Journal of Applied Sciences Research, 2(11): 837-843, 2006

The Microbial Fauna Associated with the Larvae of *Oryctes Monocerus*

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Abstract: *Oryctes monoceros*, were cultured freshly to isolate and identify the micro organisms associated with them. The microbes were cultured from both the gut and body surface of the samples on nutrient Agar (for bacteria) and potato dextrose agar (for fungi). They were incubated at a temperature of 37°C for 24-48 h before observation. A variety of micro – organism were isolated from the samples. Some of the organisms were found to be pathogenic which include *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Bacillus cereus*. The non – pathogenic microbes recovered were *Bacillus subtilis,* and *Bacillus firmus*. The presence of pathogenic microbes as a microflora of *Oryctes monoceros*, therefore pose to be a threat to the well being of man and animals such as birds and fishes that feed on the grub. Preventive measure such as refrigeration is therefore recommended to avoid the implication of disease from feeding on these contaminated Insect.

Key words: Microorganism, *Oryctes monocerus*, pathoganic microbes, coliform

INTRODUCTION

Insect have played an important part in the history of human nutrition in Africa, Asia and Latin America[7] They were equally important resource for the Indians of Western North America, who like other indigenous groups expended much organisation in harvesting them[26]. Hundreds of species have been used as human food. Some of the important species or groups include grasshoppers, caterpillars, beetles, grubs, winged termites, bees, wasps, brood larvae and pupa as well as winged ants, cicadas and a variety of aquatic ones mostly found in fresh water.[10].

The multidimensional impacts and consumption of insects among certain tribes the world over has prompted myriads of research into the scientific and economic evaluation of some of these insects, which are locally available for use.[2].

The larvae of the rhinoceros beetles have been used widely as food in Africa and Asia. The larvae are found in all sorts of refuse, raphia trunks and palm trees. Of all the three species reported as food in Africa, *Oryctes monoceros* (*Scarabaeidae*: *Dynastinae*) breeds in dead standing coconut and oil palm in West Africa and decaying coconut logs in eastern Africa, *Oryctes boas* (*Scarabaeidae*: *Dynastinae*) breeds in rotting vegetation and manure heaps and *Oryctes owariensis* (*Scarabaeidae*: *Dynastinae*) in dead standing oil palms, coconut and raphia trunks.[10].

They are more frequently searched by children and women, the larvae are larger than the palm weevil *Rhyncophorus Phoenecis* commonly called "Itun" by the Yoruba's. They are washed thoroughly and fried[13].

Since insects are used as food by man, this research work is centered or aimed at documenting the microflora associated with *Oryctes monoceros Olivier*, with the aim of advising on any health implication.

MATERIALS AND METHODS

The samples of *Oryctes monoceros.Oliv* (*Scarabaeidae*: *Dynastinae*) were all collected from Itokin, a town in the South Western axis of Nigeria, which is on the boundaries of Lagos and Ogun State, It is about 20km from Ijebu – Ode in Ogun State and also about 15km to Ikorodu in Lagos State, it lies on latitude 6^{0}N and longitude 3^{0}E and has an elevation of 141ft.

The fresh samples were taken into the laboratory and a proximate analysis was carried out to determine the percentage of Crude protein, ether extract, Ash and moisture in each of the samples, after which fresh samples were again collected and taken into the laboratory for a complete microbial analysis, the fresh samples were cultured internally and externally on five different media which included the Eosin methylene blue agar, the nutrient agar, potato dextrose agar, MacConkey agar and the yeast extract agar and were incubated at 37°C for between 24-48 h according to the method of Banjo *et al.*,[3].

Furthermore, processed and dried versions of the samples (*Oryctes monoceros*, were taken to the laboratory for analysis, the processed samples being the fried and condimented *Oryctes monoceros*. These were cultured 4 times at intervals of 3 days within each day under room conditions and refrigeration conditions. These means that

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some of the samples were kept in the refrigerator from where it was cultured at intervals whilst the rest of the samples was kept in the room from where it was cultured at intervals. Results were analyzed every 3 days so as to detect any new growth and isolates. There after, the biochemical characterization of each microorganism isolated was determined. Various counting methods were used to determine the microbial load of each isolate, the total viable count was done on nutrient agar, the coliform count was carried out on MacConkey agar and Eosin methylene blue agar while the fungal count was carried out on potato dextrose agar.

Cultured Media Used:

- Eosin Methylene blue agar
- Nutrient agar
- MaConkey agar
- Potato dextrose agar
- Yeast Extract Sugar

Microbial Count: The pure culture of each colony was obtained by using a sterile wire loop. A sterile wire loop was used to streak each separate colony onto a new solidified PCA and YPDA plates, which were then incubated at 35°C for 24 h. Stock culture of each organism were made and kept on agar slant at 4°C and these were sub-cultured from time to time.

Coliform Bacterial Count: Estimation of the coliform count was done using the 3 tubes methods 10.0 ml, 1.0ml and 0.1ml each of appropriate diluents were pipetted separately into sterilized broth contained in screw capped tubes with already inserted Durham tubes. The test tubes were inoculated and incubated at 35°C for 2 days. Production of the gas by the coliform (if present) could be read from the Durham tubes. No gas production means absence of coliform.

Identification of isolate: The bacterial isolates were identified by carrying out following morphological and biochemical tests.

Gram staining: The pure isolates were stained according to Gram's technique as described by Baker[1].

Catalase tests, methyl Red Voges Proskawer (MRVP), Nitrate Reduction, Sugar Fermentation Carbohydrates) Test, Oxidase Test: These were carried out according to specifications by Olutiola *et al*.,[1].

Spore staining, Starch Hydrolysis, Citrate Utilisation, Urease Test: These were carried out according to specification by Harrigan and McCance[23].

Motility Test: This was by hanging drop technique as described by Humphries[20].

Indole Test: This was carried out according to specifications b Cruickshank *et al*.,[9] Beech *et al*.,[5].

Identification of Fungi: In identifying the fungi, slide preparation of the moulds were according to Beech, *et al*.,[5].

RESULTS AND DISCUSSIONS

In all, micro-organisms of the coliform group which are most time disease agents were amongst organisms isolated, they includes *Escherichia coli*, *Klebsiella aerogenes*, *Proteus* vulgaricus and *Staphylococcus aureus* amongst a host of others which were present in safe proportions at the initial stage of the experiment but later increased.

It was observed that the bacteria isolates were mostly Gram positive bacteria. *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aureginosa*, and *Aerobacter aerogenes* were the gram negative isolates.

Table 1 shows the results of the analysis of the fresh sample i. e. it shows the total viable count and, the coliform count expressed in colony forming unit (Cfu/g) of the fresh samples of *Oryctes monoceros* gut and body surface) The total viable count was performed on nutrient agar, coliform count done on MacConkey agar and Eosin methlylene blue while fungal count was carried out on potato dextrose agar.

Table 1: Results of Analysis – Fresh Sample of *Oryctes monocerus*

Sample Code	Table Viable Count cfu/g	Coliform Count cfu/g	Fungal Count cfu/g	Micro organisms isolated: Bacteria	Fungi	Coliform bacteria
Outer Skin of *Oryctes monocerus*	6.8 x105	7.0 x 105	NIL	*Bacillus firumus, Bacillus subtilis, Staphylococcus aureus Proteus Vulgorricus,*	------	*Escherichia coli Klebsiella aerogenes*
Inner or gut of *Oryctes monocerus*	7.5 x 105	12.0 x 105	NIL	*Proteus Vulgaricus, Bacillus aureus Bacillus Subtilis Staphylococcus aureus*	------	*Pseudomonas aureginosa, Escherichia coli Aerobacter aerogenes*

Table 2: Results of Micro-organisms Isolated at Various Days of Storage for *Oryctes monocerus* Olivier

	Organism Isolated under Room temperature storage			Organism isolated under Refrigerator Condition.		
	Bacteria	Fungi	Coliform bacteria	Bacteria	Fungi	Coliform bacteria
0 DAY	*Bacillus subtillis, Bacillus*		*Escherichria coli,*	*Bacillus cerus,*		
	firmus, Staphylococcus			*Bacillus Subtillis,*		
	aureus, Serratia merscens			*Staphylococcus aureus.*		
3rd DAY	*Proteus vulgaricus,*	*Fusarium*	*Pseudomonas*	*Bacillus cereus, Bacillus*		
	Staphylococcus aureus	*oxysopium*	*aureginosa*	*Subtillis,*		
	Bacillus lichoniformis		*Escherichia coli*	*Staphylococcus aureus*		
	,Micrococcus Sp					
6th DAY	*B. fimus, Proteus*	*Penicillum oxacillin,*	*Ps. aureginosa,*	*S. aureus, B. Subtillis,*	*Fusarium*	
	P. vulgaricus, S. aureus, B.	*Cladosporum Sp,*	*E. coli,*	*B. firmus,*	*oxysporium.*	
	B. Subtillis	*Aspergillus, niger,,*				
	Serratia marscenes,	*Penicillum*				
		Chrysogenum.				
9th DAY	*B. Subtitis, B. firmus,*	*Fusarium*	*E. coli,*	*S. aureus, B. Subtilis,*		
	Proteus vulgaricus, S.	*conpactium,*	*P. aureginosa,*	*B. firmus*	*Fusarium*	
	aureus,	*A. niger*	*Klebsiella aerogenes*	*oxysporium,*	*Pseudomonas*	*aureginosa*
	Proteus morgami,,	*Cladosporum Sp.*				
	Micrococcus Sp.					

Table 3: Microbial Load of the Prepared Sample of *Oryctes monocerus* at Various Days of Storage

	0 Day		3rd Day		6th Day		9th Day	
	Room Temperature	Refrigerator	Room Temperature	Refrigerator	Room Temperature	Refrigerator	Room Temperature	Refrigerator
Total Viable Count (Cfu/g)	4.8×10^6	0.4×10^6	7.5×10^6	0.4×10^6	12.7×10^6	0.5×10^6	18.0×10^6	0.5×10^6
Coliform Count (Cfu/g)	2.5×10^6	0.2×10^6	1.2×10^6	0.2×10^6	3.8×10^6	0.2×10^6	9.5×10^6	0.2×10^6
Fungal Count (Cfu/g)	NIL	NIL	0.1×10^6	NIL	0.4×10^6	0.1×10^6	0.8×10^6	0.1×10^6

Table 4: Proximate Analysis of Sample

% Crude Protein	% Other Extract	% Ash	% Moisture	Vitamin & Minerals	% Vitamin & Mineral
58.30	14.58	0.87	88.34	Rich in Zinc thiamin and riboflavin	100g > 100% of daily requirement

Table 2 shows the micro-organisms isolated from prepared samples of *Orcytes monoceros* under room temperature and under refrigerator condition at intervals of 3 days. A progressive increase in total microbial population and count was observed.

Table 3 shows the microbial load of the processed *Oryctes monoceros* at various days of storage, the load was initially minimal but later generally increased as the experiment progressed though at a reduced rate under refrigerator conditions.

Table 4 shows that the proximate Analysis of the various samples and also suggests that *Oryctes monoceros* is very high in Crude protein and also in moisture content, these suggests or explains why it is very nutritious and also why it spoils easily after preparation.

Table 5 Shows the biochemical and morphological characterization of the types of organisms isolated from the samples *Oryctes monoceros*, They are isolated from the gut and the outer skin or the body surface. The media used in the biochemical characterization of the isolates

Table 5: Biochemical Characterization of the Isolates

Media Used	Grain stain	Shape	Motility	Catalase	Oxidase	Urease	Coagulase	Indole	Methyl red	Voges Proskaeur Test h
NA	0	R	0	0	-	0	-	0	0	-
McC	-	R	0	0	-	-	-	0	0	-
NA	0	R	0	0	-	-	-	-	-	-
NA	0	R	0	0	-	-	-	-	-	-
DCA	-	R	0	0	-	-	-	0	0	-
NA	-	R	0	0	0	0	-	-	-	-
NA	0	R	0	0	0	0	-	-	-	-
McC	-	R	0	0	-	0	-	0	0	-
MSA	0	C	-	0	-	0	+	-	-	-
NA	0	R	0	0	-	0	-	0	-	-
NA	0	R	0	0	0	-	-	0	-	-
MRS	0	C	0	0	0	-	-	-	-	-

Media Used	Casein hbydrolysis	Glatin hbydrolysis	Strach hbydrolysis	Citrate utilization	Glucose	fructose	Lactose	Manmitol	Raffinossse	Arabinossse	*Probable Identification*
NA	-	-	0	A/G	A/G	A/G	A/G	A/G	A/G	A/G	Proteus vulgarius
McC	-	0	-	A/G	A/G	A/G	A/G	A/G	A/G	A/G	*Escherichia coli*
NA	0	0	0	0	A/G	A/G	A/G	A/G	A/G	A/G	*Bacillus firmus*
NA	-	-	0	0	A	A	A	A	A	A	*Bacillus subtilis*
DCA	-	-	0	0	A	A/G	A/G	A/G	A/G	A	Klebsiella aerogenes
NA	0	0	0	0	A	A	A	A	A	A	*Pseudomonas aureginosa*
NA	0	0	0	0	A/G	A/G	d	A/G	WA	*WA*	*Bacillus cereus*
McC	0	-	0	0	A/G	A/G	A/G	A/G	A/G	A/G	*Aerobacter aerogenes*
MSA	-	0	0	0	A	A	A	A	A	A	*Staphylocossus aureus*
NA	0	0	0	-	A	WA	A	WA	A	A	Bacillus lichenformis
NA	0	0	0	A	A	A	A	A	A	A	*Serratia marscenes*
MRS	0	-	-	A	A	A	A	A	A	A	*Micccrococcus sp.*

KEYS

+	=	Positive	R	=	Rod	McC	=	MacConkey Agar
-	=	Negative	C	=	Cocci	DCA	=	Deoxychocolate Agar
A	=	Acid Production	WA	=	Weak Acid Production	MRS	=	Man de Rugga and Shape Medium
A/G	=	Acid / Gas Production	NA	=	Nutrient Agar			

included nutrient agar, MacConkey agar, Deoxychocolate agar, and Man de Rugga and shape medium.

Table 6 Describes the identity of the fungi that was cultured on potato dextrose Agar. The fungi recovered from all samples were *Aspergillus niger*, *Fusarium oxysporium*, *Fusarium compacticum Cladosporium sp*, *Penicillum oxalicum* and *Penicillum chysogenum*.

Some insects serves as Vectors or passive intermediate hosts of vertebrate pathogens such as bacteria, protozoa, virus or helminthes[17]as it is evident in the results obtained from the analysis carried out on the insect *Oryctes monoceros*, From the study, it was evident that *O. monoceros* carry on an appreciable microbial flora which are mainly bacteria. The bacteria flora which colonize *Orcytes monoceros* included *Staphylococcus aureus*, *Proteus vulgaricus*, *Escherichia coli* and *Klebsiella aerogenes* the finding is similar to the previous report of Duquid *et al*.[12] and Baumagartner[6] who isolated similar species of bacteria on dried fish and Banjo *et al*.[3,4] who isolated these species on the insects *Alphitobius diaperinus* and *Hermetia illucens* respectively. but in contrast to the work of Osinubi[24] who isolated *Salmonella sp*, *Shigella sp* and *Enterobacter sp.* on dried fish. The reason for this microbial growth might be due to the habitat of the larvae rotten vegetation and decaying palm logs and also improper handling of the food materials during processing, careless exposure of the food to the environment by street hawkers and inadequate sanitary measures in our markets.

Table 6: Description of Fungi Isolates

Fungi Isolated	Description of the Isolates
Fusarium compacticum	Flocose in texture, whitish – cream and deep rose – red to burgundy in reverse. Chlamydospores are abundant in chains or cluster, rough golden yellow.
Clasdoporium sp	The growth is very slow and remain relatively small, seldom exceeding a diameter of 1-2 cm. There is little or no aerial mycelium, the surface of the colonies is olive – green to olive – brown and powdery with spores.
Fusarium oxysporum	The texture is floccose and whitish – cream in colour. Chlamydospores are abundant and usually on hyphae. The reverse is pale to bluish – violet in colour.
Aspergillus niger	Blackish – brown often with yellow mycelium. Reverse is greenish – yellow to yellow – orange. Its head is globose, splitting with age. Its motulae is long, closely packed and brownish.
Penicillum oxalicum	The texture is velutinous, sporulation very heavy. The observe is grayish – green while the reverse is pale yellow. The stipes is long and smooth. The penicillum is asymmetrically biverticillate, metulae closely appressed, phialides acrose, Collula very short. The conidia is ellipsoidal, large, smooth, pale green.
Penicillum chrysogenum	It is 2.7 to 4.5 cm in diameter. The texture is sulcate and velutionous. It is bluish – green to (dark) green in obverse. Its reverse is yellow (occasionally creamy). It has a short, smooth stipe. The penicillum is penicillin is tervenicillate, philades, ampulliform, collula very short, to spherical, smooth and greenish

The insects in addition might have come into contact with the soil at one time or the other during processes to sun drying it and this account for the presence of *Bacillus* which is known to be soil inhabitant. This group of micro-organisms is as well transmitted by air so continued exposure of the foods could also result in contamination from this source.

Another factor that might explain the presence of *Bacillus cereus* could be due to the fact that these bacteria are endospore formers and as known, endospore formers have a high resistance to heat[18]. So the bacteria could survive the frezing processes involved in the processing of the *Orcytes monoceros*.

The isolation of *Bacillus sp* as one of the microflora of these grubs is similar to the report by Frazier[16] who implicated *Bacillus sp* as the cause of sponginess in dried beef or beef hams.

The predominance of *Staphylococcus aureus* in the sample (*Orcytes monoceros)* is probably due to the fact that reports had shown that apparently healthy humans including the fish and meat sellers and also grub sellers are healthy carriers of the organisms in most parts of the world including Nigeria[21].

Similarly, the presence of *Escherichia coli* which is an enteric pathogen on all the samples might be due to the presence of enteric organisms in the water used for domestic purposes in many parts of Nigeria[22]. Which has been used in processing these specimens.

The presence of M*icrococcus* a gram – positive cocci could be due to the fact that gram – positive cocci are relatively resistant to reduced water potential and tolerate drying and high salt fairly well.[8] since they are also reported to be associated with fresh meat[16] they could have been on the *Orytes monoceros* and survived the heating and frying processes.

The occurrence of *Aspergillus sp*. as the main fungal isolates along with *Penicillum* and *Fusarium oxysporium*, tallies with the work of Faparusi[15] who isolated similar species from dried fishes and Banjo *et al*.[3] on the body and gut of the insect *Alphitobius diaperinus*. It could be attributable to the fact that during the drying and smoking processes, there is a considerable reduction in the water activity of these food products. And these encourage the growth of these moulds especially the *Aspergillus* which have been reported to be able to grow in high concentration of sugar and salt, indicating that they extract water required for their growth from relatively dry sources.[25].

Aspergillus niger was also isolated, similarly De las casas *et al*.,[11] recovered Aspergillus sp from mealworm and they were detected to produce poisonous toxins called mycotoxins[14]. These toxins could cause infection in livestocks.

The recovery of pathogen micro – organism as microflora of these organisms shows that use of these organisms as food posses a risk. Microbes such as *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaricus* and *Aerobacter aerogenes* are liable to cause various infectious diseases in humans and also careful breeding of the insect and proper processing to reduce the microbial load is necessary before consumption by man and other animals.

Conclusions: If insects become widely accepted as food item in developing and industrialized countries, the economic implications are obvious. They would form a whole new class of foods made to order for low – input small business and small farm production. International trade in edible insects would almost certainly increase. However more attention should be directed toward assessing the risk factors in the edible insect groups. From the experiments and tests carried out, it is obvious that the arthropods serves as subtle vectors or passive hosts of vertebrate pathogens such as bacteria and fungi which can cause devastating infections in man.

However, the long history of human use suggests with little evidence to the contrary, that the insects intentionally harvested for human use or consumption do not pose any significant problem[17].

The contamination of dried food products particularly food products like the *Oryctes monoceros* including smoked one, may be due to improper processing, handling during retail and purchase and exposure to air. The contamination of these products might lead to disease conditions in consumers when the food is consumed, thus it is suggested that careful attempt must be made to reduce as much as possible, contamination due to exposure of the products to air and indiscriminate handling. This can be achieved by placing the products in show glass for retail or otherwise packaging and sealing or tying them up in transparent cellophane bags. The retailers of the partially dried and fried grubs varieties should also be advised to ensure proper heating of the products to eliminate as much microbes or spores of the microbes as possible.

During retail, they might use a utensil like a knife or pincers to handle the products instead of bare hands.

All these measures strictly adhered to, will go a long way in reducing the microbial load of these products as well as contamination due to varied and constant handling of the products.

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