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THE MICROBIAL FLORA OF THE DIFFERENT GUT REGIONS OF THE VARIEGATED GRASSHOPPER *ZONOCERUS VARIEGATUS* (L) (ORTHOPTERA: PYRGOMORPHIDAE

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ABSTRACT

The microbial flora of the gut regions and gut contents of the variegated grasshopper Zonocerus variegatus instars was studied using the pour plate technique. The gut sections (Fore-, mid-, and hind-gut) harboured a variety organisms mainly bacteria, fungi and mould. Yeasts species isolated were Candida, Saccharomyces and Pichia spp. Aspergilus, Penicillium, Fusarium and Rhizopus spp. Both gram positive and gram negative bacteria were isolated. The gram negative bacteria isolated were mainly rods and members of the family Enterobacteriaceae including Proteus, Alcaligenes, Escherichia and Serratia. These species were more widely distributed in and more frequently isolated from the gut regions and their contents than the gram positive bacteria which were represented by Sreptococcus, Lactobacillus and Staphylococcus species. Gram positive bacteria were isolated from the gut extracts of the 3rd and 4th instars but were found in some of the gut regions of the 6th and adult instars. Mould population decreased gradually as the grasshopper was moulting from the 3rd to adult instars. Ranking according to microbial load was adult> 6th >4th >3rd, a reflection of their gut capacity since the microbes were part of the diet. The number and types of microbes in the gut regions of these instars are similar but significantly different in terms of total microbial load (P≥0.05). The results of this study could provide a lead into the proper understanding of the physiological processes involved in the digestion of plant materials by the insect.

Keywords: *Z.variegatus*, microbial, bacteria, mould, yeast ,gut

INTRODUCTION

Zonocerus variegatus (Orthoptera: Pyrgomorhidae), the variegated grasshopper is an insect that is aposematically coloured, sequesters toxic chemicals from plants (Chapman et a.,l 1986).The insect commonly lives in dense groups and has a repellent gland, which is situated in the 1st and 2nd abdominal segments (Idowu, 1995). The grasshopper is a polyphagous with the early instars feeding on *Chromolaena odorata* (Siam weed) while the later instars prefer *Manihot esculenta* (Cassava) (Chapman *et al.*, 1986).

Literature review has shown that relatively few studies on the physiology of *Z.variegatus* has been carried out, indicating, that very little is known on the physiology of its digestive process (Idowu,1994). Although, Balogun (1972) and Modder (1977) reported the presence of glycosidase enzymes in the gut homogenate of *Z.variegatus*, the functions of these enzymes and the mechanisms of digestion of the food eaten by the grasshopper are yet to be studied. In addition to their ability to secrete enzymes for the digestion of their food, some insects are known to harbour microorganisms in their digestive tract that assist in the digestion of components of the insect's food (Mead *et al* 1988). For example, the gut of the adult locusta *Schistocerca gregaria* has a wide range of bacterial types with the predominant flora in the midgut and hindgut regions being members of the family *Enterobacteriaceae* and motile *Streptococci* (Hunt and Charnley 1981). There is however no information in the microbial flora of the gut of *Zonocerus variegatus*. This study is therefore aimed at studying the ecology of the microbial flora of the gut region of some instars of *Z.variegatus*, as well as the microbial of their gut content.

Materials and Methods

Collection and Preparation of Specimen: Twenty

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20) grasshoppers were used in this study (5 for each instar). The freshly collected grasshoppers from the field were kept in aerated containers at refrigerated temperature until dissection to prevent regurgitation of gut contents. They were killed by breaking the cervical membrane and thus severing the ventral nerve cord. Each grasshopper was surface sterilized by swabbing with a tincture of iodine followed by 70% ethanol. The body cavity was opened by a ventral longitudinal incision. The gut was partitioned in situ into 4 sections by means of double ligatures placed between the foregut and midgut, midgut and hindgut, midgut and gastric caeca, using a flamed forcep. The different regions were separated by cutting between the ligatures. The hindgut was freed of malpighian tubules so as to exclude microogrnism inhabiting the tubule which are not necessarily concerned with digestion of food materials. The gut contents of the various parts were emptied into labelled petri dishes while the walls were washed thoroughly with distilled water to free it from any adhering material. The gut contents were then added to the distilled water used for washing the gut walls. Using a glass homogeniser with a Teflon pestle, each gut section was then highly homogenized in 1ml of sterile distilled water. They were decanted into labelled screw-capped bottles containing 4ml sterilized water.

Microbiological analyses: From all samples, 1ml sub-sample was homogenized in 9ml sterile water and ten fold serial dilutions were made. 0.1ml aliquot of each sample was inoculated by the pour plate technique using the following: Potato Dextrose Agar (PDA, Difco, USA) to which 0.01% (w/v) sterile oxytetracycline was added after autoclaving. This was used for enumeration of fungi. Nutrient agar (NA) and deMann Rogosa Sharpe medium (MRS) (Oxford, England) were used for enumeration of bacteria. PDA plates were incubated at 30^{oC}, 5days while NA and MRS plates were incubated at 37^{oC} for 5 days.

Viable Counts:At intervals depending on the medium, the colony forming units (cfu) were determined using the pour plate method. Bacterial counts were made on plate count agar while fungal counts were made on PDA medium (adjusted to $pH^{3.5}$ with lactic acid).

Characterization of the Microflora: Purified colonies were grouped according to their colony morphology and cell characteristics. Yeasts and moulds were identified from their micromorphology after staining with cotton blue lactophenol. Yeasts isolates further identified according to Kreger-Van rij (1984) by pseudomycelium formation and pattern of sugar fermentation (glucose, galactose, maltose, lactose and raffinose) as well as growth at 37^{oC}. The bacterial isolates were identified using Bergey's manual of Systematic bacteriology (Sneath *et al*, 1986) and the methods of Hugh and Leifson (1953) and Harrigan and MacCance (1976).

Statistical Analysis: A comparism of mean microbial population obtained from the different instars was carried out by means of ANOVA and t-test ($P \geq 0.05$).

RESULTS

Microbial Counts: The highest number of colony forming units were found in the adult instar, for all groups of organisms. Counts of yeasts ranged from 1.4×10^4 to 4.3×10^4; bacterial from 2.3×10^4 to 8.3×10^4 and mould counts from 0.3×10^4 to 1.6×10^4 (Table 1).
The 6th instar had the next highest numbers of microorganisms with yeasts counts ranging from 1.7×10^4 to 2.8×10^4 in both the foregut and gastric caeca; mould counts ranged from 0.2×10^4 to 1.2×10^4 and bacterial counts ranged from 0.7×10^4 to 4.7×10^4. However moulds were not detected in the fore and mid gut as well as the gastric caeca of the 6th instars. Yeasts count in the 4th instar ranged from 0.4×10^4 to 2.0×10^4; bacterial from 0.3×10^4 in the midgut and hindgut to 2.6×10^4 in the gastric caeca and mould from 0.1×10^4 in the mid- and hind-gut contents to 0.4×10^4 in the gastric caeca. Moulds were not detected in the fore- and hindguts of the 4th instar. The 3rd instar generally had the lowest number of microorganisms. Yeast counts ranged from 0.2×10^4 to 0.8×10^4; bacterial from 0.3×10^4 to 2.1×10^4 and mould from 0.4×10^4 in the foregut and gastric caeca to 1.3×10^4 in the contents of the hindgut. There was no significant difference between the total microbial counts of the adult and 6th instars in all the different gut regions ($P \geq 0.05$). Also, no significant difference was recorded for the total population of microbes obtained in the gut of the 3rd and 4th instars ($P \geq 0.05$).

Table 1: Total Viable Microbial Counts (cfu/ml, 10^4) in the gut regions of instars of *Zonocerus variegatus*

Gut Regions	Microbial Counts (10^4)											
	Adult	6th instar	4th instar	3rd instar	Adult	6th instar	4th instar	3rd instar	Adult	6th instar	4th instar	3rd instar
	Yeasts				Moulds				Bacteria			
Foregut	2.1	2.8	1.6	0.4	1.6	n.d	n.d	0.6	8.0	1.0	1.9	1.3
Foregut content	2.2	2.4	1.1	0.2	n.d	0.4	0.2	0.9	5.0	2.2	0.8	0.3
Midgut	1.4	1.8	0.8	0.3	0.3	n.d	0.2	0.7	3.0	4.7	0.3	0.7
Midgut content	2.5	1.7	1.7	0.2	n.d	0.9	0.1	1.0	8.1	0.7	1.4	1.0
Hindgut	4.3	2.7	2.0	0.4	n.d	0.2	n.d	0.8	2.8	3.6	0.3	1.0
Hindgut content	2.1	2.5	0.4	0.8	n.d	1.2	0.1	1.3	3.7	3.1	1.5	2.1
Gastric caeca	3.9	2.8	0.6	0.2	n.d	n.d	0.4	0.6	2.3	1.9	2.6	0.5

n.d: Not detected

Characterization of Micro flora: Yeast isolates were identified as *Candida, Saccharomyces* and *Pichia*. One of the *Saccharomyces sp*. was identified as *S.cerevisae*. Four genera of moulds were isolated: *Aspergilus, Pencicillum, Fusarium* and *Rhizopus sp*. The gram-positive non-motile rods and cocci were identified as *Staphylococcus* (catalase +ve cocci producing acid from glucose but not from lactose). *Streptococcus* (catalase –ve cocci producing acid from both glucose and lactose) and *Lactobacillus* (catalase –ve rods producing acid from both glucose and lactose). Gram-negative motile and non-motile rods were members of the family *Enterobacteriaceace* and identified as *Proteus, Alcaligenes, Escherichia, Enterobacter, Pseudomonas* and *Serratia sp*. The list of isolated micro flora in the different instars is shown on Table 2.

Distribution of micro flora: Table 3 shows the distribution of the various yeasts, mould and bacterial isolates in the various gut regions of the four instars examined. Among the yeasts isolates *Candida sp* was the most widely distributed organism in all the gut regions of the different stages of the insect examined and most frequently isolated. This was followed by *S.cerevisiae, Pichia sp* being the least commonly isolated. There was no particular trend in the distribution of the mould flora. Gram-positive bacteria were not isolated from the 3rd and 4th instars while they were isolated in some regions in both the adult and the 6th instar, occurring more in the adult than in the 6th instar. Gram-negative bacteria were more frequently isolated than the gram positive bacteria. Of the *Enterobacteria, E.coli* was most commonly distributed in all the regions of the 3rd and 4th instar while *Alcaligenes* was widely distributed in the gut regions of the 6th instar.

DISCUSSION

A variety of microorganisms were isolated from the gut regions of the four developmental stages of *Z.variegatus* examined in the present study (Tables 2 & 3). These results show that the gut of the grasshopper is rich with a variety of microoganisms. Comparable results were obtained by Hunt and Charnley (1981) and

Table 2: List of isolated microflora (bacterial and fungi) from *Z.variegatus*

Bacteria		Yeasts		Moulds	
Isolates code	Identity	Isolates code	Identity	Isolates code	Identity
BI	Proteus sp	YI	Candida sp	MI	Aspergillus
BII	Alcaligens sp	YII	Saccharomyces cerevisae	MII	Penicillum sp
BIII	Streptococcus sp	YII	Saccharomyces sp	MIII	Fusarium sp
BIV	Esherichia coli	YIV	Pichia sp	MIV	Rhizopus
BV	Lactobacillus sp				
BVI	Enterobacter sp				
BVII	Pseudomonas				
BVIII	Staphylococcus sp				
BIX	Serratia sp				

Table 3:Distribution of isolated micro flora in the gut regions of instars of Z.variegatus.

	Adult	6th instar	4th instar	3rd instar	Adult	6th instar	4th instar	3rd instar	Adult	6th Instar	4th instar	3rd instar
Gut Regions		**Yeasts**				**Moulds**				**Bacteria**		
Foregut	YI	YI,YII	YI,YII	YI	MI	-	-	MI	BI,BII,BIII	BI,BVI	BII,BIII	BII,BIV
Foregut content	YI,YII,YIII	YI,YIV	YI,YII	YI	-	MII	MII	MI	BV,BVII,BVIII	BII,BIII	BII	BII,BIV
Midgut	YII	YII,YIII	YI,YIV	YII	MI	-	MIII	MI	BII,BIV,BV	BII,BIX	BIV,BVI	BIV,BIX
Midgut content	YI,YII	YI,YII	YI,YIII	YII	-	MII	MIV	MIII	BI,BII,BIII	BII,BIII	BII,BIV	BII,BIV
Hindgut	YI,YII	YI,YII	YI,YIII	YII	-	MIII	-	MIII	BVI	BII,BIII,BVI	BIV,BVI	BII,BIV
Hindgut content	YI	YI,YIV	YI,YIII	YI,YII	-	MIII	MIV	MIII	BV,BVI	BVI,BIX	BII,BIV	BII,BIV
Gastric caeca	YI,YII	YI,YII,YIII	YIV	YI	-	-	MII	MII	BV,BVI	BII,BIII	BII,BV	BII

Key: See Table 2

Mead *et al* (1988) for *Schistocerca gregaria* and *Melanoplus sanguinipes* respectively. The isolated microorganisms were randomly distributed in the gut regions and gut contents (Table 3) except for moulds that were virtually absent in the different gut regions of the adult instar. However, there was similarity in the types of microorganism found in all the instars. Results of microbial counts generally did not show any significant differences among the adult and the 6th instars on one hand and the 3rd and 4th instars on the other hand ($P \geq 0.05$). This differences could be due to differences in diets among the instars. The early instars, 1st – 4th are known to prefer *Chromolaena odorata* while the later instars, 5th to adult feed and grow very well on *Manihot esculenta* (Chapman *et al.*1986).

Morphological studies on the alimentary tract of *Z.variegatus* showed a progressive increase in the size and volume of the different gut regions of the grasshopper moults from the 1st to the adult instar (Idowu, unpublished). Earlier, Idowu (1996) reported that the amount of secretion stored and discharged by the repellent gland of *Zonocerus* is a function of the size and volume of the gland lumen. Microorganisms are usually taken into the gut of insects along with food (Hunt & Charnley 1981). Therefore, the types and numbers of microorganisms observed in this study are probably a reflection of the types and amounts of food ingested by the different instars which is also reflection of the capacity of their gut regions. The numbers of the isolated microflora could also be due to contamination from the insect's surrounding. There is a need for a subsequent study on the relationship between microorganisms found in the gut of this insect and those on their food plant(s) and its environment.

It is worth noting the predominance of gram-negative rods in almost all the cultures used (Table3). This is similar to what obtains in other acridids (Mead et. al. 1988). There was also an absence of gram-positive bacteria in all the gut sections of the 3rd and 4th instars as against their significant presence in the 6th and adult instars. *Z.variegatus* is a polyphagous insect but with preference for particular food plant species such as *Manihot esculenta* by the later instars 5th – adult while the early instars, 1st –4th are known to prefer *C.odorata* (Chapman et al 1986).

Microbes have high nutritive potentials (Martin & Kukor 1984). They are a rich source of protein having high levels of other macronutrients as well, such as lipids, carbohydrate and are also good source of critical macronutrients such as unsaturated fatty acids and vitamins (Martin & Kukor 1984). The fungal enzymes in the gut of termites are known to be very useful for the digestion of plant materials (Breznak *et. al.* 1994). Proteus sp. Isolated in this work is known to produce proteolytic enzymes. *Lactobacillus* sp. Also found in the later instars examined in this are known to produce glucosidases (Martin & Kukor 1984). The ability of *Z. variegatus* to tolerate and digest cyanogenic glucoside contained in *M.esculenta* has been a puzzle to scientist (Idowu, 1994). Moreso since the grasshopper lacks any enzyme capable of hydrolyzing the cyanide in its gut. It is therefore possible that apart from being a rich source of nutrients to the insects, the microflora in the gut of the grasshopper such as *Lactobacillus* sp. may play a role in the digestion of the cyanogenic glucoside present in *M.esculenta* consumed.

Results of this study has shown that the guts of the instars of *Z.variegatus* are rich in different types of microbes. Although, the function of these microbes still await further elucidation, results of studies on other insects suggests that the microbes might be of immense benefit to the insect particularly in the digestion of its food material.

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