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

M. Vazquez-arista[1], G. L. Basurto-cadena[2], R. Vargas-becera[2] and R. E. Hinoiosa-rebollar[3]

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 faecium*, *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)

In white rot fungi and probably in insects, cellulose is digested by extracellular enzymes to glucose that can be used to meet the carbon and energy requirements of an organism (Martin, 1991)

The functions of endosymbiotic microorganisms in beetles are to synthesize some nutritionally essential substances that are lacking in their diet or to make possible the use of some components of the food that beetles would be unable to digest (Crowson, 1981).

Many wood-eating insects use cellulolytic enzymes that are either endogenously secreted or that are produced by symbiotic microorganisms such as protozoa, bacteria, and fungi (Becker, 1977; Breznak, 1982; Orpin and Anderson 1988; Coughlan, 1992) Other wood or foliage-eating insects are completely noncellulolytic and derive no benefit from the cellulose consumed in their diets; instead, they rely on the degradation of other polysaccharides such as starch for their nutrition (Martin, 1983)

The presence of cellulolytic bacteria in adults and larval guts of *P truncatus* from Tanzania suggests that this insect could be able to digest cellulose (Vazquez-Arista et al , 1997)

Materials and Methods

Insects

Three colonies of *P truncatus*, from Mexico, Tanzania, and Togo, were maintained as previously described (Vazquez-Arista 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 1ml 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

[1] Centro de Investigacity de Estudios Avanzados del IPN (CINVESTAV-IPN), Departamento de Biotecnolog y Bioquica, Unidad de Biotecnolo ge Ingenier Genica de Plantas

[2] Instituto de Ciencias Agrolas, Departamento de Alimentos, Universidad de Guanajuato, Mico

[3] Departamento de Microbiology , Escuela Nacional de Ciencias Biolicas del Instituto Politnico Nacional, Mico

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 nutritive 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 nutrient agar (23 g/liter). These pure cultures were used for further assays. Microorganisms were conserved as stock cultures by inoculation of a pure culture loopful in test tubes with inclined nutrient agar (23 g/liter). The streak-plate method was used for inoculation. Petri plates and test tubes were incubated at 29±1℃ 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.

Table 1. Bacteria isolated and identified from *P. Truncatus*

Origin of *P. truncatus*	Source	Code	Bacteria
Mexico	adult guts	M1	*Streptococcus faecium*
	adult guts	M2	*Pseudomonas fluorescens*
	larval guts	M3	*Streptococcus dysgalactiae*
	larval guts	M4	*Pseudomonas fluorescens*
	dead insects	M5	*Streptococcus faecium*
	dead insects	M6	*Streptococcus faecium*
Tanzania	adult guts	Z1	*Pseudomonas fluorescens*
	adult guts	Z2	*Streptococcus faecium*
	larval guts	Z3	*Pseudomonas fluorescens*
	larval guts	Z4	*Streptococcus faecium*
	dead insects	Z5	*Streptococcus faecium*
	dead insects	Z6	*Burkholderia cepacia*
Togo	adult guts	G1	*Pseudomonas fluorescens*
	adult guts	G2	*Streptococcus dysgalactiae*
	larval guts	G3	*Streptococcus dysgalactiae*
	larval guts	G4	*Pseudomonas fluorescens*
	dead insects	G5	*Burkholderia cepacia*

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 habits, whose activities are of considerable practical importance to humans. The genus *Enterococcus* includes streptococci that

are primarily of fecal origin and are considered pathogenic for humans (Klare et al., 1995), with some strains of considerable antibiotic resistance (Chadwick et al., 1996; Henning et al, 1996; Mato et al, 1996). Then, as *S. faecium* is a human pathogen, it could be considered as an opportunistic pathogen of *P. truncatus* too, and can be responsible of its death during starvation periods. The same consideration should be made with the presence of *Burkholderia cepacia*, isolated from dead insects, because it is now demonstrated that this bacterium is an opportunistic pathogen in patients with fibrocystic lung disease, due to its increasing association with fatal pulmonary infections (Reboli et al., 1996; Revets et al., 1996).

The presence of *S. dysgalactiae* in the digestive system of *P. truncatus* seems to have no connection whit any digestive process, because it is known that this bacterium is involved in the mastitis of dairy cattle, which is an inflammation of the bovine mammary gland (Simpson, 1995).

Because of the uniformity of the bacterial presence among the three colonies of *P truncatus*, make difficult to establish that they have different origin, as it was suggested by Vazquez-Arista et al. (1997).

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