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Isolation and Characterization of Bacteria Present in the Gut of Dead Females *Glossina morsitan Submorsitans* Using Culture Dependent and Independent Methods

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Authors' contributions

This work was carried out in collaboration among all the authors. Authors LJI and ABA designed the study. Authors LJI, ABA and ISN wrote the protocol and the first draft of the manuscript. Author LJI managed the analyses of the study and carried out the laboratory analysis. Authors LJI, ABA and ISN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study aimed to isolate and characterize bacteria present in the gut of death female *Glossina morsitan submorsitans* using culture dependent and independent methods. Thirty-four fresh dead female flies were obtained from the insectary of Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna. The flies were surface sterilized and the mid-guts were dissected under aseptic conditions. The gut bacterial cultures were done for pregnant and non-pregnant dead female flies (6weeks- 15weeks old) a total of 20 isolates. The culture dependent methods identified six bacterial genera namely, *Serratia* species, *Staphylococcus* species, *Escherichia* species, *Myroides* species, *Providencia* species and *Alkaligenes* species. Molecular analysis only identified four (4) out of the six (6) isolates; as 4(50%) *Serratia mercesens*, 1(12.5%) *Alcaligenes faecalis*, 2(25%) *Myroides odoratimus* and 1(12.5%) *Providencia.* This is not surprising because the microorganisms were not included in the targeted spectrum of the Polymerase Chain Reaction. Our study recorded for the first time the presence of *Myroides odoratimus* in the gut of *G. M. submorsitans* in Nigeria. Some of

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these bacteria are pathogenic to the flies and may be the cause of mortality of laboratory reared flies. We recommend intensive research on tse tse gut bacterial flora to give insight into their use as potential vector bio-control tool.

Keywords: Bacteria; glossina; isolation; characterization.

1. INTRODUCTION

African Trypanosomiasis is transmitted by tsetse flies which belong to the genus *Glossina*. For the disease to be transmitted to susceptible host, the parasite (trypanosomes) must first be established in the midgut after an infective blood meal [1]. The success of the establishment and maturation of trypanosomes plays a key role in the disease transmission cycle. However, the capacity of the fly to be infected and transmit trypanosomes depend on several factors such as the tsetse fly species, the genetic variability within a given species and the presence of symbiotic microorganisms in the fly [1,2].

Lindh and Lehane [3] together with Aksoy [4] reported that tsetse midguts harbor various microorganisms which include pathogens and other useful endosymbionts. All field caught tsetse flies harbor bacteria of the intestines which are maternally transmitted by symbiotic bacteria and also acquired from the environment [3]. Bacteria of the genera *Enterococcus, Acinetobacter*, and *Enterobacter* were discovered to be inside the gut of these flies, in spite of feeding on blood that is free of bacteria, the reason for this is unknown but it could be that the flies sometimes feed on other nutrients apart from the blood which can be the source of the bacteria [5]. The high rate and unlikeness of bacteria in tsetse flies is not expected since these flies feed on just one kind of food, blood all through their lifetime [6]. One of the sources of gut bacteria in tsetse was the skin of the animals used as host during *In-vivo* feeding. The bacterium *Serratia marcescens* causes mortality of tsetse flies. Poinar [7] pointed out that the flies pick up the bacterium on the surface of the host skin when taking a blood meal. Both Simo [8] and Farikou [9] observed that the bacterium *Serratia marcescens* ingested by the flies multiply in their guts.

The diversity of the tsetse flies gut bacteria acquired in the environment differ significantly within and between tsetse populations, with most of the acquired microbes constituting less than 1% of tsetse's cumulative of bacteria occurring in the intestines [4]. The source of these gut acquired microbes, and their functional contribution in relation to the mechanism of their host tsetse flies, is still unclear, neither do we fully understand whether the microbes are playing roles of pathogens or simply found in the natural environment [4].

To achieve effective control of these vectors and the parasites they transmit, scientist require large number and a continuous supply of uninfected tsetse flies of standard physiological quality through all seasons for research and laboratory trials of insecticides. One way to achieve this is the establishment of laboratory colonies of tsetse flies where they are bred in captivity [1,4]. Unfortunately, this is often difficult especially in Africa owing to the technical and structural deficit. The absence of closed viable colonies of tsetse flies in Nigeria to provide materials for scientific research has been a hindrance to an effective study of disease associated with tsetse fly. Therefore, the disease has continued to impact negatively on human and animal health leading to huge economic losses estimated at several million dollars [10]. Therefore, our study is aimed to isolate and characterize bacteria present in the gut of dead female *glossina morsitan submorsitans* using culture dependent and independent methods.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted with flies obtained from the tsetse breeding laboratory of the Nigerian Institute for Trypanosomiasis Research, Kaduna. The history of the flies has been given by Ahmed [11]. The colony was maintained at a temperature of 23ºC -25ºC and relative humidity of 75-80%. The colony of the *Glossina morsitans submorsitans* was established in 2008, with 6,10500 pupae brought from Cote d' Ivoire [12].

2.2 Experimental Flies Used for the Study

Eighty-eight dead *Glossina morsitans. submorsitans* were collected from the breeding room of N.I.T.R, Kaduna, and transported to the Department of Biological Sciences, Kaduna State

University, Kaduna State. Thirty-four of the dead flies were used for the culture dependent and independent midgut microbiota characterization.

2.3 Sex Determination

The sex of the flies was recognized by the presence of Hypopygium on the males and its absence in the female flies [13].

2.4 Bacteriological Analysis

2.4.1 Preparation of culture media

Basic enrichment differential and identification media were used for the isolation and identification of the micro-organisms; Brain heart infusion (BHI) agar and Brain heart infusion broth (15g/LH20), peptone water (25.5g/1Ltr H20), MacConkey agar (52g/1L H20), Urea agar (15g/1Ltr H20), Simon's citrate agar (24.3g/1LtrH20), Nutrient agar (28g/1Ltr H20) and Triple Sugar Iron (TSI) agar (65g/1Ltr H20). These media were aseptically prepared according to the manufacturer's instructions.

2.5 Dissection of the Tsetse Flies

The processes of the fly dissection were performed in a laminar flow hood, with the dissection microscope mounted inside the laminar flow chamber; using dissecting pins the surface-sterilized flies were dissected under the microscope (Magnification ×60) on a slide from sterile phosphate buffered saline; the wings and legs were carefully removed from the body by the use of forceps, the fly was placed on the dorsal side and held by the thorax for the removal of the mid-guts; the first segment of the abdomen on the ventral side was grasped with the aid of forceps and gradually pulled back to open the abdomen [14]. After dissection, the mid-guts were removed, washed in sterile phosphate buffered saline, and aseptically transferred into a 2.0ml Eppendorf tube containing 200u1 of BHI broth. This procedure was repeated until the required mid-guts were gotten. The tubes were thoroughly mixed with a micro-pestle, homogenized by vortexing.

2.6 Bacteria Enumeration

2.6.1 Serial dilution

Material: Stock Solution (Crushed guts), Test tubes, Pipettes, beaker and distilled water.

Four test tubes were labelled A, B, C and D. 9ml of distilled water was poured into the labelled test tubes (A, B, C and D). 1ml of the stock solution was transferred into the test tube labelled A and mixed properly to avoid cells settling at the bottom. 1ml was transferred from test tube A into the test tube labelled B and mixed properly to avoid cells settling at the bottom. 1ml from the test tube B was transferred into the test tube labelled C and mixed properly and so on until the test tube labelled D. 1ml from the test tube C was transferred into the test tube labelled D and mixed properly to avoid cells settling at the bottom. This gave four dilutions 10-1, 10-2, 10-3 and 10-4 [15].

2.6.2 Isolation of bacteria

The molten agar in the water bath (DK 600) was kept for three hours. The agar was poured after it had been cooled to 50°C. Phosphate buffer pH 7.2 was used to dilute the suspension. Contamination was avoided by pouring plates in a laminar-airflow cabinet, the mouth of the flask was flamed before moving on to the next plate; plates were filled according to regulators' recommendations and incubated at 37°C for 48 hours. Discrete colonies were counted using the colony counter. After counting, bacterial colonies from the incubated cultured plates were carefully subculture on fresh BHI (Brain heart infusion) agar, MCA (MacConkey agar) and TSIA (triple sugar iron agar) plates to obtain pure isolates and incubated for 48 hours at 28°C to obtain pure bacterial isolates [16].

2.7 Morphological and Biochemical Characterization of Bacterial isolates

The bacterial isolates from dissected mid-gut of the flies were identified based on their colonial morphology and cellular morphology.

• Gram reaction – This helps to make the bacteria visible and helps to know the type of bacteria present, which is dependent on the shape of the bacteria and the staining properties. Gram-positive shows a purple color, gram-negative shows a pinkish/reddish color [17]. The gram stain helps to differentiate gram-positive from gram-negative bacteria based on different staining with a crystal violet-iodine complex and the use of a counterstain safranin; the cell walls of gram-positive organisms absorbs the chemical after treatment with alcohol and appear purple, unlike gram-negative organisms that decolorize after treatment which appears pink; the method is

useful for determining bacterial contamination of tissue culture samples or for discovering the gram stain status and morphology of bacteria isolated from combined or isolated cultures of bacteria, on the surface of the microscope slide materials were fixed by heating; crystal violet usually absorbs all cells blue/purple color; the iodine solution (mordant) was poured to form a crystal violet-iodine (CV-I) complex; the cells appear blue [18]. The decolorization process differentiates gram-positive from gram-negative cells [19].

2.8 Biochemical Characterization

2.8.1 Catalase test

Colonies of the bacterial growth were emulsified on a clean grease-free slide placed in a Petri dish; three percent drop of (10 Volumes) of H_20_2 was added. Bubbles (effervescence) indicated a positive reaction; the absence of bubbles indicated a negative result [20].

2.8.2 Citrate utilization test

Suspected bacterial colonies were inoculated with a sterile straight wire loop into Simmon citrate medium. Incubation was done at 37°C overnight, colour changed from green to blue indicated a positive result while no change of color from the green color of the agar indicated a negative result [21].

2.8.3 Indole test

The colony of the tested bacterium was transferred with a sterile lope into peptone water and was incubated at 37^0C overnight. Three drops of Kovac's reagent (5.0ml) were mixed with the broth culture.; the presence of a red ring color at the surface layer within 10 minutes indicated a positive result, while the absence of red ring color at the external layer within 10 minutes indicated a negative result [21].

2.8.4 Triple sugar iron (TSI) agar test

Using a sterile wire, the tested bacterial colony was stabbed into the butt and from the bottom of the slope, a serpentine streak was made; the medium was incubated at 37°C overnight before the examination; a change to the yellow color of the slope and butt indicated fermentation of the sugars (glucose, sucrose, and lactose); the blackening of the medium indicated 'hydrogen sulfide production, while the presence of cracks or hollow spaces in the medium indicated gas production from glucose fermentation; a pinkish slant indicated that the organism was a non - lactose fermenter; no change in the color of the medium (butt and slope) indicated that the sugars were not fermented while a crack or hollow space at the region of the butt indicated CO2 production from glucose fermentation [22].

2.8.5 Urease test

Through the help of a sterile straight wire the tested bacterial colony was inoculated into urea agar slants and was incubated at 37^0C for 24hours; colour change from yellow to red-pink indicated a positive result and no changed in the color of medium showed a negative result [23].

2.8.6 Oxidase test

Two (2) drops of 1% freshly prepared Tetraethyl paraphenylenediamine dihydrochloride (oxidase reagent) was made on a filter paper in a clean petri-dish; the suspected bacterial colony was smeared with a glass rod or an applicator stick; a blue-purple color development within 10 seconds was recorded as a positive result and nonappearance of blue-purple color was considered a negative result [21].

2.9 Molecular Analyses of Isolates

Pure isolates were aseptically transferred to the sterile nutrient broth and sent to the Bioinformatics services, Ibadan for DNA extraction, PCR, Gel (Agarose electrolysis), Sequencing and Blast (Basic local alignment search tool). This was based on PCR and Sanger Sequencing investigation. The DNA Extraction and PCR using the 16SrRNA gene were carried out at Bioinformatics Services Molecular Laboratory, Queen Elizabeth way, Ibadan, Oyo State, while sequence analysis was carried out in the International Institute of Tropical Agriculture (IITA) Bioscience Center.

2.9.1 DNA extraction

Extraction of DNA was performed using Qiagen DNA extraction kit. Extraction was done according to manufacturer's instructions. The pure samples were mixed with 180µl buffer then 20µl proteinase K were added to make a solution which was mixed thoroughly by vortexing. Incubation was done at 56^0C. Again, 200µl buffer was poured and properly mixed through vortexing for 15 seconds then 200µl of ice cold iso-

propanol was properly mixed through vortexing. Combined substance was pipetted into a DNeasy mini spin column attached to a 2ml tube and centrifuged at 8000rpm for 2minutes. Both flow and collection tube were thrown away and DNeasy mini spin column was attached to a new 2ml collection tube. 500µl buffer AW1 was added to it and centrifuged for 2minutes at 8000rpm. Both flow through and collection tube were discarded and DNeasy mini spin column was attached to a new 2ml tube. 500µl buffer AW2 was added and was centrifuged at 14,000rpm for 4 minutes. Both flow and collection tube were thrown away. DNeasy mini spin column was placed in a sterile 2ml micro centrifuge tube and 200µl buffer AE was pipetted directly into DNeasy membrane which was incubated at room temperature for 2minutes and centrifuged at 8000rpm to elute. Elution was repeated to increase the yield of the DNA [24].

2.9.2 PCR amplification of DNA

The total volume of PCR content used was 10µL. The following are the premix used for the process 10× PCR buffer 1.0, 25mM Mgcl2 1.0, 5pMol forward primer 0.5, 5pMol reverse primer 0.5, DMSO 1.0, 2.5Mm dNTPs 0.8, Taq 5u/ul 0.1, 10ng/µl DNA 2.0, H2O 3.1. This primer sequences of the 16SrRNA gene were used

Primer Sequence

16SF 5′- GTGCCAGCAGCCGCGCTAA 3′
16SR AGACCCGGGAACGTATTCAC

Initial and final denaturation temperature was set at 94˚C and 94^0C for 5min and 30seconds respectively. The temperature for annealing was set at 56^0C for 30 seconds. The extension was done at 72^0C for 45 seconds. Number of circles was 36. The final extension was set at 72^0C for 7min. Holding temperature was set at 10^0C for. From the reaction above, the amplicon was loaded on 1.5% agarose gel and the gel picture was attached as PCR. The ladder used was 1kb plus ladder from Invitrogen condition. The PCR product was purified using the following protocols Two (2vol) (20ul) of absolute ethanol was poured into the PCR product and incubated at room temperature for 15minutes; it was spun down at 10000rpm for 15minutes; the supernatant was decanted; two (2vols) (40ul) of 70% ethanol was added; the supernatant was decanted again; and left to air dry. Ten (10ul) of ultrapure water was added; the amplicon was checked on 1.5% agarose [25].

2.9.3 DNA sequencing

Sanger method was adopted for the sequencing according to the selective inclusion of chain terminating dioxynecleotide by DNA polymerase chain reaction during replication. The 10µM stock solution of the sequencing primer was diluted 1:15 with sterile H_2O. 2µl of the diluted sequencing primer was pipetted into appropriately labeled, thin-walled PCR tubes; two (2µl) of the Big Dye ready reaction termination mix was pipetted into each tube; one (1µl) of each PCR product was added to the appropriate tube, ensuring that all 3 reaction components were mixed; these are 1/4 size reactions (5µl total reaction volume compared with 20µl total reaction volume suggested in the ABI BigDye sequencing manual) [26].

The following cycling program was run

Step	Temperature (°C)	Time
1	96	10 s
2	50	5 s
3	60	2 min
4	repeat steps 1, 2 and 3 a further 29 times	
5	4	Forever

The tubes were removed from the thermal cycler. Fifteen (15µl) of sterile H_2O was added to each tube to make up the reaction volume to 20µl (This helps to prevent severe dye blobs). Two (2µl) of 3M NaOAc, pH 4.6, and 50µl EtOH were added to the labeled 1.5ml Eppendorf tubes; twenty (20µl) sequencing reaction was added to the appropriate tube containing the salt/EtOH mixture; it was mixed by vortexing and incubated at room temperature for 15 minutes.

The tubes were spined for 15-30 min at maximum speed. After spinning, the supernatant was removed by the use of a pipette; the pellets were washed with 500µl of 70% EtOH added to each tube and again spined at maximum speed for a further 10 min; the EtOH was removed in other to reduce dye blobs in the sequencing reactions and the pellets were dried in the vacuum dryer; and stored at -20^0 C until it was ready for loading on a sequencing gel (Each pellet was resuspended in 3-4µl of loading buffer on the same day as they are to be run) [27].

This was accomplished to control the nucleotide arrangement of the exact microorganism secluded by automatic PCR cycle- Sanger Sequencer™ 3730/3730XL DNA Analysers from

Applied Biosystems; the results were obtained as nucleotides. Sequence examination from subsequent nucleotides base sets was performed by basic local alignment search tool (BLAST) analysis by direct blasting on the American database [28].

3. RESULTS

3.1 Morphological and Biochemical Characteristics of Presumptive Bacteria Isolated from the Gut of Dead *Glossina morsitans submorsitans* Obtained from NITR Insectary, Kaduna

Serratia species were seen in cultures of 6,7,8 and 10 weeks old dead flies. *Staphylococcus* species were found in 7,9,10,12,13,14 and 15 weeks old flies. *E. coli* was seen in 11 weeks old flies. *Myroides* species were seen in 9- and 11-weeks old flies. *Alkaligens* species were seen in 12 and 13 weeks and *Providencia* species were obsered in weeks 14 and 15. Only flies in weeks 6 and 8 harbour *Serratia* species while all the flies in the remaining age groups had infections with two bacterial species each in different combinations (Table 1).

3.2 Molecular Identification

3.2.1 PCR amplification

Four (4) types of bacteria were identified. Of the eight isolates identified, 4(50%) were *Serratia mercesens*, 1(12.5%) was *Alcaligens faecalis,* 2(25%) were *Myroides odoratimimus* and 1(12.5%) was *Providencia rettgeri* (Table 2). The gel electrophoresis of the PCR products revealed the bands of the isolate on 850bp of the 1kb marker used (Plate 1).

3.2.2 PCR products blast sequences

The sequence of *Serratia mercesens* strain Jii-1 with accession number MN647531, *Myroides odoratimus* strain Jii-2 with accession number MN647532, *Providencia rettegeri* strain Jii-3 with accession number MN647533 and *Alkaligens feacalis* strain Jii-4 with accession number MN647534 were identified and all the sequences were deposited to the gene bank . Table 2 shows the identified bacteria: *Serratia mercesens, Myroides odoratimus*, *Providencia rettegeri*, and *Alkaligens feacalis*). Homology characteristics obtained from the sequences BLAST results from this table (Table 2), the homology in terms of percentage identical ranged from 83 to 99.2 percentage pairwise, E- values 0.00 to 1.76, and percentage GC content 52.70 to 54.50.

4. DISCUSSION

4.1 Isolation and Characterization of the Gut Bacteria

The midgut of tsetse fly (*Glossina sp*ecie) is inhabited by both inherited and environmentally transmitted bacteria that impacts on several aspects of the fly's mechanism. However, not much is known on the role of the bacterial communities of the midgut- relating to fitness of the flies in laboratory-maintained colonies [29]. This study was conducted to determine why *G. m. submorsitans* from a laboratory colony kept at NITR were dying despite evidence that they had successfully taken blood meals offered from vertebrate hosts. The communities of bacteria living inside the midgut of tsetse flies were characterized using culture dependent and independent methods. The aim is to determine whether there is an association between presence of midgut-associated bacterial communities and fly mortality.

Bacteria were isolated from the gut of the flies and identified biochemically and molecularly.

The overall bacterial species are assigned to four families *Enterobacterioceae*, *Staphylococcacaceae*, *Flavobactriaceae* and *Alkaligenaceae*. The dorminant family is *Enterobacterioceae*, represented by the genera *Serratia* sp, *Escherichia coli* and *Providencia sp*. The remaining three bacterial families had one genus each.

While the culture-dependent methods identified six bacterial organisms namely, *Serratia* species, *Staphylococcus* species, *Escherichia coli, Myroides* species, *Providencia* species, and *Alkaligenes* species, the molecular method only identified four isolates, (*Serratia mercesens, Alcaligenes faecalis, Myroides odoratimus,* and *Provencia retgerri).* Both *Staphylococcus* species and *Escherichia coli* identified by the culture method were not detected by the molecular method. Though the molecular method is presumed to be more acurate than the culture method, the reason for failure of the PCR to detect *E. coli* and *Staphylococcus* could be that the microorganisms are not included in the targeted spectrum of the PCR [30]. Another reason could be the sensitivity of 1 CFU per specimen (as for well culturable pathogens) is difficult to attain by PCR [30].

Table 1. Morphological and Biochemical characteristics of bacteria isolated from the gut of dead *Glossina morsitans submorsitan*s obtained from NITR Insectary, Kaduna

Gram Morphology		**Mot**	**Cat**	**Oxd**	**Ur**	**Ind**	**Cit**	**Coag**	**MSA**	**S**	**H2S**	**Gas**	**Probable identification**
Age of dissected Fl reaction													
6,7,8 and 10 Wks -	Rod	+	-	-	-	-	+		+	+	-	+	*Serratia species*
- 7,9,10,12,13,14,&15	Cocci	-	+	-	-	+	+	+	+	+	-	+	*Staphylococcus.species*
11 Wks -	Rod	+	+	-	-	-	+	-	+	+	-	+	*E. coli*
- 9 and 11 Wks	Rod	+	-	-	-	-	+		-	+	-	+	*Myroides. Species*
- 12 and 13 Wks	Rod	+	+	+	-	-	+		+	-	-	-	*Alkaligens. species*
14 and 15Wks -	Rod	+	+	+	-	-	+		+	-		+	*Providencia.species*

Key: +Positive, -=Negative,Mot=MotilityCoag=Coagulase , Cat= Catalase, Oxidase,Ur= urease, Ind=Indole, Cit=Citrate,Msa=Manitol salt agar 41

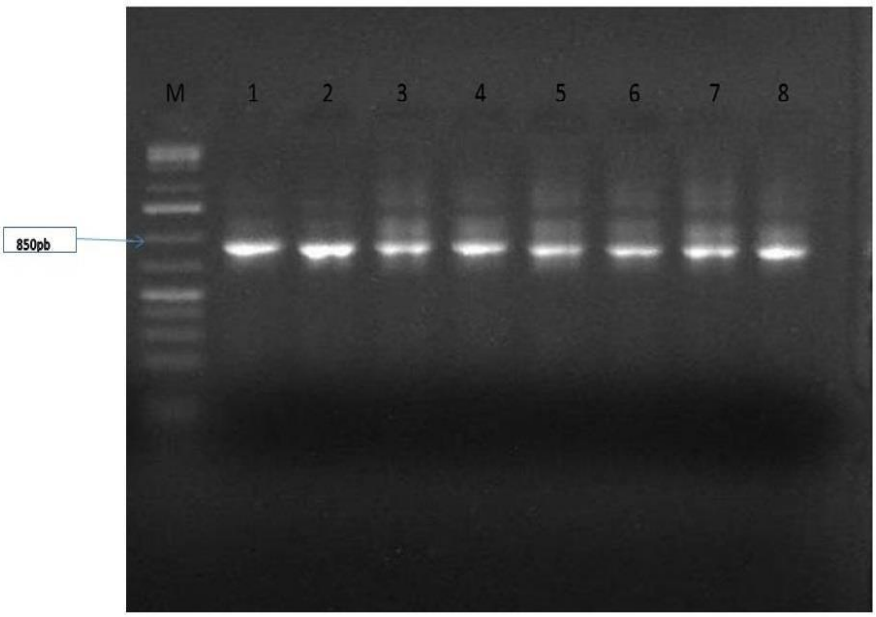

Plate 1. Gel electrophoresis showing 850bp DNA bands of PCR amplification of the Isolates on a 1kb Marker

Keys: *M= DNA ladder; 1,2,7 and 8 = Serratia marcescens strain Jii-1; 4 Providencia sp. Strain Jii-3; 3= Alkaligenes feacalis strain Jii-4; 5 and 6= Myroides odoratimimus strain Jii-2*

Table 2. Summary of BLAST characteristics of bacteria Isolated from the gut of dead *Glossina morsitans submorsitans* obtained from NITR Insectary, Kaduna

S/N	Sample code	Organism	Identical (%)	Pairwise(%)	E-value	GC (%)	Name/Accession number
1	1		96.40	96.40	0.00	54.10	JX469435
	2	*Serratia*	99.20	99.20	0.00	53.00	MF716676
	7	*marcescens*	84.40	84.40	1.07E-153	54.50	JX020764
	8		94.90	94.90	0.00	53.30	JX469436
2	4	*Providencia sp.*	96.00	96.00	0.00	52.70	KX981304
3	3	*Alkaligenes faecalis*	83.40	83.40	0.00	54.00	KC12884
4	5		97.50	97.50	0.00	47.80	KJ4011113
	6	*Myroides odoratimimus*	87.00	87.00	1.73E-176	45.10	KJ4011113

Key: GC (Genomic Contents), E-Value (Expected value)

The occurrence of six bacterial species in the gut of the tsetse studied is surprising and their origin unknown since the flies were fed on rabbits *in-vivo* throughout their life span. Other workers [31,32], studied wild mosquitoes could also not understand the origin of the diverse bacteria they encountered, but attributed it to variations in the environmental conditions and source of food. Poinar [31] demonstrated with *Glossina m. morsitans* and *G. pallidipes* that it is possible for the flies to pick bacteria during feeding if they are present on the ears of rabbits used for feeding. Another possibility for bacterial diversity in tsetse gut was provided by Simo [8] and Farikou [9], that such could arise from habit of the flies feeding on variety of animal hosts carrying various bacteria on their hair and skin, thereby increasing the tendency of the flies being infected by these bacteria. While this explanation may explain diversity in wild flies, it cannot account for the bacterial diversity observed in the present study because the flies were held in captivity and fed only on live rabbits.

While the tsetse species used in the present study is *G. m. submorsitans*, a member of the savanna group, most of the studies on the gut microbiota of tsetse were conducted on members of the riverine *Palpalis* group. For example, both *Providencia specie* and *Staphylococcus species* were isolated from *G. p. palpalis* in Cameroun [33]. In Burkina Faso, species of *Serratia* were isolated from *G. p. gambiense* [34. In Kenya, *G. f. fuscipes* harbours *Staphylococcus species*, *Providencia* and *Serratia marcescens*. Gei [33] and Geiger [34] also observed *Serratia* species from *Glossina p. palpalis* in Angola. Geiger [6] also reported presence of nine species of bacteria (*Acinetobacter*, *Enterobacter*, *Providencia*, *Pseudomonas*, *Enterococcus, Lactococcus, Staphylococcus*, *Chryseobacterium* and *Sphingobacterium*) in *G. p. palpalis*, *G. pallicera*, *G. caliginea*, and *G. nigrofusca*. All the flies from where the bacteria were isolated were members of the *Palpalis* group except *G. nigrofusca* that belong to the *Fusca* group. *Enterobacteriaceae* is a large family of Gram-negative bacteria that inhabit the guts of mammals and inverbterates that includes over 30 genera and more than 100 species [6]. Three of the isolated bacteria in this study, namely *Escherichia*, *Providencia*, and *Serratia* belong to this family.

5. CONCLUSION

In conclusion, findings from this study enhances our knowledge of the micro-biota of environmentally acquired gut bacteria and their possible link with mortality in a laboratory bred colony of tsetse. The species *Myroides odoratus*, previously isolated from the gut of a lepidopteran, has been isolated in the gut of *G. m. submorsitans* for the first time in Nigeria.

6. RECOMMENDATIONS

Based on the findings from this study, we recommend further studies to be conducted on tsetse fly gut bacterial flora to gain insights into their use as potential vector bio-control tool. One way of achieving this is by identifying the toxic enzymes produced by these bacteria that cause deleterious effects on the female survival and productivity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lindh j, Jenny M, Michael J. The tsetse fly Glossina fuscipes harbours a surprising diversity of bacteria other than symbiont. Antonie Leeuwenhoek. 2011;7720:1.
2. Mbewe NJ, Sole CL, Masiga DK, Yusuf AA. Efficiencies of stationary sampling tools for tsetse fly Glossina fuscipes in western Kenya. Act Trop. 2021;223-235.
3. Lindh JM, Lehane MJ. The tsetse fly Glossina fuscipes fuscipes (Diptera:Glossina) harbours a surprising diversity of bacteria other than symbionts. Antonie Leeuwenhoek. 2011;99:711-20.
4. Aksoy E, Telleria EL, Echodu R, Wu Y, Okedi LM, Weiss BL et al. Analysis of multiple tsetse fly populations in Uganda reveals limited diversity and species-specific gut microbiota. Appl Environ Microbiol. 2014;80(14):4301-12. DOI: 10.1128/AEM.00079-14, PMID 24814785.
5. Colman DR, Toolson EC, Takacs-Vesbach CD. Do diet and taxonomy influence insect gut bacterial communities? Mol Ecol. 2012;21(20):5124-37. DOI: 10.1111/j.1365-294X.2012.05752.x, PMID 22978555.
6. Geiger A, Fardeau ML, Njiokou F, Ollivier B. Glossina spp. Gut bacterial flora and their putative role fly-hosted trypanosome development. Cell Infect Microbiol. 2013;3:1-8.
7. Poinar GO, Wassink HJ, Leegwater-van der Linden ME, van der Geest LP. Serratia marcescens as a pathogen of tsetse flies. Acta Trop. 1979;36(3):223-7. PMID 43085.
8. Simo G, Njiokou F, Mbida Mbida JA, Njitchouang GR, Herder S, Asonganyi T, et al. Tsetse fly host preference from sleeping sickness foci in Cameroon: epidemiological implications. Infect Genet Evol. 2008; 8(1):34-9. DOI: 10.1016/j.meegid.2007.09.005, PMID 17977803.
9. Farikou O, Njiokou F, Simo G, Asonganyi T, Cuny G, Geiger A. Tsetse flies blood meal modification and trypanosome identification in two sleeping sickness foci in the forest of southern Cameroon. Acta Trop. 2010;116(1):81-8. DOI: 10.1016/j.actatropica.2010.06.002, PMID 20541513.

10. Bukachi SA, Wandibba S, Nyamongo IK. The socio-economic burden of human African trypanosomiasis and the coping strategies of households in the South Western Kenya foci. PLOS Negl Trop Dis. 2017;11(10):e0006002.
DOI: 10.1371/journal.pntd.0006002, PMID 29073144.
11. Ahmed AB. Laboratory rearing of tsetse flies. NITR annual scientific report. 1990;73.
12. Abubakar A, Abdullahi RA, Jibril HZ, Samdi SM, Mohammed H, Sabo E, et al. Patterns of wild pupae emergence from 2000-2009 at the insectary of the Department of Vector and Parasitology Studies, Nigerian Institute for Parasitology Research (NITR), Kaduna, Nigeria. Curr Resour J Biol Sci. 2010;2(6):407-9.
13. FAO. La situation mondiale de l'alimentation et de l'agriculture. Edition collection FAO. Agriculture; 2002.
14. Bouyer J, Yoni W, Bila C, Desquesnes M, Guerrini L [technical guide]. The Dissection of Tsetse flies. 2000;1:1-16.
15. Hedges AJ. Estimating the precision of serial dilutions and viable bacterial counts.642. Int J Food Microbiol. 2002;76(3):207-14.
DOI: 10.1016/s0168-1605(02)00022-3, PMID 12051477.
16. Kadhem HS, Abed Ali AA, Hassan OM. Isolation and identification of bacteria isolated from different parts of cell phones. World J Exp Biosci. 2016;4:29-31.
17. Van Den Abbeele J, Claes Y, van Bockstaele D, Le Ray D, Coosemans M. Trypanosoma brucei spp. development in the tsetse fly: characterization of the post-mesocyclic stages in the foregut and proboscis development in the steste fly. Parasitology. 1999;118(5):469-78.
DOI: 10.1017/s0031182099004217, PMID 10363280.
18. Kayser FH. Haemophilus and pasteurella. medical microbiology. 10th ed. Stuttgart: Georg Thieme Verlag. 2005;300-1.
19. WHO. World Health Organization: manual of basic techniques for health laboratory. 2nd ed. Geneva: World Health Organization; 2003.
20. Yumoto I, Ichihashi D, Iwata H, Istokovics A, Ichise N, Matsuyama H, et al. Purification and characterization of a catalase from the facultatively psychrophilic bacterium Vibrio rumoiensis S-1(T) exhibiting high catalase activity. J Bacteriol. 2000;182(7):1903-9.
DOI: 10.1128/JB.182.7.1903-1909.2000, PMID 10714995.
21. Vashist H, Sharma D, Gupta A. A review of commonly used biochemical tests for bacteria. Innovare J Life Sci. 2013;1(1):1-7.
22. Lehman D. Triple sugar iron agar protocols. Vol. 3. American Society for Microbiology. 2005;1-7.
23. Dahlén G, Hassan H, Blomqvist S, Carlén A. Rapid urease test (RUT) for evaluation of urease activity in oral bacteria in vitro and in supragingival dental plaque ex vivo. BMC Oral Health. 2018;18(1):89.
DOI: 10.1186/s12903-018-0541-3, PMID 29776416.
24. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. Vol. 3. Cold Spring Harbor: Cold Spring Harbor Laboratory Press. 1989;1625.
25. Courtois S, Frostegård A, Göransson P, Depret G, Jeannin P, Simonet P. Quantification of bacterial subgroups in soil: comparison of DNA extracted directly from the soil or cells previously released by density gradient centrifugation. Environ Microbiol. 2001;3(7):431-9.
DOI: 10.1046/j.1462-2920.2001.00208.x, PMID 11553233.
26. Shi W, Syrenne R, Sun JZ, Yuan JS. Molecular approaches to study the insect gut symbiotic microbiota at the 'omics' age. Insect Sci. 2010;17(3):199-219.
DOI: 10.1111/j.1744-7917.2010.01340.x
27. Nam YD, Jung MJ, Roh SW, Kim MS, Bae JW. Comparative Analysis of Korean Human Gut microbiota by barcoded Pyrosequencing. PLOS ONE. 2011;6(7):e22109.
DOI: 10.1371/journal.pone.0022109, PMID 21829445.
28. Akman L, Yamashita A, Watanabe H, Oshima K, Shiba T, Hattori M et al. Genome sequence of the endocellular obligate symbiont of tsets flies, Wigglesworthia glossinidia. Nat Genet. 2002;32(3):402-7.
DOI: 10.1038/ng986, PMID 12219091.
29. Griffith BC, Weiss BL, Aksoy E, Mireji PO, Auma JE, Wamwiri FN, et al. Analysis of the gut-specific microbiome from field-

captured tsetse flies, and its potential relevance to host trypanosome vector competence. BMC Microbiol. 2018; 18(Suppl 1):146.
DOI: 10.1186/s12866-018-1284-7, PMID 30470178.

30. Grif K, Heller I, Prodinger WM, Lechleitner K, Lass-Flörl C, Orth D. Improvement of detection of bacterial pathogens in normally sterile body sites with a focus on orthopedic samples by use of a commercial 16S rRNA broad-range PCR and sequence analysis. J Clin Microbiol. 2012;50(7):2250-4.
DOI: 10.1128/JCM.00362-12, PMID 22553237.

31. Pumpuni CB, Demaio J, Kent M, Davis JR, J, Beier C. Bacterial population dynamics in three Anopheline species: an impact on Plasmodium sporogonic development. Am J Trop Med Hyg. 1996; 54(2):214-8.

32. Straif SC, Mbogo CNM, Toure AM, Walker ED, Kaufman M, Toure YT, et al. Midgut bacteria in Anopheles gambiae and A. funestus (Diptera: Culicidae) from Kenya and Mali. J Med Entomol. 1998;35(3): 222-6.
DOI: 10.1093/jmedent/35.3.222, PMID 9615538.

33. Geiger A, Fardeau ML, Grebaut P, Vatunga G, Josénando T, Herder S, et al. First isolation of Enterobacter, Enterococcus, and Acinetobacter species as inhabitants of the tsetse fly (Glossina palpalis palpalis) midgut. Infect Genet Evol. 2009;9(6):1364-70.
DOI: 10.1016/j.meegid.2009.09.013.

34. Geiger A, Fardeau ML, Falsen E, Ollivier B, Cuny G. Serratia glossinae species.not, isolated from the midgut of the tsetse fly Glossina palpalis gambiensis. Int J Syst Evol Microbiol. 2010;60(6):1261-5.
DOI: 10.1099/ijs.0.013441-0, PMID 19667382.

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