



Characterization of Bacteria from the Gut of *Sitophilus zeamais* (Maize Weevil)

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ABSTRACT

Sitophilus zeamais is ranked among insects causing the greatest damage in stored to maize grains in most parts of the tropical and sub-tropical countries. The activities of the pests are so destructive that damage ranging from 20 to 90% has been recorded on the untreated maize. The current work focus on bacteriological isolation and identification of bacteria found in the gut of *S. zeamias*. Heterotrophic plate counts was carried out using pour plate method, morphological features of the bacterial by colonies appearance on the plate media and identification by microscope and biochemical test. The results showed that nutrient agar plate had Total heterotrophic bacteria count of 33×10^7 cfu/ml, Total Enterobacter count in MaCconkey agar was 17×10^7 cfu/ml and the Total coliform count in Eosin Methylene Blue agar was 32×10^7 cfu/ml. Five (5) different bacteria from the gut adults *S. zeamais* were isolated. The members of the genera isolated were Bacillaceae and Enterobacteriaceae families were isolated from *S. zeamais*. The results of biochemical tests conducted revealed the present of *E. coli*, *Enterobacter sp.*, *Acetobacter spp.*, *Bacillus spp.* and *Gluconobacter spp.* This study confirms that the gut of *S. zeamais* harboured. The bacteria isolated produces enzymes capable of degrades chitin, a structural polysaccharide in maize grains. Thus, the *S. zeamais* and the microorganisms inhabiting the gut share a highly symbiotic relationship.

Keywords: Bacteria, Colony, *Sitophilus zeamais* and *Zea mays*

INTRODUCTION

Maize (*Zea mays* L.) belongs to the family Gramineae and is one of the most important cereal crops in Africa. After wheat and rice, maize is the third most grown cereal (Danho *et al.*, 2002). It occupies less land area than either wheat or rice but has a greater average yield per unit area of about 5.5 tonnes per hectare (Ofori *et al.*, 2004). The grain is very nutritious, with about 70-72% digestible carbohydrate, 4 - 4.5% fats and oils and 9.5-11% proteins (Ofori *et al.*, 2004). Worldwide, about 66% of all maize is used for feeding livestock, 25% for human consumption and 9% for industrial purposes (Danho *et al.*, 2002)

Maize (*Zea mays*) is a grain grown by farmers in various parts of the country especially in Kaduna, Niger, Jos, Benue, Nasarawa, Kebbi and other part of Nigeria. The spoilage could limit its availability to consumers thereby causing economic loss to the farmers. Spoilage of maize reduces the nutritional, minerals and vitamins contents as well, increasing its allergic potential value and palatability of the feed. Nigeria like other developing countries, experiences serious problems with availability of quality maize, which often arise from poor storage (Armah and Asante, 2006; Danjuma *et al.*, 2018a). *Sitophilus zeamais* is implicated among insects causing the greatest damage in storage to maize grains in most parts of the tropics and sub-tropics (Danho *et al.*, 2002). The activities of this pest are so destructive that damage ranging from 20 to 90% has been recorded on the untreated maize (Issa *et al.*, 2011).

Several studies have been conducted on control of maize by *Sitophilus zeamais*, Mustafa *et al.*, (2017) in their study isolation and identification of bacteria from four important poplar pests Forty-three bacteria were isolated from the larvae and adults pest exhibiting characteristic disease symptoms of these pests in five different localities. Danjuma *et al.* (2018) study effects of leaf powder of *Citrus aurantifolia* and *Senna occidentalis* on the development of maize weevil (*Sitophilus zeamais* Motschulsky, Coleoptera Curculionidae). However,

information on isolates from the gut of *S. zeamais* (maize weevil) is limited. This prone this research work to microbiologically isolate and characterize the bacterial in gut of *S. zeamais* (maize weevil) that are responsible for enzymatic spoilage of maize grains in the study area.

MATERIALS AND METHODS

Study Area

The study was carried out in Birnin Kebbi Local Government Area of Kebbi State. It lies between latitude 10° and 13.5° N and longitude 3° and 6° W. The climate of the area is generally characterized by high temperature ranging between March and May with mean annual temperature 38°C and 42°C and the area experiencing harmattan wind between late November to early February, with temperature as low as 23°C. The study was carried out over a period of three (3) months (August to October 2018).

Sample Collection and Processing

Maize grain infested with *S. zeamais* was purchase from Birnin Kebbi central market. The *S. zeamais* were kept in biology laboratory of Waziri Umaru Federal Polytechnic Birnin Kebbi for two (2) weeks to develop. The adult *S. zeamais* were then identify. The identification key used was proposed by (Danjuma *et al.*, 2018). The adult maize weevils used were picked and place inside a jar reared on to maintain a mother stock which is used to obtain newly emerged adult of *S. zeamais* for experimental purposes. The jar was covered with a muslin cloth tied with a rubber band and kept under ambient condition for two (2) week, after which both the surviving and dead weevils were removed from the jar leaving only the grains. New generation of the weevils started emerging after 4-5 weeks (Danjuma *et al.*, 2018b). The newly emerge F1 generation were used for the experiment. In the laboratory, each *S. zeamais* was washed with 70% ethanol in a sterile conical flask (To decontaminate external surfaces (70% ethanol is bactericidal) and was transferred to sterilized flask and allowed to dry at room temperature under sterile

condition as described by Yusuf *et al.* (2018). The insects sample were placed in a sterilized stainless bowl, their surfaces were cleaned with 70% alcohol and placed on a sterile white tiles and each of the sample were homogenized using a pestle, and the homogenate was used as an inoculum. The inoculation was done in flasks containing various media that is, nutrient agar, and other enriched and selective media to isolate specialized group of organisms. These flask were kept for incubation and the medium inside was used as an inoculums for further isolation and identification.

Bacteriological Analysis

The media used for culturing were aseptically prepared in accordance with the manufacturer instruction. Nutrient agar was used for Total heterotrophic count (THC), MacConkey agar was used for Total enterobacteriaceae count and Eosin methyl blue agar used for Total *Escheria coli* count and autoclaved at 121°C for 15 minutes as described by Adebayo *et al.* (2015)

Microscope Analysis

Gram staining was carried out as described by Yusuf *et al.* (2018). The smear was allowed to air-dry and then passed over a flame in order to be fixed. After fixing, the smear was covered with a primary dye (crystal violet) and washed with water after one minute. The slide was covered with lugol's iodine and again washed with water after the same period. Acetone-alcohol was used to decolourize the smear and washed immediately with water. This was followed by application of safranin (secondary dye) and left for 30 seconds, then washed with water. Back of the slide was cleaned with cotton wool and allowed to air dry. The slides were examined through microscope using an oil immersion objective lens (x100).

Morphological Characteristic of Isolates

Colonies identifiable as discrete on the various agar were carefully examine macroscopically for cultural characteristics such as the shape, colour, size and consistency. Bacterial isolate were characterized based on macroscopic appearance and colonial morphology.

Biochemical Identification of Bacterial Isolates

The biochemical tests used in this work are Indole Test, Methyl red Test (MR), Catalase, citrate, Glucose, Lactose and Mannose as describe by Yusuf *et al.* (2018)

Indole Test Using Tryptone Water

The test organism was inoculated in a test tube of 4mL of sterile tryptone water. It was incubated at 37°C for 72 hours. Fifteen (15) drops of Kovac's, indole reagent were added and gently shaken and examined within 10 minutes for a red color in the surface layer

Methyl Red and Voges-Proskauer Test

MR-VP broth was prepared aseptically in sterile test tubes and inoculated with a pure culture of bacteria under investigation. The tubes were incubated for three days at 35°C. Five drops of methyl red reagent (for methyl red test) and Barrit's reagent (for Voges-Proskauer test) were added in each of the tube. Change of color was observed. Red and yellow color indicates positive and negative results respectively for both methyl red test and Voges-Proskauer tests

Catalase Test

Few drops of hydrogen peroxide solution were poured into a glass slide using a sterile wooden stick; several colonies of the test organism were removed and immersed in the hydrogen peroxide solution and observed for bubbles

Citrate Test Using Simmon's Citrate Agar

Slants of the medium were prepared in test tubes using a sterile wire loop. The slants were streaked with a suspension of the test organism and incubated at 35°C for 48 hours and examined for a bright-blue colour in the medium

Sugar Fermentation Test

This is used to know whether the organism can ferment sugar and produce acid. Fermentation tests were carried out using the following sugars: Glucose, sucrose, lactose, maltose, and mannitol. To each of 10ml of peptone water in the test tube, 1.5g of each sugar was separately dissolved into and labelled, 3 drops of 0.01% phenol red was added. Durham tubes were inserted in it and inverted position into the tubes for detection of gas production. The tubes were plugged with non-absorbent cotton wool and sealed with aluminium foil before being sterilized in an autoclave at 121°C for 15 minutes at 151 boiling pressure. The tubes were then aseptically inoculated with small bacterial colonies using sterile wire loop. The tubes were incubated for 24 hours at 37°C and on inoculated tube served as controls.

RESULTS AND DISCUSSION

The results in Table 1 show that Total heterotrophic count (THC) was higher in nutrient agar plate with 33×10^7 cfu/ml. The total enterobacter count in MacConkey agar was 17×10^7 cfu/ml and the total coliform count in Eosin Methylene Blue was 32×10^7 cfu/ml. This implies that the gut of *S. zeamais* harboured a widely range of bacteria. In the present study, five different species of bacteria were isolated from the adults of important poplar pest *S. zeamais* which affect maize (Tables 2). The results of biochemical tests conducted revealed the present of *E. coli*, *Enterobacter* sp., *Acetobacter* spp., *Bacillus* spp. and *Gluconobacter* spp. *Enterobacter* sp. Exhibits similar biochemical properties as reported by Madhaiyan, *et al.* (2010), where they isolated a Gram negative, motile *Enterobacter* sp. from rice. Their results indicate positive for catalase, oxidase, starch hydrolysis and growth on MacConkey, but negative for indole and urease. However, differences are observed in the motility and oxidase test, which was could be due to the absence of flagella and enzyme cytochrome c oxidase. The growth of *Enterobacter* sp. in a chemically defined medium (CDM) containing glucose, lactose, sucrose or starch as the sole carbon sources were presented in Table 3. Among the carbon sources tested, sucrose was found to be the best carbon source for the growth of the strain, followed by glucose. This slightly slow growth of the bacteria on glucose may be due to suboptimal level of cAMP, leading a situation called reversed diauxic shift, usually occur as a result of poor nitrogen sources (Bren *et al.*, 2016). While the sufficient utilization of the sucrose by the bacteria is due to the presence of an enzyme called sucrose phosphorylase. This enzyme catalyzes phosphorylation of sucrose to α -D-glucos-1-phosphate and fructose (Schwab *et al.*, 2007). Bacteria showed poor growth when lactose was supplied as sole carbon sources in the medium. This

indicates the absence of enzyme lactase (β -galactosidase) to break down lactose.

Table 1: Analysis of bacterial load from samples from gut of *S. zeamais*

Media	bacterial enumeration			Average
	10 ³ cfu/ml	10 ⁴ cfu/ml	10 ⁷ cfu/ml	
N.A	40	26	66	33
McC	20	14	34	17
E.M.B	35	29	64	32

Key: N.A- Nutrient agar, McC- MacConkey agar, E.M.B- Eosine methylene blue and cfu- coliform forming unit

Table 2: The Morphological Characteristics of the Isolates on Various Media Used.

Media used	Colony Morphological Features
N.A.	Small circular, whitish and moist colonies
	Small rough, irregular flat moist opaque colonies.
	Pin point, circular, whitish flat and translucent.
	Small whitish, circular and moist colony.
	Pin point, circular whitish, entire flat translucent and moist colonies.
E.M.B.	Small, circular, pink, entire elevated translucent and mucoid colony.
	Pin point, circular, pinkish, entire flat translucent, greenish metallic sheen and moist colonies.
McC	Pin point, circular whitish, entire flat translucent and moist colonies.

Key: N.A- Nutrient agar, McC- MacConkey agar, E.M.B- Eosine methylene blue and cfu- coliform forming unit

These bacteria were classified in four different genera: *Bacillus*, *Enterobacter*, *E. coli* and *Gluconobacter*. Ertürk *et al.* (2008) reported that *Bacillus* species are insect pathogens and have different insecticidal effects. This result is in line with the finding of Yaman *et al.* (2010) who isolated *Enterobacter amnigenus* from the bark beetle *Dendroctonus micans* and found it to be pathogenic on this pest with a rate of 72.2 % on the larvae and 37.5 % on the

adults. One of our isolates from the body of maize weevil was *Gluconobacter spp.* Some insect pathogenic species of the genus *Gluconobacter spp.* have been isolated from different insects (Yaman *et al.*, 2010) and would possibly be the bio-control agents against some insects (Sezen *et al.*, 2001).

Table 3: The biochemical Test Determination of the bacterial isolated

Bacteria	Gram reaction	Catalas	Indole	MR	VP	Citrate	Glucose	Lactose	Mannose
<i>E. coli</i>	-ve rod	+	+	+	-	+	+	+	+
<i>Enterobacter spp</i>	-ve rod	+	+	+	-	+	+	-	-
<i>Bacillus spp</i>	+ve bacilli	+	+	-	+	-	-	-	+
<i>Acetobacter spp</i>	-ve rod	+	+	-	+	+	+	+	+
<i>Gluconobacter spp</i>	-ve rod	+	-	-	+	+	+	-	+

Key: -ve (negative), +ve (positive), spp (specie), MR (Methyl red), VP (Voges-Proskauer)

This study confirms that the gut of *S. zeamais* harboured *Bacillus spp*, *E. coli*, *Acetobacter spp*, *Enterobacter spp* and *Gluconobacter spp*. Moreover, such bacteria produce

enzymes capable of degrading chitin, a structural polysaccharide in maize grains. The present study, also reported the cellulolytic and amylolytic bacteria. They

have elaborated enzymes like cellulase and amylase required digesting cellulose and starching respectively. Thus, the *S. zeamais* and the microorganisms inhabiting the gut share a highly symbiotic relationship.

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