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Microbiological Investigations on Gryllotalpa Africana [Orthoptera: Gryllotalpidae], an Edible Cricket of the Niger Delta

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Abstract: *This paper reports for the first time the bacterial distribution on Gryllotalpa africana, a mole cricket and one of the edible hexapods of the Niger Delta region of Nigeria. Children hunt it during the rainy and also at dry seasons and it is harvested as snacks in the rural settings of the region. It is eaten raw, boiled, fried or roasted along with other condiments including onions and pepper. It is a delicacy enjoyed in many other parts of Nigeria. Assessments were made to identify bacteria that are associated with different external structures of the edible mole cricket.*

The bacterial distribution on different external structures [the skin] of the adult Gryllotalpa africana (Orthoptera: Gryllotalpidae) was investigated and ten bacterial isolates were obtained; the genera Micrococus, Corynebacterium, Bacillus, Pseudomonas, Staphylococcus and Proteus. Fungal genera isolated were: Aspergillus, Alternaria, Fusarium, Penicillium and Rhizopus. Total Heterotrophic bacterial populations of the insect Gryllotalpa africana were: Head (8.00 x 10^7 cfu/g), Wing (5.12 x 10^7cfu/g), Leg (7.36 x 10^7) while total fungal count (1.00 x 10^6 cfu/g). The population of viable bacteria was higher than that of fungi. The percentage occurrence of the bacteria isolates on the three examined parts of the skin were: Head (Bacillus sp. 25%, Proteus sp. 66%, Staphylococcus sp. 5%, Micrococcus sp. 3%, Corynebacterium 1%), Wing (Bacillus sp. 35%, Proteus sp. 60%, Staphylococcus sp. 3%, Micrococcus sp. 1%, Corynebacterium 1%); Leg (Bacillus sp. 35%, Proteus sp. 45%, Staphylococcus sp. 16%, Micrococcus sp. 2%, Corynebacterium 2%). Summarily, considering the mean bacterial genera population; Proteus (57%)> Bacillus (31.7%) >Staphylococcus (8%) > Micrococcus (2%) > Corynebacterium (1.3%). The incidence of Bacillus and Staphylococcus is of great health concern.

Key words: *Gryllotalpa africana, edible insect, Niger Delta, bacterial distribution.*

I. Introduction

Mole crickets in the family Gryllotalpidae are distributed throughout temperate and tropical regions. These insects are best known for their digging forelimbs which different species possess worldwide with different modifications. The European mole cricket, Gryllotalpa gryllotalpa (Orthoptera: Gryllotalpidae) L., was introduced from Europe into the United States. Most crickets spend their days in burrows as they are mostly nocturnal they are seen at nights and are hunted at such hours by children who pour water into holes underground hunting them. As water enters into the holes, there is oxygen depletion a situation that leads to their suffocation and as they gasp for air, they are driven out of burrows and captured by their hunters. They damage vegetable gardens, seedling beds, eat seed, cereals, potato and almost all vegetables. Shoots and young plants often perish after damage [1, 2] The insect damages tobacco, all vegetables, corn, sunflower, cotton, fruits and forest trees in Turkey. G. gryllotalpa open gallery in soil and eat everything on their way while opening the gallery [1]. G. gryllotalpa is widespread in Europe, Russia, Turkey, Central Asia, Iran, Afghanistan, central and southern Asia, North Africa, America and southern Ukraine [3]. Chlorpyripos-ethyl and parathion-methyl have been used for the control of G. gryllotalpa in Turkey (URL-1). Because of hazards of the chemicals in the environment, these are no longer recommended for agricultural pest management.

Due to their edibility, in most parts of Nigeria especially in the Niger Delta, Eastern and Middle belt regions, we decided to assess the bacteria that are associated with the different parts of their body which are eaten by man. The insect, G. africana is very attractive for microbial studies; up till now, there is no study on the investigation of the bacteria composition of mole crickets. It is known that many bacteria which can be isolated from insects belong to the families Bacillaceae, Enterobacteriaceae and Pseudomonaceae. However, bacteria-insects interactions are not only pathogenic but also symbiotic. Symbiotic bacteria are ubiquitously located in insect's guts with these symbioses ranging from pathogenic to mutualistic and from facultative to obligate [4]. Bacterial symbionts are thought to enable their hosts to survive on restrictive diets by providing nutritional

supplements such as amino acids and vitamins[5]. The potential use of these organisms for biological control of insect pests has driven much of the current research on bacterial symbionts. Chagas disease, for example, is a vector-borne disease that affects 16-18 million people in regions of South and Central America. The Chagas disease vector, Rhodnius prolixus, harbors the symbiotic bacteria Rhodococcus rhodnii. It was found that the symbiotic bacteria could be genetically transformed to express an antitrypanosomal agent in the gut [6]. This discovery provides proof of principle for the use of symbionts as biological control agents. Beetles also minimize overcrowding by oxidizing aggregation pheromones into antiaggregants, both through their own and their microbial symbionts' biosynthetic pathways [7]. In members of the genus Bacillus and Paenibacillus, the HV region sequence is highly conserved within a species and has diverged sufficiently different between species, enabling identification and grouping of Bacillus and Paenibacillus species by sequence comparisons of the HV region, as it has shown for the genera Brevibacillus and Alicyclobacillus [8, 9, 10]. Here, we report on the isolation and identification of bacteria from Gryllotalpa africana.

II. Materials And Methods

Collection of samples:

The insect, Gryllotalpa africana was collected into a sterile container from Egbeda, Ikwerre Local Government area of Rivers State, Nigeria. Collection was made by squashing vegetables with volatiles; 20g of scent leaves, Ocimum gratissimum, 20g of waterleaf, Talinum triangulare. As they were attracted to the volatiles in the leaves, they came out to feed on them and they were trapped in the nets smeared with adhesive [11] laid around the holes. The crickets caught were transferred into sterile container and taken to the Post Graduate Entomology Laboratory of the Department of Applied and Environmental Biology of the Rivers State University of Science and Technology, Port Harcourt, Nigeria. This was used within 1hour of the collection for the isolation of bacteria and fungi employed in the study. The media used were Nutrient agar and Sabouraud Dextrose agar prepared according to standard specification.

Isolation of Microorganisms

The method used was the 10-fold dilution method of [12] with one gram (1g) of the different parts [Head-thorax, wing, leg and abdomen] aseptically transferred into 9ml of sterile saline in a test tube separately. The test tube was shaken vigorously to dislodge the skin-bacterial flora. Further, aliquot – 0.1ml of appropriate dilution was plated onto Nutrient agar using spread plate technique. This was incubated for 24hours at 30^0C, after which pure cultures of the different colonies were obtained. A loop full of the test organism was transferred into 10ml sterile nutrient agar broth; incubated at 28 ± 0.5^0C and stored in refrigerator at 4^0C for morphological and biochemical test.

Identification of Microorganisms

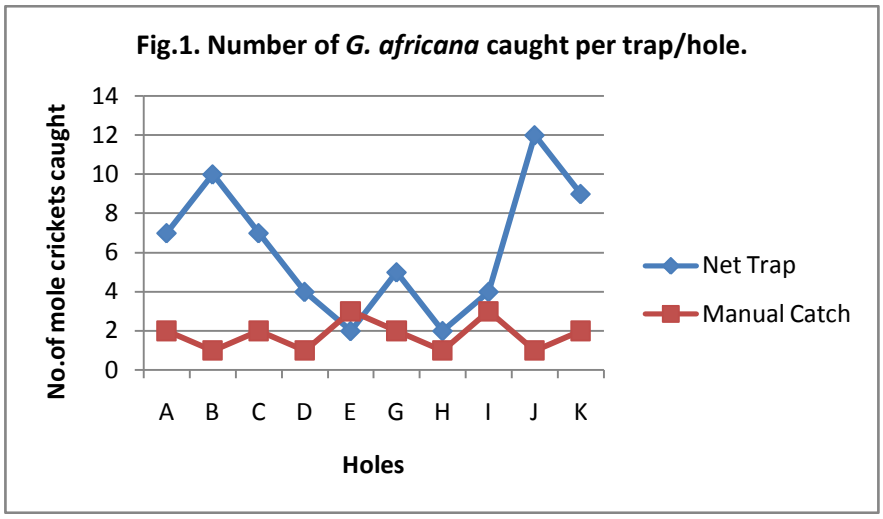
Gram reaction, motility, oxidative/fermentative (O/F) utilization of sugars, catalase, oxidase, indole production, urease, methyl red-Voges Proskauer, citrate, coagulase, hydrogen sulphide production tests were carried out according to methods described by [13]. Identification of the genera followed the scheme of APHA [1992] 14].

The method used for the isolation of fungi from the samples was carried out similarly on Sabouraud Dextrose agar incubated at 28^0C for 2-3days. Structural and morphological characteristics were used for identification of fungal isolates.

Statistical Analysis: ANOVA was used to establish significant differences among the means……. separate the means

III. Results And Discussion

The number of mole crickets caught by traps was significantly higher than those caught manually by hands [DMRT, P= 0.05][Fig. 1]. Bacterial and fungal isolates were made from different parts of the mole crickets body. Most of the mole crickets escaped in the attempt to capture them but those trapped in the nets remained intact as they held by the adhesive. Results showed that among the Staphylococcus and Bacillus isolates from the skin of the cricket were some strains of Staphylococcus aureus and Bacillus cereus.



Microbial Populations

Total Heterotrophic bacterial populations of the insect Gryllotalpa africana were: Head (8.00 x 10^7 cfu/g), Wing (5.12 x 10^7cfu/g), Leg (7.36 x 10^7) [Fig. 2] while total fungal count (1.00 x 10^6 cfu/g). **Total bacteria on the head capsule was 39%**, and this was **higher than other parts because this section is made of different components that are utilized by the insects during feeding. The number on the legs was also high as the mole utilizes this in digging its way through and thereby coming in contact with debris and soil with their** microorganisms. The population of viable bacteria was higher than that of fungi.

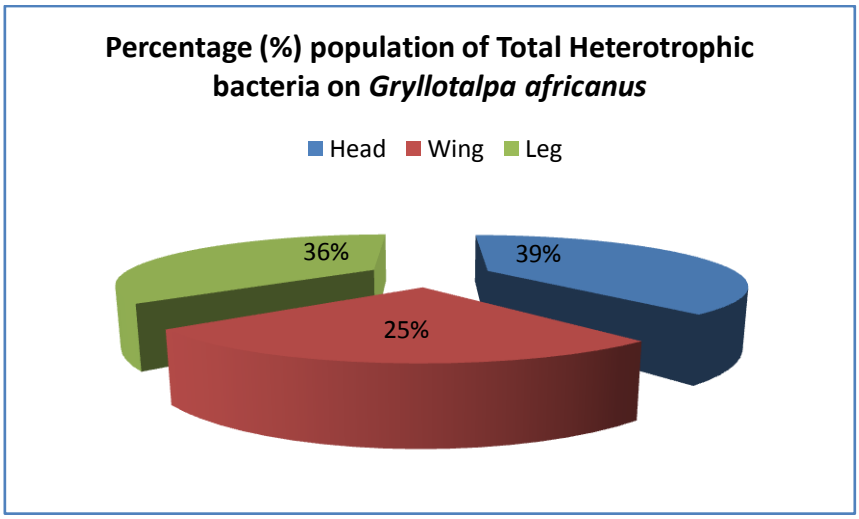


Table 1: Morphology of the Bacterial Isolates

Isolate	Shape	Texture	Color	Elevation	Translucent	Identification
A1	Bacilli	Moist	Cream	Praised	Opaque	Micrococcus sp
A2	Cocci	Dry	Cream	Flat	Apaque	Corynebacterium sp
A3	Rod	Mucloid	Cream	Raised	Opaque	Bacillus sp.
A4	Rod	Sticky	white	Raised	Transient	Proteus sp
A5	Cocci	Moist	Yellow	Smooth	Transient	Staphylococcus sp

Table 2: Biochemical Identification of Bacterial Isolates from Gryllotalpa africana.

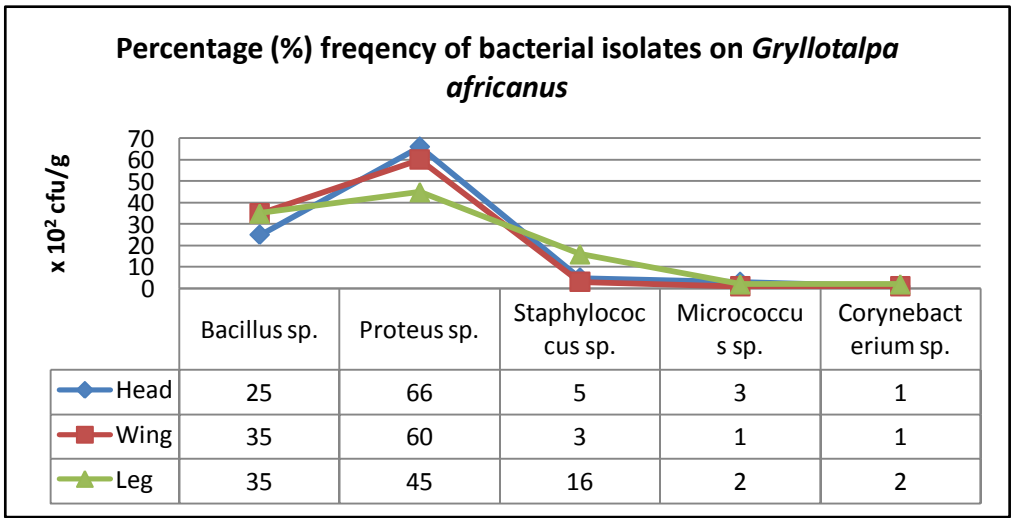
Isolates	Gram reaction	Oxidase	Citrate	Catalase	Coagulase	Methyl Red	Indole	Motility	Glucose	Lactose	Sucrose	Matase	Identification
A 1	+	+	+	+	-	-	+	-	A	AG	-	-	Micrococcus sp
A 2	+	+	-	+	-	+	+	+	AG	-	A	A	Corynebacterium sp
A 3	-	+	+	+	-	+	-	+	A	-	-	-	Bacillus sp
A 4	+	-	-	+	-	-	-	+	AG	-	-	-	Proteus sp
A 5	+	+	+	+	-	-	+	-	-	-	-	-	Staphylococus sp

+	=	Positive reaction
-	=	Negative reaction
AG	=	Acid and Gas
A	=	Acid

Ten bacterial isolates were obtained and they belong to the genera Micrococcus, Corynebacterium, Bacillus, Staphylococcus and Proteus. Among the Staphylococcus and Bacillus isolates from the skin of the cricket were some strains of Staphlococcus aureus and Bacillus cereus. The occurrence of Staphylococcus aureus on both skin of cricket is of significant medical importance. Bacillus cereus, are known enterotoxin producers [14, 15, 16]. Their presence on the edible mole cricket raises some public health concern. In places where crickets are used as food, the health risk becomes greater as in the Niger Delta, eastern and Middlebelts of Nigeria where mole crickets, some larval moths of Bunaea alcinoe, many palm weevils of both Oil and Raphia palms, such as Rhynchophorus phoenicis are eaten as snacks or in porridges, stews and soups[17, 18, 19]. Some of the edible insects of the Niger Delta are good sources of Protein that help the rural populace to augment their protein even though they have enough fish, animals, sea foods as other sources of proteins. Foods are frequently contaminated by the bacterial species (Staphylococcus aureus and Bacillus cereus) through the insect [cricket] whose habitat is within the human environment.

Table 3: Percentage occurrence of the bacterial isolates from Gryotallpa africana

Parts of Gryllotallpa africana	Bacillus sp. (%)	Proteus sp. (%)	Staphylococcus sp. (%)	Micrococcus sp. (%)	Corynebacterium sp. (%)	Total (%)
Head	25	66	5	3	1	100
Wing	35	60	3	1	1	100
Leg	35	45	16	2	2	100
Mean Value (%)	**31.7**	**57**	**8**	**2**	**1.3**	100



However, the cooking process applied to the contaminated food before consumption employed temperatures capable of eliminating Staphylococcus aureus but not Bacillus cereus**, an endospore former that may withstand such temperatures.**

Table 4: Characteristics of Fungal Isolates from Gryllotalpa africana

Isolates	Structural characteristic	Microcopic/Morphdogical characteristic	Probable organism
C_1	Dark-brown colonies with mainly surface growth, growing to cover the plate.	Conidial heads are radiate conidiophore is unbranded no rhizad, hyphae is septate	Aspergillus sp
C_2	Colonies appear pinkish in color	Sporangia spore are erect and branched	Alternaria sp
C3	White soft and cottony mass grew rapidly covering the surface of the petridish	Shore cresent shaped conidiophore, septate hyphae, with abundant microconidia	Fusarium sp
C_4	Green pigmentation with white background, powdery surface, circular in shape with an elevated centre	Conidiophore is septate erect and branched conidia spores are brush-like, hyphae is septate	Penicillium sp
C_5	Pale-brownish colony, growing whitish when young and becomes dark-brown with age.	Back pigmented sporangium, sporangiophore is unbranched with shore rhizoid, hyphae is non-septate	Rhizopus sp

Five fungal isolates belonging to the genera Aspergillus, Alternaria, Fusarium, Penicillium and Rhizopus were recovered from G. africana. The study has shown that caution should be taken in its consumption as food as it contains some pathogenic bacteria, efforts also should be made to ensure that the insect is boiled at high temperature, roasted but not to be eaten raw.

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