>adult guts</td>
<td>G2</td>
<td>Streptococcus dysgalactiae</td>
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<td></td>
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<tr>
<td></td>
<td>dead insects</td>
<td>G5</td>
<td>Burkholderia cepacia</td>
</tr>
</tbody>
</table>

The use of nutritive agar in the isolation of these bacteria, instead carboxymethyl cellulose (Vazquez-Arista et al., 1997), gave uniform results among the adults and larval stages.

The bacterium, P. fluorescens, causes food spoilage in a wide variety of agricultural products, including milk, meat, and fresh vegetables. It is responsible for a substantial proportion of postharvest losses of agricultural commodities (Liao and McCallus, 1997), and rarely considered as an animal pathogen (Brock et al., 1997b). Hence, the isolation and identification of these bacteria from the adults and larval guts of beetles from 3 P. truncatus colonies of different origin, could confirm that this insect has the ability to feed on different types of carbohydrates; that is, P. truncatus does not depend on food where starch is the main source of carbohydrates such as maize and cassava, and it may successfully establish as a pest in a large variety of crops.

On the other hand, the genus Streptococcus contains a wide variety of species with quite distinct habitats, whose activities are of considerable practical importance to humans. The genus Enterococcus includes streptococci that
References


prey Prostephanus truncatus (Horn) (Col: Bostrichidae) in the Yucatan Peninsula, Mexico. Tropical Science. 30, 153–165.


