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Isolation and Biochemical Characterizations of Mid Gut Microbiota of Culex (*Culex quinquefasciatus*) Mosquitoes in Some Urban Sub Urban & Rural Areas of West Bengal.

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Mosquitoes, in general are medically important vectors of many diseases like Malaria, Dengue and Filariasis, which are a great challenge for public health in many countries. All animals and plants establish symbiotic relationship with microbes. Mosquitoes can be considered as an holobiont units in which the host (mosquito) and its microbiota are involved in complex reciprocal multipartite interaction such as host reproduction and survival, protection against natural enemies. This naturally acquired microbial flora can modulate the mosquitos' vectorial capacity by inhibiting the development of pathogen. But enough care has not been under taken regarding the biochemical characterization of *Culex* mosquitoes (*Culex quinquefasciatus*) in West Bengal. Therefore a preliminary investigation have been undertaken for the determination of biochemical characterization such as gram staining, pattern of growth, detection of economically important enzyme as well as antibiotic susceptibility assay of midgut bacterial isolates of *Culex* (*Culex quinquefasciatus*) in some urban, sub-urban and rural areas of West Bengal.

Keyword: *Culex*, Gut-Microbiota, Biochemical Characteristics, Basal Immunity.

1. Introduction

Mosquitoes are medically important haematophagous vectors, carrying numerous maladies from lymphatic filariasis to dengue and malaria. Filariasis is caused by *Wuchereria bancrofti* and transmitted by *Culex quinquefasciatus,* an anthropophilic mosquito. The disease filariasis is highly prevalent in developing countries such as India. Moreover, Japanese encephalitis virus has been isolated from field collected *Cx. Quinquefasciatus* [1]. Symbiotic bacteria are known to play important roles in the biology of insects. All animals and plants establish symbiotic relationship with microbes. Mosquitoes can be considered as an holobiont units in which the host (mosquito) and its microbiota are involved in complex reciprocal multipartite interactions [2], such as host reproduction and survival, protection against natural enemies etc. Filaria transmitting mosquitoes *(Cx. quinquefasciatus*) are continuously exposed to microbes. This naturally acquired microbial flora can modulate the mosquito's vectorial capacity by inhibiting the development of pathogen. Various lines of data [3-6] have shown the beneficial impact of microbiota on their insect host. Bacterial endosymbionts contribute to different biological functions like supplying essential nutrients inducing resistance to pathogen & parasitoids and interfering tolerance of temperature stress. However, a very little attention has been made on the biochemical

and molecular characterization of gut microbiota of *Culex* mosquitoes based on culture dependent or independent method or both [7-10]. But to our knowledge enough care has not been paid to study on the biochemical characteristics of *Culex* mosquitoes in West Bengal.

In view of these reasons we have undertaken a preliminary investigation to know biochemical characteristics such as gram staining, pattern of growth, detection of economically important enzyme as well as antibiotic susceptibility assay of midgut bacterial isolates of *Culex* (*Culex quinquefasciatus*) in some urban, sub-urban and rural areas of West Bengal.

2. Materials and Methods

2.1 Collection of Mosquitoes

Culex mosquitoes were collected from three different ecological habitats viz urban (Dum Dum) sub urban (Serampore) and rural (Mogra) areas of West Bengal. Collection was made in the early morning (6-8 am) from different biotopes like cattle sheds (just behind the dwelling houses) by using manual aspirator. The samples were immediately transported back to the lab (within 45 minutes) in suitable collection vessels kept at uniform temperatures (35 ^{0}C) and relative humidity (80%).

2.2 Bacterial Colonization:

Only live mosquitoes collected from the field were taken for our investigation. The gut of the mosquitoes were cut open in a sterilized environment using a sterilized scalpel inside a laminar air flow hood and the gut contents were carefully transferred to a sterilized inoculation loop. Preformed sterilized Nutrient agar (peptic digest of animal tissue 5 gms/ltr.; NaCl 5 gms/ltr.; Beef extract 1.5 gms/ltr.; Yeast extract 1.5 gms/ltr.; Agar 15 gms/ ltr., distilled water 1000 mls; Final pH at 25 ^{0}C 7.4±0.2) were made and cast in sterile disposable petri plates. The gut contents were aseptically streaked onto the solidified nutrient agar plates by standard 4 way streaking protocol of Loeffler and Gaffky (1881). The plates were incubated in a BOD incubator at 35 ^{0}C±2 ^{0}C under uniform aeration (110 rpm shaker speed) for 24±24 hours until there appeared some growth colonies of the specimen microbes on the plates.

Each growth colony each was subsequently streaked on separate sterile nutrient agar slants, grown in BOD under the aforementioned conditions until growth occurred. Upon occurrence of growth the slants were stored in a 4 ^{0}C refrigerator for further assays.

2.3 Identification of Colony Characters:

The specimen microbe slants were subcultured in sterilized nutrient agar Petri plates by standard 4 way streaking protocol of Loeffler and Gaffky (1881). The plates were incubated in a BOD incubator at 35±2 ^{0}C under uniform aeration (110 rpm shaker speed) for 24 hours and the colony characteristics were noted according to visual interpretation.

2.4 Detection of Colony Character Through Gram Staining

The specimen microbe slants were subcultured in sterilized nutrient agar broth liquid media in sterile culture tubes and incubated overnight (18-20 hours) at 35±2 ^{0}C and 110 rpm shaker speed. Growth was observed visually. Under sterile conditions grease free pathological glass slides [that has been cleansed by standard ionic detergents as well as 70% alcohol (vol/vol)] was taken in an aseptic environment and 5-6 loopfulls of the broth culture of the specimen microbe was transferred on the slide by a sterile inoculation loop. The transferred culture was made into an uniform smear and left to air dry in the laminar air flow hood. Upon being dried the slide was heat fixed by passing the slide once or twice (not more than twice) over a standard methanol lamp flame. Having fixed the specimen to the slide , the slide was mounted on a staining bridge and flooded with Gram's Crystal Violet staining solution as primary stain [Solution A : Crystal Violet powder (certified 90% dye content) 2 gms; Ethanol 95% (vol/vol) 20 ml; Solution B: Ammonium oxalate 0.8 gms; distilled sterile water 80 ml; mix Solutions A and B , store for 24 hrs and filter thorough Whatmann's filter paper prior to use], Gram's Iodine as mordant [Iodine 1 gm; Potassium iodide 2 gms; distilled water 300

mls. Grind the iodine and potassium iodide in a mortar and add water slowly with continuous grinding until the iodine is dissolved. Store in amber bottles], Ethanol as decolourizer (95% vol/vol) and Saffranine as secondary stain (Stock solution: Saffranine O 2.5 gms; 95% Ethanol 100mls; Working solution: 10 mls of stock solution and 90 mls of sterile distilled water) using standard protocol of gram staining of Hans Christian Gram (1884).

2.5 Specific Biotyping

a) Amylase Production Detection Assay

Overnight (18-20 hrs.) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Starch agar medium (Starch soluble, 20 gm/ltr; Peptone, 5 gms/ltr; Beef extract, 3gms/ltr; Agar, 15 gms/ltr; Final pH at 25^{0}C 7.0±0.2) were prepared and cast on sterile disposable petri plates. The isolate suspension was streaked onto the Starch agar plates using standard 4 way discontinuous streaking protocol and incubated at 35±2 ºC for 18 to 48 hours. After incubation the growth colonies on the plates were scraped off using a sterile inoculation loop and the plates were flooded with a dilute iodine solution for 60 seconds. Excess iodine drained off. Results were observed.

b) Protease Production Detection Assay

Overnight (18-20 hrs.) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Standard count Caseinate agar medium (Casein enzymic hydrolysate 5 gms/ltr; Yeast extract 2.5 gms/ltr; Dextrose 1 gm/ltr; Sodium caseinate 10 gms/ltr.; Trisodium citrate 10 gms/ltr; Calcium chloride 2.2gms/ltr.; Agar 15 gms/ltr; Final pH at 25 ^{0}C 7.2±0.2) were prepared and cast on sterile disposable petri plates. The isolate suspension was streaked onto the medium plates using standard 4 way streaking protocol of Loeffler and Gaffky (1881) and incubated at 35± 2 ºC for 18 to 48 h. Results were observed.

c) DNase Production Detection Assay

Overnight (18-20 h.) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile DNase Test Agar w/ Toluidine blue medium (Tryptose 20 gms/ltr.; DNA powder 2 gms/ltr.; NaCl 5 gms/ltr.; Toluidine blue 0.1 gm/ltr.; Agar 15 gms/ltr.; Final pH at 25^{0}C 7.2±0.2) were prepared and cast on sterile disposable petri plates. The isolate suspension was streaked onto the medium plates using standard 4 way streaking protocol of Loeffler and Gaffky (1881) and incubated at 35 ±2 ºC for 18 to 48 h. Results were observed.

d) Gelatin Solubilization Activity Detection Assay

Overnight (18-20 h) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Gelatin iron agar medium (Peptic digest of animal tissue 25 gms/ltr; Meat extract 7.5 gms/ltr; NaCl 5 gms/ltr; Gelatin 120 gms/ltr; Ferrous chloride 0.5 gms/ltr; Final pH at 25 ^{0}C 7.0±0.2) were prepared and cast as stabs in sterile culture tubes. The isolate suspension was pierced into stab with the help of a sterile inoculation needle and incubated at 35 ±2 ºC for 18 to 48 h. Results were observed.

e) Phosphate Solubilization Activity Detection Assay

Overnight (18-20 h) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Pikovskaya agar medium (Yeast extract 0.5 gm/ltr; Dextrose 10 gms/ltr; Calcium phosphate 5 gms/ltr; Ammonium sulphate 0.5 gms/ltr; Potassium chloride 0.2 gm/ltr; Magnesium sulphate 0.1 gm/ltr; Magnesium sulphite 0.001 gm/ltr; Ferrous sulphate 0.001 gm/ltr; Agar 15 gms/ltr; Final pH at 25 ºC 7.0±0.2) were prepared and cast on sterile disposable petri plates. The isolate suspension was stabbed into the agar plates and incubated at 35±2 ºC for 24 to 120 h. Results were observed.

f) Arginine Dihydrolase Production Detection Assay

Overnight (18-20 h) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Arginine Dihydrolase agar medium (Peptic digest of animal tissue

1gm/ltr; NaCl 5 gms/ltr; Dipotassium hydrogen phosphate 0.3 gm/ltr; L-Arginine 10 gms/ltr; Bromo cresol purple 0.016 gm/ltr; Agar 3 gms/ltr; Final pH at 25 ^{0}C 6.0±0.2) were prepared and cast as stabs in sterile culture tubes. The isolate suspension was pierced into stab with the help of a sterile inoculation needle and incubated at 35±2 ^{o}C for 18 to 48 h. Then 5-6 drops (600 µl-800 µl)of the Nessler's Reagent [Mercuric iodide 3%, Potassium Iodide 3.5%, Sodium Hydroxide 12%,and Water 81.5%] was added to the culture tube. Results in colour change were observed.

g) Catalase Production Detection Assay

Overnight (18-20 h) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Nutrient agar medium were prepared and cast on sterile disposable petri plates. The isolate suspension was streaked onto the medium plates using standard 4 way discontinuous streaking protocol and incubated at 35±2 ^{o}C for up to 48 hours. A few drops (500 µl-600 µl) of 15% H_2O_2 were poured over the grown colony and observed immediately for effervescent bubble production.

h) Oxidase Production Detection Assay

Overnight (18-20 h) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Nutrient agar medium were prepared and cast on sterile disposable petri plates. The isolate suspension was streaked onto the medium plates using standard 4 way discontinuous streaking protocol and incubated at 35^{o}C±2 ^{o}C for up to 48 h. The test was performed by putting bacterial culture on a strip of sterile filter paper impregnated with 1% (wt/vol) aqueous solution of N-N-dimethyl-*p*-phenylenediamine. Immediate colour change was observed.

i) Cellulase Production Detection Assay

Cellulase production test was performed according to the method of Hankin and Anagnostakis (1977) with little modification. A loopful of young culture of the bacterial isolate was inoculated to sterile disposable petri plates containing Czapek-mineral salt agar medium ($NaNO_3$ 2 gms/ltr.; K_2HPO_4 1 gms/ltr.; $MgSO_4$. $7H_2O$ 0.5 gms/ltr.; KCl 0.5 gm/ltr.; Peptone 2 gms/ltr.; Agar 20 gms/ltr.; Final pH at 25^{0}C 6.5 +/- 0.2) supplemented with carboxymethyl cellulose (5 gms/ltr.). The plates were incubated at 35^{o}C +/- 2^{o}C for 96 hours. After incubation the plates were flooded with 1% solution of hexadecyltrimethylammonium bromide (HDTMA). Any zone formation was observed.

j) Lipase Production Detection Assay

Overnight (18-20 hrs.) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Tributyrin agar medium (Peptic digest of animal tissue 5 gms/ltr; Yeast extract 3 gms/ltr; Agar 15 gms/ltr; Final pH at 25^{0}C 7.5±0.2) supplemented with 10% (vol/vol) sterile Tributyrin oil were prepared and cast on sterile disposable petri plates. The isolate suspension was streaked onto the medium plates using standard 4 way discontinuous streaking protocol and incubated at 35±2 ^{o}C for up to 48 hours. Any zone formation was observed.

k) Indole Acetic Acid (IAA) Production Detection Assay

IAA quantification was performed following the method of Gord**on** and Weber (1951). Bacterial strain was grown overnight (18-20 h) at 35±2 ^{o}C on Nutrient broth with or without tryptophan (500 µg /ml) and then the bacterial cells were removed from the culture medium by centrifugation. One ml of the supernatant was mixed vigorously with 4 ml of Salkowski's reagent (150 ml conc. H_2SO_4, 250 ml H_2O, 7.5 ml 0.5 M $FeCl_3.6H_2O$ as per proportion) and the absorbance was measured at 535 nm with Jasco UV-Visible spectrophotometer (Model V600) using a quartz cuvette (Kozima).

2.6 Antibiotyping

Sterile Mueller Hinton agar (Beef infusion 300 gms/ltr; Casein acid hydrolysate 17.5 gms/ltr; Starch 1.5 gms/ltr; Agar 17 gms/ltr; distilled water 1000 mls; Final pH at 25^{o}C 7.3±0.1) was prepared and poured in sterile disposable petri plates. The microbe culture was cultured overnight (18-20 h.) in Mueller Hinton broth

liquid cultures at 35±2 ºC at 110 rpm shaker speed. Growth was observed. About 0.2 ml (200µl) of the culture suspension was taken and uniformly spread over the solidified Mueller Hinton Agar by the standard protocol of Spread plate technique. The suspension was allowed to soak for 20 minutes at 25 ºC under sterile conditions. Subsequently standard antibiotic discs (Himedia) of commercially available concentrations were used to check the antibiotic sensitivity of the isolates using the Bauer-Kirby (1966) method of determining antibiotic sensitivity. Nine different antibiotics were used for each isolate [(Ticarcilin (10µg); Colistin (10 µg); Polymyxin B (30 units); Tetracyclin (30 µg); Imipenem (10 µg); Ciprofloxacin (5 µg); Netilin (30 µg); Gentamycin (10 µg) and Amikacin (30 µg)]. Experiments were performed in triplicate.

2.7 Growth Curve Assay of the Isolate

Fresh overnight grown (18-20 h) cell suspension was inoculated to 250 ml conical flask containing 75 ml of Nutrient broth and incubated at 35±2 ºC on incubator shaker (at 110 rpm). The growth responses were measured as a change in optical density at 600 nm at every 2 h of interval for a duration of 36 h using Jasco UV-Visible spectrophotometer (Model V600) using a quartz cuvette (Kozima) at 600 nm.

3. Observation /Result

3.1 Colony Characteristics of Each Isolate After Isolation

The three isolates showed a variety of colony characters which are listed in the following table:1

Table 1: Colony characteristics of isolates

Colony parameters	Colony characteristics		
	Urban (Dum Dum)	**Suburban (Serampore)**	**Rural (Mogra)**
Colony shape	Circular diffused	Round	Round
Colony size	Large	Medium	Small
Colony opacity	Opaque	Opaque	Translucent
Colony texture	Glossy	Glossy	Glossy
Colony colour	Yellowish white	Off white	Light Yellow
Colony surface morphology	Smooth	Myceloid	Smooth

3.2 Gram Characteristics of Each Isolate After Gram Staining

The Gram characteristic of each isolate was observed /determined after Gram staining. The results are listed on the following table: 2

Table 2: Gram characteristics of isolates

Cell parameters	Cell characteristics		
	Urban (Dum Dum)	**Suburban (Serampore)**	**Rural (Mogra)**
Cell Gram character	Gram Positive	Gram negative	Gram negative
Cell morphology	Rod shaped with round edges	Rod shaped with round edges	Rod shaped with tapered ends
Cell size	Very small	Small	Very small
Cell association	Occurred individualy	Occurred in chains	Occurred in chains

3.3 Specific Biotyping Assays:

These assays were performed to assess the economic and commercial viabilities of the isolates. Observation shows that the ability of the isolates to secrete different types enzymes which can be commercially harnessed and marketed. (Table -3)

Table 3: Biotyping assessment of isolates

Biotyping assays	Biotyping parameters (*"+" means positive result, "-" means negative result)*		
	Urban (DumDum)	**Suburban(Serampore)**	**Rural(Mogra)**
Amylase production detection assay *	+	++	-
Protease production detection assay	+	+	+
DNase production detection assay	-	-	-
Gelatin solubilization activity detection assay	-	-	-
Phosphate solubilization activity detection assay	-	-	-
Arginine dihydrolase production detection assay *	++	+	+
Catalase production detection assay	+++	+++	+
Oxidase production detection assay	-	-	-
Cellulase production detection assay	-	-	-
Lipase production detection assay	+ (Growth occurs)	+(Growth occurs)	-
Indole Acetic acid (IAA)production detection assay	-	-	-

The multiple "+" signs indicate the intensity of the colour formed with respect to the respective control setups.

3.4 Antibiotyping

The results are listed in the following table-4.

Table 4: Assessment of antibiotic resistance

(a) *Culex* gut bacteria isolate from urban (Dum Dum) area

Name of antibiotic	Zone of inhibition(in cm)	Mean Zone of inhibition(in cm)	Resistance type
Ticarcillin	0.2	0.2	Resistant
	0.2		
	0.2		
Colistin	No inhibition	-	Resistant
	No inhibition		
	No inhibition		
Polymyxin B	0.1	0.1	Resistant
	0.1		
	0.1		
Tetracyclin	0.45	0.43	Resistant
	0.42		
	0.45		
Imipenem	0.6	0.6	Resistant
	0.6		
	0.6		
Ciprofloxacin	1	1	Moderately Resistant
	1		
	1		
Netilin	0.9	0.92	Resistant
	0.92		
	0.92		
Amikacin	0.33	0.33	Resistant
	0.32		
	0.32		
Gentamycin	0.21	0.22	Resistant
	0.21		
	0.23		

(b) *Culex* gut bacteria isolate from sub-urban (Serampore) area

Name of antibiotic	Zone of inhibition(in cm)	Mean Zone of inhibition(in cm)	Resistance type
Ticarcillin	0.25	0.24	Resistant
	0.25		
	0.24		
Colistin	0.3	0.29	Resistant
	0.28		
	0.28		
Polymyxin B	0.18	0.18	Resistant
	0.18		
	0.17		
Tetracyclin	0.85	0.82	Resistant
	0.8		
	0.85		
Imipenem	0.64	0.65	Resistant

	0.65		
	0.65		
Ciprofloxacin	1.1	1.13	Moderately Resistant
	1.1		
	1.15		
Netilin	0.51	0.52	Resistant
	0.51		
	0.52		
Amikacin	0.43	0.43	Resistant
	0.42		
	0.42		
Gentamycin	0.71	0.72	Resistant
	0.71		
	0.73		

(c) *Culex* gut bacteria isolate from rural (Mogra) area

Name of antibiotic	**Zone of inhibition(in cm)**	**Mean Zone of inhibition(in cm)**	**Resistance type**
Ticarcillin	0.82	0.81	Resistant
	0.82		
	0.81		
Colistin	0.1	0.1	Resistant
	0.1		
	0.1		
Polymyxin B	0.21	0.22	Resistant
	0.22		
	0.22		
Tetracyclin	0.7	0.7	Resistant
	0.7		
	0.7		
Imipenem	1.4	1.4	Intermediate to sensitive
	1.4		
	1.4		
Ciprofloxacin	1	1	Moderately Resistant
	1		
	1		
Netilin	0.65	0.66	Resistant
	0.67		
	0.65		
Amikacin	0.5	0.5	Resistant
	0.5		
	0.5		
Gentamycin	0.6	0.6	Resistant
	0.6		
	0.6		

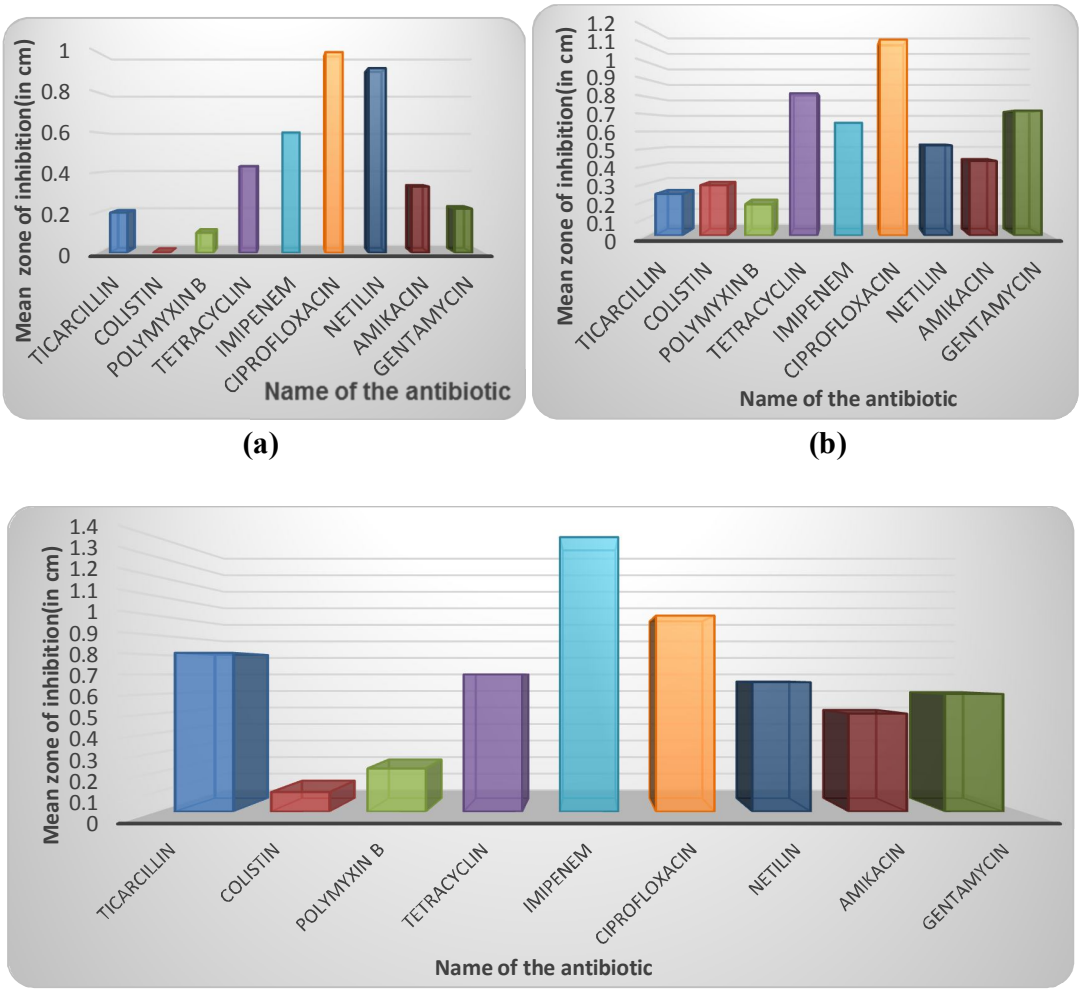

Fig 1: Graphical representation of mean zone of inhibition of mid gut isolates for different antibiotics from urban (Dumdum) (a), sub urban (Serampore) (b) and rural (Mogra) (c) *Culex* mosquitoes.

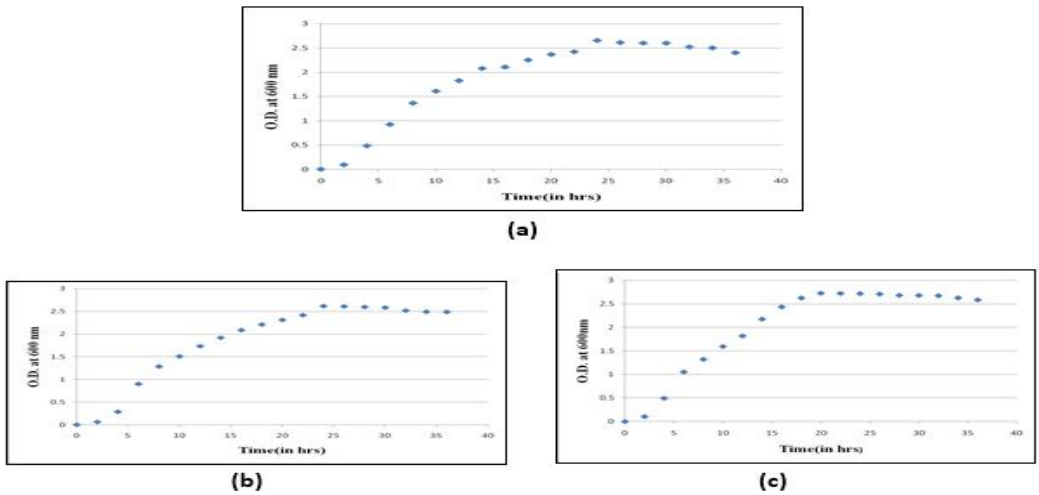

Fig 2: Growth curve of gut bacterial isolate from urban (Dum Dum) (a), sub-urban (Serampore) (b) and rural (Mogra) areas (c).

Table 5: Measurement of growth rate of isolates

Time (hours) (x-axis)	Optical density at 600 nm (y-axis)		
	Urban (DumDum)	Suburban(Serampore)	Rural(Mogra)
0	0.0000	0.0000	0.0000
2	0.0927	0.0618	0.1052
4	0.4833	0.2843	0.4952
6	0.9241	0.8990	1.0541
8	1.3625	1.284	1.3227
10	1.6087	1.507	1.5943
12	1.8263	1.732	1.8183
14	2.0771	1.917	2.1771
16	2.1059	2.086	2.4350
18	2.2501	2.209	2.6251
20	2.366	2.311	2.7258
22	2.4211	2.416	2.7223
24	2.6541	2.616	2.7181
26	2.6110	2.609	2.708
28	2.6008	2.596	2.6808
30	2.5991	2.583	2.6782
32	2.5201	2.516	2.6729
34	2.5008	2.491	2.6274
36	2.4018	2.486	2.5838

4. Result and Discussion:

Our findings provide comprehensive information about colony character, gram staining properties and biochemical characteristics of mid gut microbiota of *Culex* mosquitoes collected from urban, sub urban and rural areas of West Bengal. Our data (Table-1) revel that the gut-bacterial colony character of sub urban and rural areas are more or less round and urban one is circular. Observation from Gram staining (table-2) indicated that bacterial isolates from sub- urban and rural areas are gram negative and preferably of enterobacteriaceae family. On the contrary urban isolates are Gram positive in nature.

The growth of bacterial isolate of all the three areas displayed exponential fashion of growth rate (fig: 2-a, b, c). It has been reported that the rapid growth of mid gut micro biota may help in blood digestion in mosquitoes gut [1]. Data (Table -5) also reveal that if the bacterial culture is to be utilized for the isolation of economically important enzymes, the culture needs to be sub cultured within about 20 h.

These bacterial isolates demonstrate their unique property of secreting economically important enzymes. The different biochemical tests (Table-3) reveal that protease, arginine dihydrolase and catalase are produced by all the three bacterial isolates from urban, sub urban and rural localities. However, amylase and lipase are secreted by bacterial isolate from urban (Dum Dum) and Sub urban (Serampore) areas of West Bengal. These biochemical properties to secrete novel enzymes project these bacterial isolates as potential candidates for commercial importance. As for example, amylase producing bacteria can be used for production of amylase immobilized in beads and are used in detergents to degrade starch stains in cloths. Protease producing bacteria has the potential for degrading proteinaceous wastes. Lipase recently has been incorporated in nearly all synthetic commercial detergents as a lipidaceous stain remover similarly as amylase. Arginine Dihydrolase and Catalase can be harnessed from the isolates and marketed as molecular biological tool for research.

Different biochemical tests (Table-4) indicated that midgut microbiota of *Culex* mosquitoes obtained from urabn (Dum Dum) and sub urban (Serampore) and are found to be highly resistant

to the antibiotics such as Ticarcillin, Colistin, Polymyxin B, Tetracyclin, Imipenem, Netilin, Amikacin and Gentamycin and they are moderately resistant to antibiotic Ciprofloxacin. On the other hand rural (Mogra) *Culex* gut microbiota show resistance to the antibiotics viz. Ticarcillin, Colistin, Polymyxin B, Tetracyclin, Netilin, Amikacin and Gentamycin, moderately resistant to antibiotic Ciprofloxacin and sensitive to Imipenem. These results reveal that the availability of mid gut microbiota of mosquitoes may indicate the presence of these bacteria within the host (Human being/ cattle) of mosquitoes. Mosquitoes are known to illicit a specific immune response against parasite, Gram positive and Gram negative bacteria. Some of the immune responsive genes are expressed in response to both protozoa and bacteria [1]. Several studies [11,12,13] have indicated that midgut microbiota of *Culex* mosquitoes stimulate basal immune activity which in turn inhibit the growth of parasites viz *Wuchereria*, *Plasmodium* etc. It can suggest that this microbiota may also illicit basal immunity of the host (human/ cattle).

Culex mosquitoes usually live in highly contrasting environments where biotic (like competition or the food chain) and abiotic (like temperature or humidity) factors can influence the population of gut microbiota [14]. The mid gut bacterial diversity along with the biochemical characteristic is closely associated with the complex potential interaction between the symbiotic microbes and host. Current technologies are not sufficient to pinpoint all the fluxes of matter and energy between microorganisms and their hosts. However, a little investigation has been done on the beneficial functions provided by bacteria, especially those living intracellularly as endosymbionts [15]. It can be concluded that the study on gut microbiota along with their different biochemical characteristics may open new windows for better understanding of *Culex*-midgut microbiota interaction.

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