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Full Length Research Paper

Molecular characterization of midgut bacteria of *Aedes albopictus* exhibiting swarming motility property

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***Aedes* mosquitoes are responsible for the transmission of arboviruses including dengue, chikungunya, zika, and yellow fever throughout the world. Mosquito gut harbors a variety of microbes, known to play a potential role in various physiological processes such as food digestion and pathogen development. Many bacterial species exert various movements like swarming, gliding and twitching which vary with individual bacterial species. Swarming is one of the movement patterns of bacterial cells in which newly growing cells direct themselves towards the edges of the colony on the solid agar plate. We have isolated a bacterial colony, which exerts swarming activity and moves away from its inoculation point following a translucent path. After repeated sub-culturing of the colony we isolated two different bacterial species which were found to be highly associated to each other in co-culture. Various techniques like biochemical analysis, 16S rRNA gene sequence based analysis, MALDI-TOFF MS and Scanning Electron Microscopy (SEM) were applied for in-depth characterization of isolates. The isolates were identified as *Staphylococcus saprophyticus* and *Brevibacillus agri.* Both bacterial isolates showed swarming activity in co-culture on the solid agar surface, but lost the activity when tested individually. Moreover, the swarming activity was even not recovered when both purified bacterial isolates were mixed at different concentrations. This demonstrated that an integral connection exists between the two species which is responsible for their swarming activity in co-culture.**

Key words: Swarming, midgut, microbiota, *Aedes albopictus*, pathogens.

INTRODUCTION

Many bacterial species exert various movements like swarming, gliding and twitching which varies with individual bacterial species. Various types of movement properties have been reported in many prokaryotic cells, mainly in bacteria (Cong et al., 2011; Fraser and Hughes, 1999; Harshey, 2003; Kim et al., 2003; Merino et al.,

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2006). The swimming movement pattern is the individual bacterial cells behavior, whereas, swarming is shown by bacterial colony (Cong et al., 2011). Many bacterial species have the characteristic to grow and spread on the surface such as nutrient agar or eukaryotic cells in a tissue, from where they take up the nutrient and water. Swarming is one of the movement patterns of bacterial cells in which new cells direct themselves towards the edges of the colony (Kaiser, 2007). In swarming motility, the bacterial cells required to get in touch with solid substrate and this interaction between bacterial cells and solid surface make them swarming proficient (Kearns, 2010). Swarming reduces competition between bacterial cells for the nutrient and speeds up colony growth (Kaiser et al., 2007). Some other surface phenomena feature like host invasion and biofilm formation has been shared in the swarming movement as in *Proteus mirabilis*, which facilitates urinary tract colonization and encouraging biofilm formation on catheters (Cong et al., 2011; Allison et al., 1994; Stickler et al., 1998).

Aedes aegypti and *Ae. albopictus* are the most important vectors of arboviruses including dengue, chikungunya, zika, and yellow fever throughout the world. Mosquito gut harbors a diverse variety of microbes, which interact with disease-causing parasites and influence vectorial capacity of mosquito vectors (Yadav et al., 2015a, b, Chandel et al., 2013; Moro et al., 2013; Minard et al., 2013; Dong et al., 2009, Rani et al., 2009). The midgut bacterial communities of *Anopheles gambiae* decrease the susceptibility to *Plasmodium falciparum* infection (Dong et al., 2009). However, it has been also reported that the presence of some bacterial species promote the *Plasmodium* infection (Boissière et al., 2012). The bacterial species *Serratia marcescens* enhances the susceptibility to dengue and chikungunya viral infection (Apte-Deshpande et al., 2012, 2014). Chikungunya viral infection changes the midgut bacterial diversity in *Ae. albopictus* and increases the bacterial genera of Enterobacteriaceae family and decreases *Wolbachia* and *Blattabacterium* (Boissière et al., 2012, Zouache et al., 2012, Minard et al., 2013).

The midgut microbiota of insects also interacts to each other either in cooperative or competitive manner (Minard et al., 2013). An interspecific competition was identified by bacterial species *Serratia marcescens* on *Sphingomonas* and Burkholderiaceae family members isolated from *Ae. aegypti* (Terenius et al., 2012). The bacterial diversity was observed low in mosquito's midgut in the presence of *S. marcescens* and the same result was found in the case of desert locust *Schistocerca gregaria* (Dillon et al., 2002). However, in *Ae. albopictus* a convincing association-ship was shown by two bacterial genera *Asaia* and *Acinetobacter* (Minard et al., 2013).

It has been demonstrated that bacterial species interact to each other inside the mosquito's midgut (Minard et al., 2013). However, the interspecific interaction between the bacterial isolates responsible for swarming motility was not described previously. The aim of this study was characterization of bacterial isolates, exhibiting swarming motility in co-culture isolated from the midgut of *Ae. albopictus*. The information about the midgut bacterial interaction and behavior is limited. Hence, the present study might be a step towards understanding the bacterial interaction of mosquito's midgut, which plays various important roles in defense, food digestion and pathogen development (Minard et al., 2013; Dong et al., 2009).

MATERIALS AND METHODS

Sample collection and isolation of midgut bacteria

Larvae and pupae of *Aedes* mosquitoes were collected from Missamari Military Farm, Sonitpur, North East India, and pupae were transferred to the cages covered with net for emergence. 24 h after emergence, female mosquitoes were removed and anesthetized with chloroform for confirming the species *Ae. albopictus* on the basis of morphological characteristics using taxonomic pictorial keys (Rueda, 2004). The mosquitoes were dissected for the isolation of midgut bacteria according to the protocol previously described by Chandel et al. (2013). The dissected midgut was transferred in the sterile Phosphate-buffered saline (PBS) and homogenized with sterilized micro-pestle. The homogenate was pour plated on nutrient agar and incubated at 37°C for 24 h (Chandel et al., 2013). The bacteria colony designated as MS1-8 was further serially diluted up to 10^{-8} and two morphological distinguished bacterial species designated as MS1-8-I and MS1-8-II was obtained.

Characterization of bacterial isolates

Phenotypic characterization

The isolated bacteria were characterized based on colony morphology (like shape, size, colour, margin, opacity, elevation) and Gram staining (Rani et al., 2009). Motility of individual bacterial species was studied using the hanging drop method under a light microscope (*Leica* DM E compound microscope). Biochemical tests such as nitrate reduction, urease activity, citrate utilization, H_2S production, Voges-Proskauer (VP), Methyl Red (MR), β–galactosidase activity, ornithine and lysine utilization activity and carbon source utilization profile were also performed according to manufacturer's instruction (HiMedia kit).

Matrix-assisted laser desorption / ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis

The MALDI-TOF-MS identification system was used for the identification of MS1-8-I and MS1-8-II. The fresh bacterial culture was directly deposited on a MALDI-TOF MTP 96 target plate (Bruker Daltonics GmbH) and inactivated with 96% formic acid (1 µl) followed by overlaying 1 µl of matrix solution (α-cyano-4-hydroxycinnamic acid in 50% acetonitrile, and 2.5% trifluoroacetic acid). Finally, proceeds with air-drying at room temperature for crystal formation of matrix-sample and spectra measured and recorded using Microflex III mass spectrometer (Bruker Daltonics) with a 337- nm nitrogen laser. The obtained raw spectra were compared with reference Biotyper database using Biotyper

Table 1. Restriction enzymes used for cutting 16S rRNA gene of all isolates with their cutting sites.

S/N	Restriction enzyme	Cutting site
1	MvaI	5' CC↓WGG 3' 3' GGW↑CC 5'
2	EcoRI	5' G↓AATTC 3' 3' CTTAA↑G 5'
3	SphI	5' GCATG↓C 3' 3' C↑GTACG 5'
4	MspI	5' C↓CGG 3' 3' GGC↑C 5'

software, *ver*. 3.0 (Prakash et al., 2014).

PCR amplification and sequencing of 16S rRNA gene

Genomic DNA was isolated from overnight grown bacterial culture, according to modified protocol of Sambrook et al. (2001). Ribosomal 16S RNA gene from genomic DNA was amplified according to the protocol described by Yadav et al. (2015 b) using an universal primer set 16S1 (5'-GAGTTTGATCCTGGCTCA-3') and 16S2 (5'-CGGCTACCTTGTTACGACTT-3') (Alam et al., 2006). PCR cleanup kit (Chromous Biotech Pvt. Ltd., Bangalore, India) was used for the purification of amplified PCR product followed by manufacturer's instruction and was sequenced on ABI 3500xL Genetic Analyzer (Applied Biosystems Inc. Foster City, CA) at Chromous Biotech Pvt. Ltd, Bangalore, India. Forward and the reverse sequence were aligned to get a complete sequence.

Sequence analyses

Obtained 16S rRNA gene sequences of bacterial isolates were analyzed using EzTaxon server (http://www.ezbiocloud.net/eztaxon) and BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and closely related sequences were recovered from GenBank database for phylogenetic analysis. Multiple sequence alignment was performed using the Clustal W and unaligned sequences at the end were trimmed out. MEGA 6.0 package was used to perform phylogenetic relatedness using neighbor joining and Kimura 2 distances parameter method with thousand bootstrap replicates (Tamura et al., 2013). The sequence of 16S rRNA gene of *Kocuria palustris* (Y16263) was used as out group. Sequences were submitted to GenBank under the accession numbers (KT315584 and KT315585).

Scanning electron microscopy (SEM)

Overnight broth culture of bacteria was centrifuged at 5000 rpm for 10 min followed by two times pellet washing with PBS. Glutaraldehyde (2.5% in PBS) was added to cell pellets, mixed gently and incubated at 4°C for 6 h for fixation. Supernatant was discarded after centrifugation followed by three times pellet washing with PBS. Bacterial specimens were dehydrated using gradually increased concentrations of ethanol (30, 50, 80 and 100%). While using 30, 50 and 80% ethanol, the ethanol was added to pellet, which was mixed, and incubated at room temperature for 10 min? It was then centrifuged and the supernatant was removed, but in case of 100% ethanol, samples were directly transferred to pre-sterilized cover slip and allowed to air dry. Dry samples were platinum coated and examined under (SEM) JEOL-JSM-6390LV Scanning Electron Microscope (Yadav et al., 2015b).

PCR-RFLP analysis of 16S rRNA gene

A total of 4 different restriction enzymes having different restriction sites (MvaI, EcoRI, SphI, and MspI) were used for the analysis of restriction fragment length polymorphism (RFLP) patterns of 16S rRNA gene of bacterial isolates (Table 1). Purified 16S rRNA PCR amplicons were used for the digestion through restriction enzymes according to the manufacturer's instruction (Thermo fisher scientific, UK). Restriction digested PCR amplicons were visualized on 2% agarose gel.

Movement property study using isolated bacterial species

Overnight grown bacterial cultures of both MS1-8-I and MS1-8-II were mixed in a gradually increasing proportion followed by inoculation on solid agar plates for checking the regaining of movement property. Prior to mixing, the optical density of both bacterial cultures was measured at 630 nm to find out the concentration of bacteria in each culture. The optical density of each bacterial culture was equally maintained and mixed in a specific proportion, that is, increasing the concentration of one culture (10, 20, 30, 40, 50, 60, 70, 80 and 90) and decreasing the concentration of another culture (90, 80, 70, 60, 50, 40, 30, 20 and 10). The mixed culture was inoculated on the agar plate at 0, 24 and 48 h intervals. Zero hour samples were inoculated immediately after mixing of the individual isolate, whereas, for 24 and 48 h mixed bacterial culture was incubated at 37°C at 120 rpm and inoculated on the agar plate after 24 and 48 h. The inoculated plates were incubated at 37°C for 12 to 24 h and movement properties of bacterial colonies were observed.

Both the overnight broth cultures MS1-8-I and MS1-8-II were mixed on the solid agar plate surface. For this purpose, one culture was inoculated onto agar plate through spreader and was incubated at 37°C for 3 to 4 h. Later on, the other culture was point inoculated in the center of the lawn of first culture and incubated at 37°C for 24 to 48 h. This procedure was followed for the both bacterial species, respectively.

RESULTS

The bacterial isolate MS1-8 was isolated from the midgut of an Asian tiger mosquito *Ae. albopictus*. Initially, MS1-8 appeared as a single bacterial colony on a solid agar plate but repeated sequencing of 16S rRNA gene revealed the presence of mixed sequences. Further, microscopic examination under a light microscope, Gram staining and Scanning Electron Microscopy (SEM) demonstrated that the colony designated as MS1-8 was a mixture of two different type of bacterial isolates (round *vs.* rod) which were characterized by using various techniques.

Phenotypic characterization

Phenotypic characteristics of bacterial isolates were

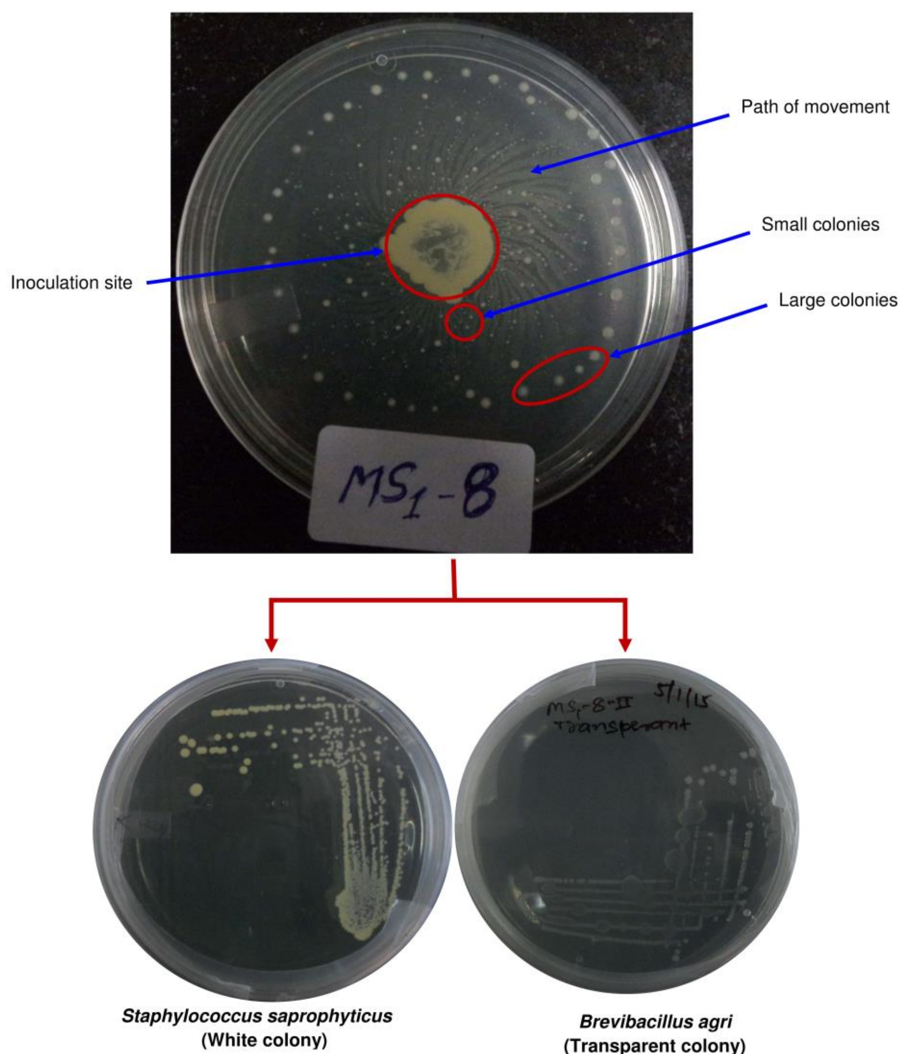

Figure 1. Bacteria colony of MS1-8 exhibiting movement property: I. Small colonies of MS1-8 appeared near to inoculation site and large colonies at peripheral site and movement of colonies follows a transparent pathway. II. White purified bacterial colonies MS1-8-I. III. Transparent purified bacterial colonies MS1-8-II.

initially studied for the first line of characterization. The isolated parental bacterial colony MS1-8 showed swarming property when inoculated on the center of nutrient agar plates and left their path from the inoculation site as shown in Figure 1. During movement, small colonies started from their inoculation site and became larger when reached to periphery and it followed a translucent path from inoculation site to the periphery. The parental bacterial colony MS1-8 was the mixture of two highly associated bacterial species MS1-8-I and MS1-8-II which were separated and purified later. On the solid surface of the agar plate, the bacterial colonies of MS1-8-I appeared as a white, round shape, small, about 2 mm in diameter, convex, opaque, and smooth margin. Like MS1-8-I, bacterial colonies of MS1-8-II were also in round shape, small, about 2 mm in diameter and smooth margin but it was thin, flat and translucent (Figure 1). Both the bacterial species MS1-8-I and MS1-8-II were Gram-positive. During observation of nutrient broth culture under light microscope, the bacterial species MS-1-8 appeared like small cocci, which was not moving in broth culture while, MS1-8-II appeared as a rod shape and was very fast moving.

The comparative biochemical reactions of bacterial isolates MS1-8-I, MS1-8-II and MS1-8 are summarized in Table 2. All bacterial isolates MS1-8-I, MS1-8-II, and MS1-8 have some common biochemical reactions. They were able to hydrolyze the Esculin and urea. Phenylalanine deamination and Arginine decarboxylation activity were also detected by these three isolates. These

Table 2. Biochemical characteristics of bacterial Isolates MS1-8-I, MS1-8-II and MS1-8.

Test	MS1-8-I (White)	MS1-8-II (Transperant)	MS1-8 (Mixed)
ONPG	+	-	+
PYR	+	-	-
Lysine utilization	+	+	V
Ornithine utilization	+	+	V
Arginine utilization	-	-	-
Urease	+	+	+
Phenylalanine deamination	V	V	V
Nitrate reduction	-	-	+
H_2S production	V	+	+
Citrate utilization	-	+	+
Voges Proskauer's	+	-	-
Methyl Red	-	-	-
Indole	-	-	-
Malonate utilization	-	-	-
Esculin hydrolysis	+	+	+
Arabinose	-	-	+
Xylose	-	-	-
Adonitol	-	-	-
Rhamnose	-	-	-
Cellobiose	-	-	+
Melibiose	-	-	+
Saccharose	+	-	+
Raffinose	-	-	+
Trehalose	+	-	+
Glucose	+	-	+
Arabinose	-	-	+
Sucrose	+	V	+
Sorbitol	-	-	+
Mannitol	+	-	+
Lactose	-	-	+
Oxidase	-	+	+

+, Positive; -, negative; V, variable.

isolates exhibited negative result for utilization of xylose, adonitol, rhamnose, malonate and methyl red. They were not capable of domination of tryptophan, hence showed a negative result for Indole test. The bacterial isolates MS1-8-I and MS1-8 have the ability to utilization of carbohydrates like saccharose, trehalose, glucose, sucrose, and mannitol, while the strain MS1-8-II was not utilizing this carbohydrate as a food sources. Apart from these mentioned carbohydrates, the isolate MS1-8 showed a positive response to utilization of some other carbohydrates like arabinose, cellobiose, melibiose, raffinose, arabinose, sorbitol and lactose. MS1-8 and MS1-8-II also have the capability to use citrate as a carbon source, while MS1-8-I does not have this ability. Only in MS1-8-I, PYR enzyme activities were detected and it also produces the acetoin which confirms positive activity for Voges-Proskauer's reaction. MS1-8-II and MS1-8 have the capability for the production of H_2S and also have the ability for the production of cytochrome oxidase (Table 2).

Matrix-assisted laser desorption / ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis

MALDI-TOF mass spectrum analysis is recently very useful, easy and discriminatory tool for identification of bacterial species (Lista et al., 2011). The obtained score values were compared to Biotyper database for identification of bacterial species. The score value of

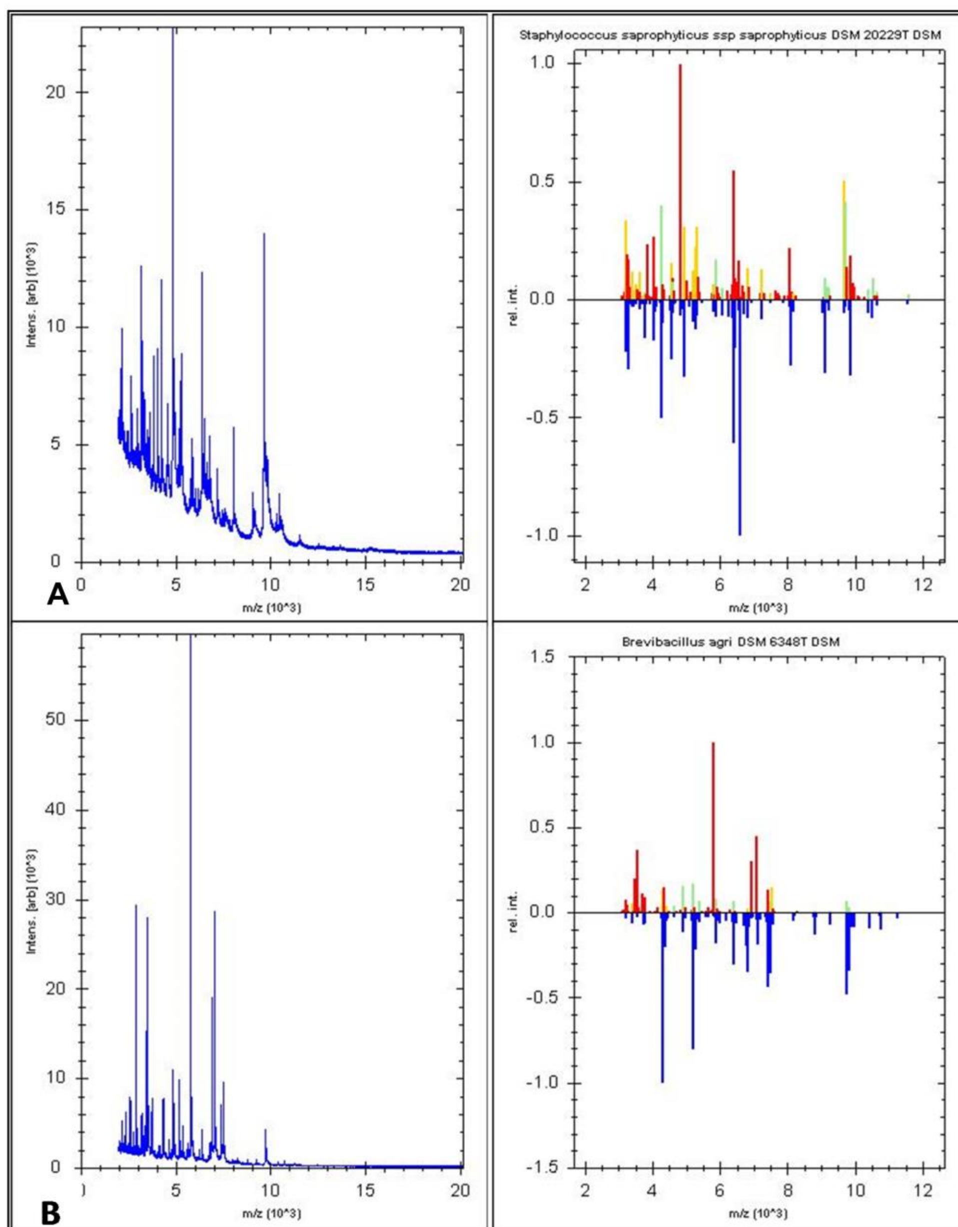

Figure 2. MALDI-TOF mass spectrum analysis based bacterial identification: **A.** *Staphylococcus saprophyticus;* and **B.** *Brevibacillus agri.*

MS1-8-I and MS1-8-II was 1.4 and 1.64 respectively and according to result matching with Biotyper database they were identified as *Staphylococcus saprophyticus* and *Brevibacillus agri* (Figure 2A and B).

16S rRNA gene sequence based analysis

Identification of bacterial species from MALDI-TOF mass spectrum analysis system was finally confirmed with the result of 16S rRNA gene sequence analysis. The obtained sequences of MS1-8-I and MS1-8-II were compared with homologous sequence of Genbank database. The sequence of MS1-8-I showed highest similarity with the sequence of *S. saprophyticus* in EzTaxon server (100%) and NCBI database (100%) and MS1-8-II with *B. agri,* EzTaxon server (99.41%) and NCBI database (100%). The phylogenetic tree was constructed using homologous sequences downloaded from Genbank database in which *Kocuria palustris*

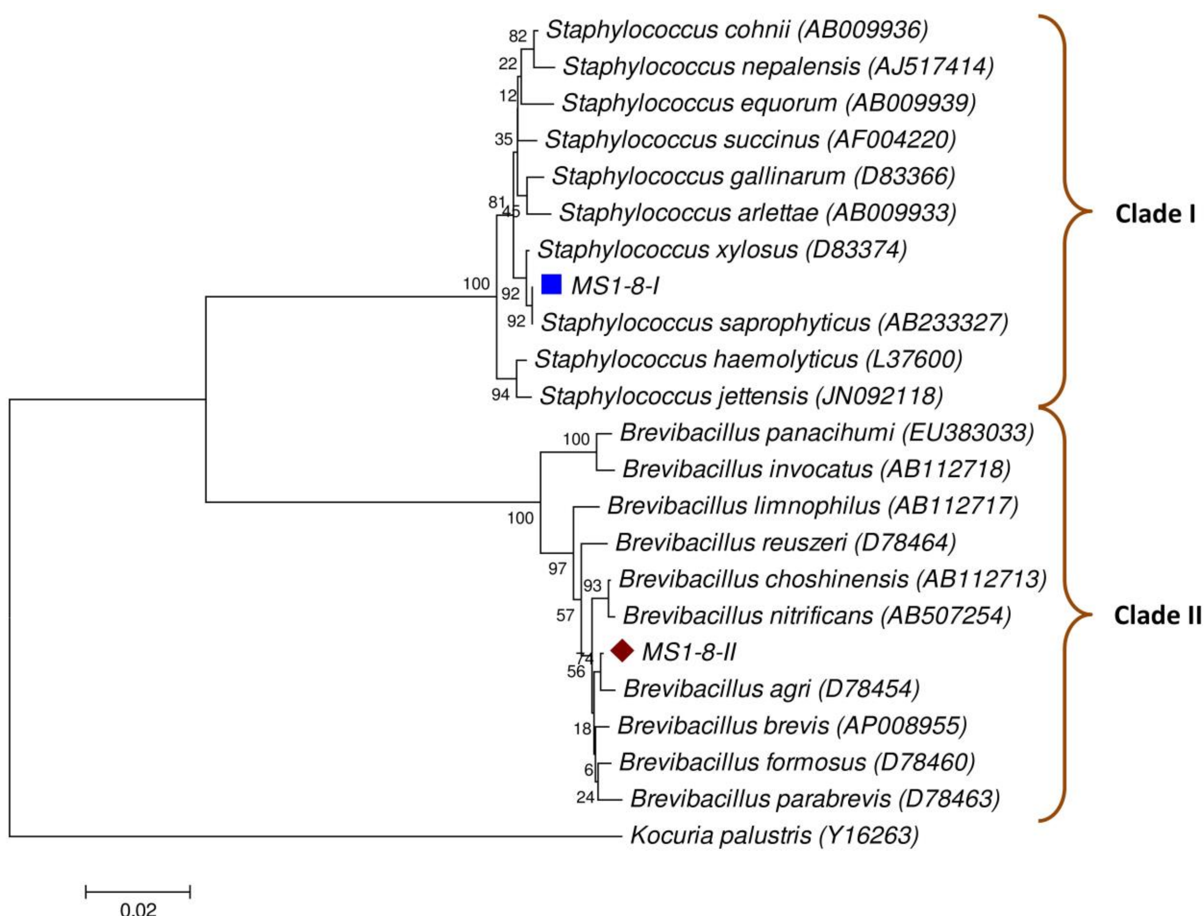

Figure 3. Dendogram based on 16S rRNA gene sequencing constructed by neighbor joining method-using Kimura 2 distances parameter.

(Y16263) was taken as an outgroup. The phylogenetic tree was divided into two mega clusters, genus *Staphylococcus* and *Brevibacillus* (Figure 3).

Scanning Electron Microscopy (SEM)

Bacterial samples were also analyzed through Scanning Electron Microscopy (SEM) at 5000X magnification (Figure 4). The image of MS1-8 containing two types of bacterial cells, one a small cocci and second a long rod shape (Figure 4a). These types of bacterial cells were also appeared in the Gram staining. The bacterial isolate MS1-8-I was identified as *S. saprophyticus* and appeared as a small round shape (Figure 4b) while bacterial isolate MS1-8-II appeared as a long, rod shape and was identified as *B. agri.* The appearence of only two types of bacterial cells in the image of MS1-8, confirmed that the parental bacterial culture MS1-8 was the mixture of two type of bacterial species *S. saprophyticus* and *B. agri* (Figure 4a, b and c).

PCR-RFLP analysis of 16S rRNA gene

According to the examination of broth culture of MS1-8 under a light microscope, Gram staining and SEM image analysis, it was confirmed that the bacterial culture MS1-8 was the mixture of only two different bacterial species and was identified as *S. saprophyticus* (MS1-8-I) and *B. agri* (MS1-8-II), respectively. For further validation of the above finding, we performed the Restriction Fragment Length Polymorphism (RFLP) analysis of 16S rRNA gene of all the bacterial isolates using four different restriction enzymes (MvaI, EcoRI, SphI, and MspI). In the all agarose gel image, the GeneRuler 100 bp Plus DNA Ladder (Thermo fisher scientific, UK) was loaded in the first lane followed by control (uncut 16S rRNA gene), MS1-8-I, MS1-8-II, and MS1-8, respectively. Figure 5A was the gel image digested through enzyme MvaI, where three bands were seen in third lane (MS1-8-I) and two in forth lane (MS1-8-II). All these bands were also seen in the fifth lane (MS1-8). Figure 5B was the image digested by the enzyme EcoRI where two bands appeared in the

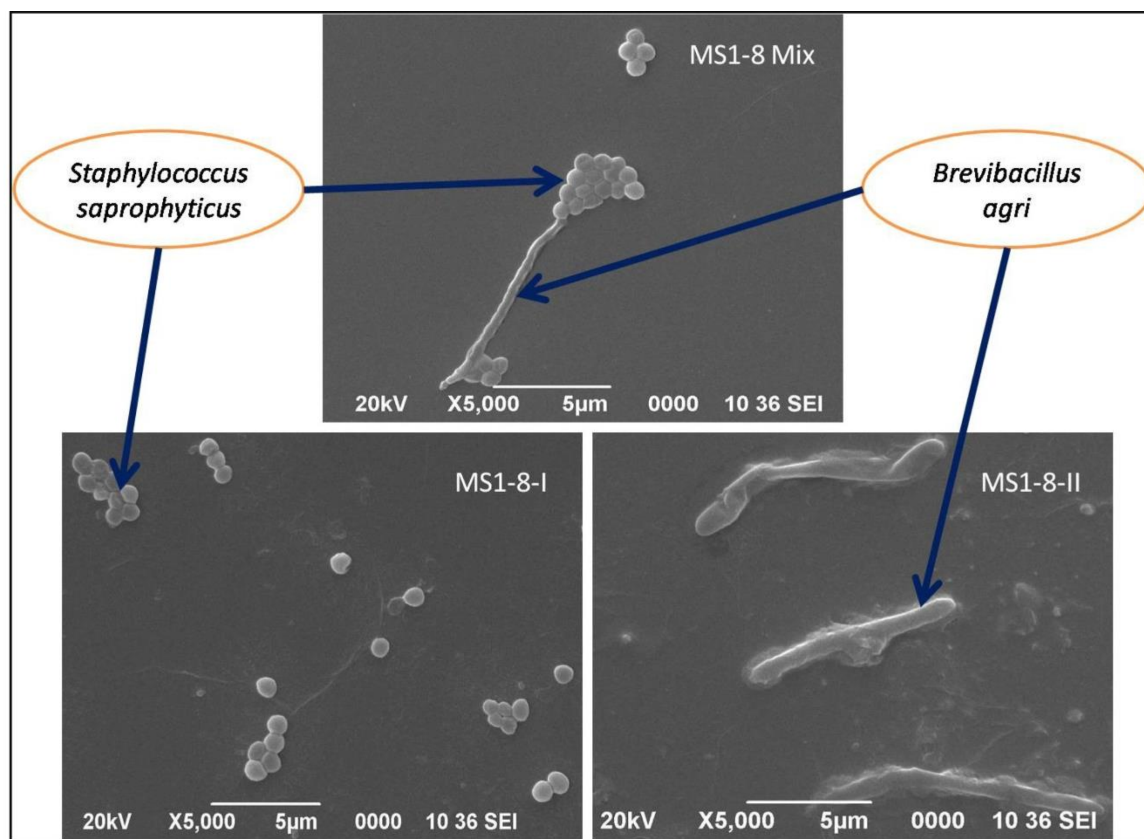

Figure 4. Scanning electron microscopy (SEM) image of bacterial samples at 5000X magnification: (A) Top image MS1-8, (B) below left MS1-8-I (*Staphylococcus saprophyticus*) and (C) below right MS1-8-II (*Brevibacillus agri*).

third lane (MS1-8-I), while in the fourth lane (MS1-8-II), PCR product was uncut and only a single band of 1500 bp size appeared and all these bands were also seen in the fifth lane of sample MS1-8. In Figure 5C, SphI enzyme was used for the digestion but this was unable to cut PCR product, due to which only a single band of about 1500 bp appeared in all lane of MS1-8-I, MS1-8-II, and MS1-8 samples. Last figure 5D was the image digested by the enzyme MspI and the band appeared in third and fourth lane (MS1-8-I and MS1-8-II respectively) was also seen in the fifth lane (MS1-8) (Figure 5).

Movement property study using isolated bacterial species

We have tried to find out the regaining of movement property after mixing of the both pure bacterial species MS1-8-I and MS1-8-II in a specific proportion. The growth and movement properties were observed after 12 to 24 h of incubation and we observed that no any movement properties were regained in the mixed bacterial species MS1-8-I and MS1-8-II, inoculated after 0, 24 and 48 h. It has been also observed that the movement properties were also not regained in the plate where the one bacterial species was inoculated on the lawn of another species.

DISCUSSION

The swimming property of many bacteria in the liquid medium is well studied; however, moving on top of the solid surface of some bacteria is not well characterized. The movement of the bacteria on the solid surface is known as swarming. For the movement of bacteria on a solid surface, various mechanisms, like reduction of surface tension for spreading by secreting some surfactant, increasing flagella, cell-cell interactions and formation of a multicellular group rather than an individual are being employed (Kearns, 2010). In our study, the midgut bacteria isolated from *Ae. albopictus,* when inoculated on center of the nutrient agar plate, exhibited swarming property. These bacteria demonstrated swarming property because they migrated away on the solid surface from their inoculation point (Kearns, 2010). The purified bacterial isolates were initially characterized morphologically and biochemically followed by MALDI-

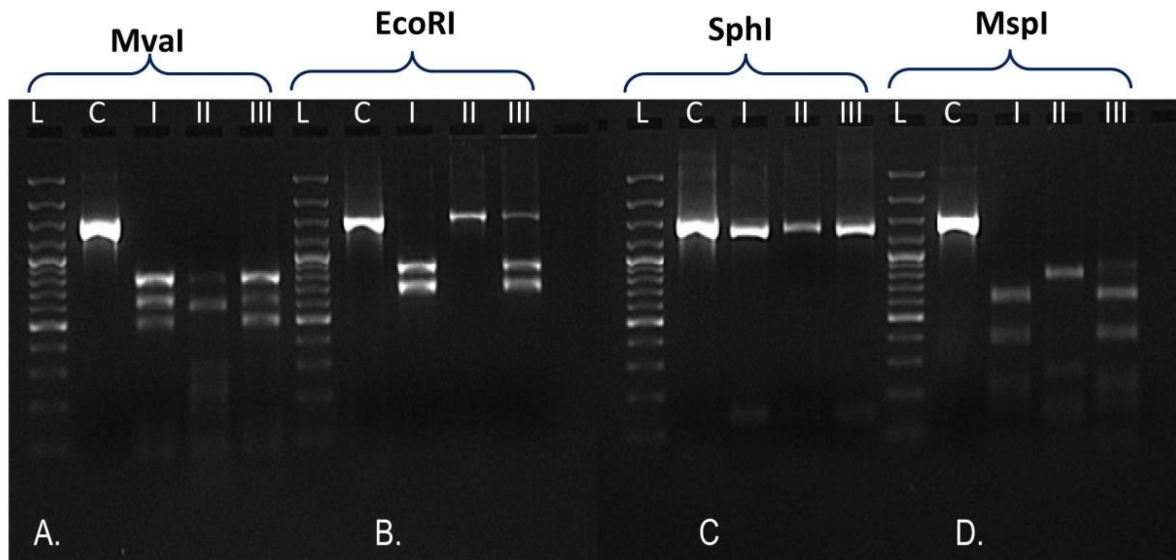

Figure 5. RFLP band pattern of all three bacterial isolates using four different restriction enzymes (MvaI, EcoRI, SphI and MspI). L, ladder; C, control (uncut 16S rRNA gene); I, digested 16S rRNA gene of MS1-8-I; II, digested 16S rRNA gene of MS1-8-II; III, digested 16S rRNA gene of MS1-8.

TOF-MS analysis and 16S rRNA gene sequencing and the result revealed that purified bacterial isolates were *S. saprophyticus* and *B. agri.* The midgut bacteria MS1-8 was the mixture of two bacterial species *S. saprophyticus* and *B. agri*, which was confirmed by the observation of broth culture under microscope, SEM image and RFLP band pattern analysis. Both the bacterial species *S. saprophyticus* and *B. agri* were highly associated during the swarming motility and were not separated easily. Only the bacterial colony MS1-8 showed the swarming property and individually purified bacterial species MS1-8-I and MS1-8-II and the mixture of these purified individuals were not able to show this property. The colony movement property in bacteria MS1-8 might have occurred due to the production of surfactant, which might be secreted by sharing resources and regulated by the quorum sensing property. Sufficient amount of bacteria should be needed for the production of beneficial surfactants, which is required for swarming (Lindum et al., 1988; Ochsner et al., 1995; Eberl et al., 1996; Magnuson et al., 1994). According to our result it is clear that the bacterial isolates MS1-8-I and MS1-8-II were found in very high association in the MS1-8 colony and this highly association-ship of bacterial species might provide the facility for the production of sufficient amount of beneficial surfactants. After the mixing of purified bacterial isolates, this property was not regained, this occurred because they might not be in such a proportion which is required for the production of sufficient amount of surfactant. It has been reported that different bacterial colonies produce different swarming patterns, which depends on the condition of environment where they are growing (Shimada et al., 2004, Hiramatsu et al., 2005).
The bacteria MS1-8 was isolated from midgut of *Ae. albopictus* mosquito where environmental condition of mosquito's midgut was highly different from the environmental condition of the laboratory. Bacterial species residing in the mosquito's midgut were highly associated to each other and fulfilled their requirements for such type of properties till purified two bacterial species in the laboratory conditions. After purification, both bacterial isolates independently were grown on media and lost all connections between them, which was in co-culture MS1-8 (prior to separation). This might be fact that after purification swarming property was not regained in individuals or in the mixture of the two purified bacterial species.

Apart from a surfactant, cell-cell interactions, and flagella formation is also an important requirement for swarming property. In our study, in the broth culture of purified bacterial species, *Staphylococcus saprophyticus* is Gram positive, cocci, and non-motile while the *B. agri* is Gram positive, rod shape, and highly motile. It has been reported that bacteria *S. saprophyticus* do not have flagella while in *B. agri,* it is present (Lara-Mayorga et al., 2010; Joshi et al., 2013). The presence of flagella in *B. agri* might play an important role in swarming property of parental isolated co-culture MS1-8, which might be supported by *S. saprophyticus.*

A lot of bacteria have been isolated from various mosquito's midgut and its role in various important functions like; food digestion, pathogen growth inhibition

etc. have been also studied (Minard et al., 2013). The identified bacterial species *S. saprophyticus* and *B. agri* in our studies have already previously reported from different mosquito's midgut. The genera *Staphylococcus* was normally isolated from the midgut of *Culex, Anopheles* and *Aedes* mosquitoes, while *Brevibacillus* was scarcely isolated (Chandel et al., 2013; Moro et al., 2013; Ramirez et al., 2012; Cirimotich et al., 2011a; Zouache et al., 2011; Rani et al., 2009; Favia et al., 2007; Fouda et al., 2001; Pumpuni et al., 1993; Apte-Deshpande et al., 2012; Ngwa et al., 2013). Different essential functions of midgut microorganisms in various fields have been depicted but swarming properties were not previously reported.

It has been previously described that the midgut bacteria provides viable defense against invading pathogens. The midgut bacteria are responsible for the inhibition of sporogonic development (Pumpuni et al., 1993; Straif et al., 1998; Gonzalez-Ceron et al., 2003; Dong et al., 2006; Cirimotich et al., 2011b). The bacterial species interact with host-pathogens and bacterial species itself and plays important role in vectorial capacity of mosquitoes. The bacterial species inside the midgut interact with each other, but the information about these interactions, its behavior is limited. Using midgut bacteria in vector-borne disease control, it is important to find out more information regarding bacterial species interaction. In this study, we have characterized the bacterial species responsible for the swarming motility, which was an example of bacterial interaction. The information obtained from this study might be a step towards the understanding of midgut bacterial interaction, which will help in development of a novel, cheap and eco-friendly approach towards the prevention of any vector-borne disease.

Conflict of Interests

The authors declare that they do not have any conflict of interest.

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