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Research Article

Characterization of Bacterial Isolates found in Breeding Habitats of Mosquitoes in Dehradun City, Uttarakhand

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A B S T R A C T

The breeding habitat is crucial for mosquito population dynamics, since it is the location where many important life cycle processes occur such as oviposition, larval development, and emergence take place. The present study mainly aimed to characterize microbial bacteria from the breeding habitats of mosquitoes in Dehradun city. The water sample was collected from mosquito breeding habitats of Dehradun during June to October 2013. For the characterization growth, morphology, staining properties, physiological, biochemical and antibiotic sensitivity test were conducted. Morphologically the isolates are sticky round and of diameter range of 4.1-6.1 mm. Gram staining showed isolates were gram positive and categorized as rod shaped in SEM. Isolates exhibited no growth on Mac'Conkey and EMB agar media. NaCl tolerance was found ≥ 6% and pH tolerance in the range of 5.5-7.7. Among the four bacterial isolates, all of them were positive for catalase test and starch hydrolysis test; and negative for citrate, indole and lipase hydrolysis test. R1 and R2 isolates were positive for nitrate test. Only R4 bacterial isolate showed positive result for urease test. R1 and R4 isolates showed positive result for oxidase test and R2 and R3 were negative for this test. All of the isolates produced both acid and gas utilizing glucose, sucrose and lactose as a carbohydrate source. R1 produced both acid and gas and R3 produced only acid but no gas from mannose in the medium. R1 isolate was found sensitive for chloramphenicol and tetracycline. R2 showed sensitivity against all the antibiotics used for this assay. R3 and R4 were sensitive to chloramphenicol and tetracycline, and thus characterized as Bacillus sp. All these four bacterial isolates (R1, R2, R3 and R4) were present in almost all the breeding grounds of mosquitoes in the study area which reflected that these bacteria must have some role in the oviposition and larval development of mosquitoes.

Keywords: Mosquitoes, Bacterial Isolates, Dehradun, Uttarakhand

Introduction

Mosquitoes belong to the group Diptera and transmit the diseases, such as Malaria, Dengue, Filariasis, Yellow fever, Chickungunya, West Nile virus, Encephalitis etc. Mosquitoes originating from specific breeding habitats because transmission normally occurs within a certain radius (within flight range of adult vectors) from breeding habitats.[1] The female mosquito lays eggs in natural habitats such as irrigation drainage system etc.[2,3] The breeding habitat is crucial for mosquito population dynamics, since it is the location where many important life cycle processes occur such as oviposition, larval development, and emergence take place.[4]

Different physical, chemical and environmental factors are known to influence mosquito oviposition behavior, and consequently their ultimate site selection. These are either attractant, repellent or stimulant.[5] Microorganisms, especially bacteria, have often been encountered in mosquito larvae and mosquito breeding sites.[6,7] Microorganisms play an important role as the oviposition attractant.[8] Henceforth, the bacterial diversity should be characterized to determine their relationship with the breeding habit of mosquito vectors.

According to the reports of Health Department, Uttarakhand (India), in district Dehradun, during the last decade, the epidemiological pattern of airborne diseases transmission has been unstable and seasonal from place to place. More serious is the effect of deforestation in hilly areas in present scenario and thereby its effect on the temperature governing sporogony like that reported in Anopheles species.[9]

In the view of this, it is important to identify the breeding habitats of mosquitoes at grass root level to find the association of larvae along with the bacterial strain as the mosquitoes use the bacterial cues for oviposition, and so if role of bacteria can be manipulated from the breeding sites then it would help to prevent the egg laying of mosquito species which in turn would be useful to control the vector population. Hence the present study aimed at preliminary stage to isolate and characterize the breeding water bacteria from the breeding habitats of mosquitoes in Dehradun city.

Material and Methods

Collection of Water Sample from Mosquito Breeding Habitats

The water sample was collected from different mosquito breeding habitats of Dehradun city during the month of June to October 2013.

Processing of Water Sample for Microbial Culture

Serial dilution of water sample (up to 10^{-4}) was made with sterile distilled water. Each dilution was plated on nutrient agar (peptone: beef extract: NaCl: agar at 5:3:3:1 g/l) plates and incubated at 30±1°C in a BOD incubator for 24 hours to obtain the isolated colonies. After the incubation, there were visible bacterial colonies. Then pure culture was made by quadrant streaking technique.[10] Pure culture was maintained on agar slants for further characterization and identification.

Characterization of Microbes

For the characterization of microbes, the standard microbiological methods were followed.[10-12] The isolates were streaked on NA slants, Mac'Conkey, EMB (Eosin Methylene Blue) agar media and inoculated in Nutrient Broth (NB), then incubated at 30 ± 0.1ºC for 72 hours and growth of the isolates were recorded. Morphological characters of the colony like shape, size, color, margin and opacity were recorded. For the study of staining characters, vegetative cells were stained with gram stains and were also processed for scanning electron microscopy images.

Physiological and Biochemical Characterization

For physiological test, extracellular enzymatic activity like Starch hydrolysis test and Lipase test were performed. To characterize the isolate biochemically, Catalase test, Oxidase test, Citrate utilization test, Nitrate reduction test, Indole production test, Urease test, NaCl tolerance, Carbohydrate metabolism (acid- gas production) tests were performed.[13] Antibiotic sensitivity test of the bacterial strain was also performed using antibiotic discs of various antibiotics like ampicillin (10mcg), chloramphenicol (30mcg), ciprofloxacin (5mcg) and tetracycline (30mcg) following the protocol of Brown.[14]

Result

The colony of R1 isolate was sticky, round shaped, off-white in color with smooth margin and 6 mm in diameter. R2 colony was also found sticky, almost round shaped, off- white in color with slightly serrated margin and 6.1mm in diameter. The R3 bacterial colony was sticky, round shaped, smooth and white in color and 5.6 mm in diameter. The colony of the R4 isolate was sticky, round shaped, off white in color with slightly serrated margin and 4.1 mm in diameter. The vegetative spores were observed under microscope, featuring rod shaped spores in scanning electron micrograph (Table 1 and Fig. 1).

Gram staining showed that the bacterial isolates were Gram positive and rod shaped having spores. The isolates showed no growth on Mac'Conkey and EMB agar media. All these bacterial isolates could tolerate up to 65˚C temperature. R1 and R4 isolate could tolerate up to 6% NaCl whereas R2 and R3 could tolerate up to 8% NaCl in the nutrient broth medium. The pH tolerance range of R1, R2, R3 and R4 isolates were 6.0-7.1, 5.8-7.7, 5.5-7.7 and 6.0-7.0 respectively (Fig. 2).

Table 1.Morphological features of the bacterial isolates

Morphological Features	R1	R2	R3	R4
Nature	Sticky	Sticky	Sticky	Sticky
Color	Off white	Off white	White	Off white
Shape	Round	Almost round with slightly serrated margin	Round and smooth	Round and slightly serrated
Diameter	6 mm	6.1 mm	5.6 mm	4.1 mm

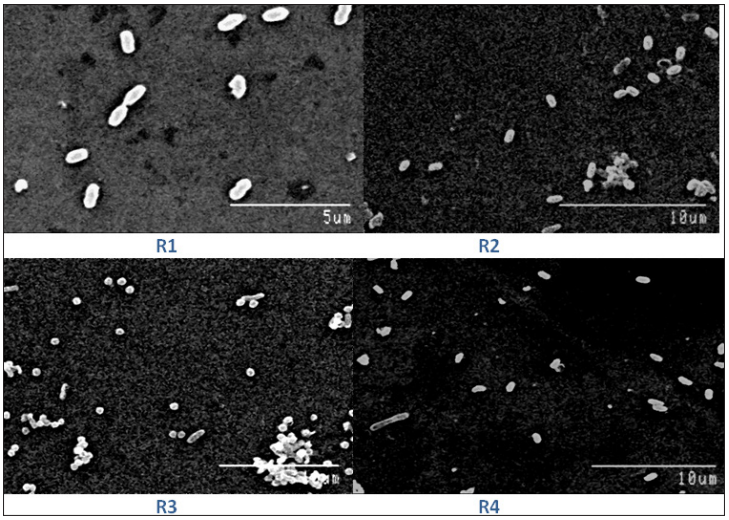

Figure 1.Scanning Electron Micrograph of the Bacterial isolates (R1, R2, R3 and R4)

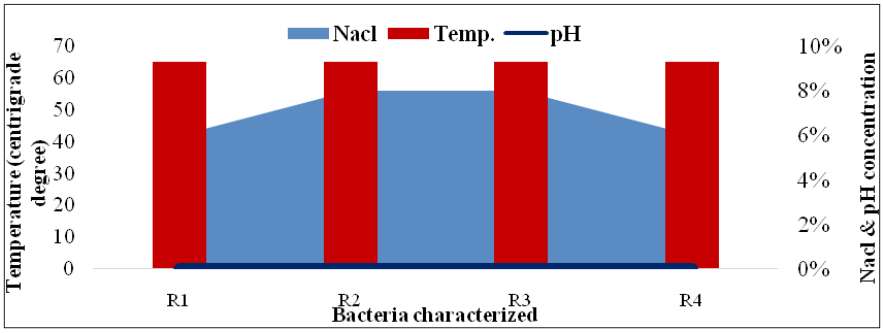

Figure 2.Physiological test of the Bacteria characterized

Table 2.Biochemical test of the Bacteria characterized

Bio chemicals	R1	R2	R3	R4
Catalase	+	+	+	+
Citrate	-	-	-	-
Nitrate	+	+	-	-
Indole	-	-	-	-
Urease	-	-	-	+
Starch	+	+	+	+
Lipase	-	-	-	-
Oxidase	+	-	-	+

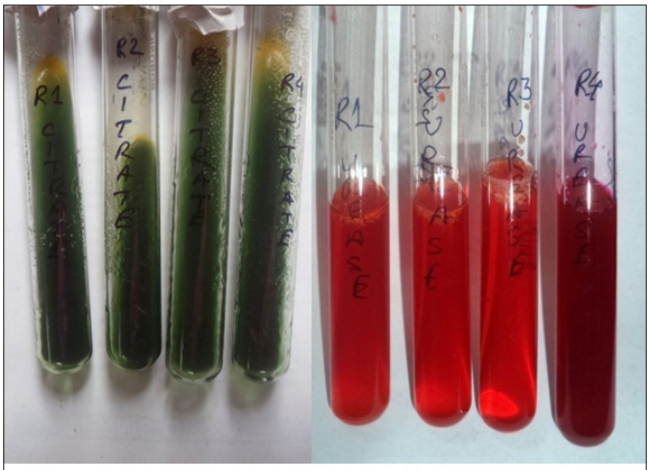

Figure 3.A.Citrate test and B.Urease test of the Bacteria (In Citrate test R1, R2, R3 and R4 no growth and yellowish- green color of the medium indicated negative result for the test. In Urease Test R1, R2, R3 are unable to change the pale yellowish pink color of the medium whereas R4 produces an alkaline reaction in the medium and a pinkish-red color appeared)

Table 3.Fermentation test of the Bacteria characterized

Fermentingelements	R1		R2		R3		R4	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Glucose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Mannose	+	+	-	-	+	-	-	-

Table 4.Antibiotic sensitivity test

Antibiotic	R1	R2	R3	R4
Ampicillin	R	S	R	R
Chloramphencol	S	S	S	S
Tetracyclin	S	S	S	S

Note: R= Resistant and S= SensitivityAfter analyzing.

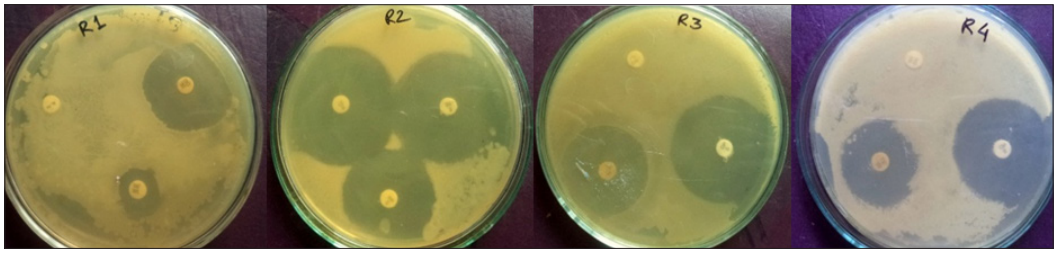

Figure 4.Antibiotic sensitivity test with Chloromphenicol, Tetracyclin and Ampicillin with the isolates

Among the four bacterial isolates, all of them were positive for catalase test and starch hydrolysis test; and negative for citrate, indole and lipase hydrolysis test. R1 and R2 isolates were positive for nitrate test. Only R4 bacterial isolate showed positive result for urease test. R1 and R4 isolates showed positive result for oxidase test and R2 and R3 were negative for this test (Table 2 and Fig. 3).

All of the isolates produced both acid and gas utilizing glucose, sucrose and lactose as a carbohydrate source. R1 produced both acid and gas and R3 produced only acid but no gas from mannose in the medium (Table 3).

R1 isolate was sensitive for chloramphenicol and ciprofloxacin. R2 showed sensitivity against all the antibiotics used for this assay. R3 and R4 were sensitive to chloramphenicol, ciprofloxacin and tetracycline (Table 4 and Fig. 4).

The water samples of different locations of same habitats at Dehradun, four isolates viz., R1, R2, R3 and R4 were investigated and characterized as Bacillus sp. following Bergey's Manual (1964).[15]

Discussion

The breeding water quality is an important determinant of whether or not the female mosquitoes will lay their eggs and the mosquito larvae will successfully complete their life cycle.[16] It is already known that the biological and physicochemical parameters play an important role in the oviposition and larval development of the mosquitoes. Water microbes play the role of oviposition attractant in many places. Bacillus cereus had been reported as a significant oviposition attractant.[17-18]

The breeding water bacteria are able to produce several microbial by- products that may be acidic, alkaline or neutral, which may change the physicochemical properties of the breeding water and thus influence the oviposition and larval development of different mosquitoes. Different mosquito species prefer different physicochemical parameters for their development and so the microbial population in the breeding water also varies with respect to different mosquito breeding grounds. In a study of north- western Thailand, between two Anopheles mosquitoes; An. vagus were found to prefer high pH for its larval development while An. dirus larvae were found in habitats with lower pH values which were directly proportional to the microbial population of the breeding ground of these two different species.[19]

The breeding water bacteria sometimes have various enzymes which help to degrade complex biomolecules and produce simple particles that may be taken by the mosquito larvae to fulfill their nutritional requirement. Some bacterial isolates present in the breeding water may also be incorporated within the larval gut and thus help in the larval development. Here, R1, R2, R3 and R4 isolates, all could hydrolyze starch to simple carbohydrate which can be used by the larvae present in the water as carbohydrate source. The symbiotic gut bacteria are an important factor for the larval development that helps in the increase of the adult vector population, thus helping in disease propagation. A bacterium, Thorsellia had impact on mosquito mid gut had been reported from Kenya.[20-23]

The bacteria got incorporated within the mosquito gut via food from the breeding water. Thorsellia can be used as a potential candidate for paratransgenesis, the genetic modification of symbiotic microbes in the mosquitoes for producing anti- malaria parasite molecules.[24] In addition, bacterial species from an infusion of alfalfa reported to produce chemical stimulants of oviposition in Ae. aegypti and Cx. quinquefasciatus.[25]

Conclusion

It was found that all these four bacterial isolates (R1, R2, R3 and R4) were present in almost all the breeding grounds of mosquitoes in the study area which reflected that these bacteria must have some role in the oviposition and larval development of the mosquitoes. So if we can control the bacterial population, it will be easier to control the vector population without using harmful chemicals. These bacterial isolates may also be used in paratansgenesis in near future. Malaria, dengue and various air borne diseases issued big health problems in many parts of the world and it is the need of the day to continue research in this area to find new ways to prevent transmission. Though it is only a preliminary study, but this can help us to control as well as to eradicate the vector population in its immature stage.

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Conflict of Interest: None

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