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Isolation and Characterization of Internal Bacteria from the Mosquito, *Culex pipiens* from Egypt

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Abstract: ***A total of 11 bacterial isolates were isolated from internal body of the mosquito,Culexpipiens(eggs, 4th larval instars, pupae, adult males and adult females)in one gonotrophic cycle.Two bacterial isolates were Gram +ve stained (Staphyllococcusepidermidis and Bacillussubtilis) and the other nine bacterial isolates were Gram –ve stained. Phenotypic, biochemical biotyping and differential media were used to characterize and identify our isolate. These methods could differentiate our isolates to species level.In addition, many of the obtained strains were proved to have economic and commercial importance. Bacterial antagonism was studied and it was noticed that B. subtiliswas antagonistic to bothE. coli and S. aureus; while S. flexneriwas antagonistic to S. typhimurium;and* S. epidermidis *was antagonistic to the multi-drug resistant strain,S. aureus.***

Keywords: *Culexpipiens*, bacterial isolates,bacterial antagonism.

1. Introduction

Insects are considered holobiont units in which the insect host and its microbiota are involved in complex reciprocal multipartite interactions (Rosenberg and Zilber-Rosenberg, 2011). Insects contain large communities of diverse microorganisms that probably exceed the number of cells in the insect itself(Dillon and Dillon, 2004). One of these microorganisms is bacteria that present on integument, inhabit digestive tract and in some cases inhabit unique structures within insect body (Chen *et al.,* 2000 and Fukatsu*et al*., 2000). The bacterial association in insects plays significant role in host insect morphogenesis, food digestion, antifungal toxin production, pheromone production, pH regulation, vitamin synthesis, temperature tolerance, resistance against parasitoid development and detoxification of noxious compounds as well as nutrition, reproduction, development or protection against enemies (Dillon and Dillon, 2004, Genta*et al.*,2006 and Douglas, 2011).In addition to fecundity and viability of insect host the bacterial communities play a role on the establishment of parasites within the host gut (Pumpuni*et al*., 1993) as well as on maturation of the innate immune system of the host (Weiss *et al.*, 2011).

Moreover, symbiotic relationships between mosquitoes and several microorganisms most probably have important implications in mosquitoes' evolutionary success, including their widespread distribution. Furthermore, the resident microbiota of mosquito vectors may inhibit the development of pathogens they transmit (Cirimotich*et.al.*, 2011). In this context several microbes may offer opportunities to successfully manipulate the vector competence of mosquitoes to reduce their abilities to transmit human pathogens.

The aim of our study is to understand the microbial community structure as a step to enable us to understand the organisms that play significant roles in the maintenance of these communities and to identify interaction between the microbiota communities isolated from both immature and mature stages of the mosquito *Culexpipiens*.

2. Materials and methods

2.1. Colonization of *Cx. pipiens*

A colony of the mosquito, *Cx. pipiens* was maintained under controlled laboratory conditions (27 ± 2 °C, 60-70% RH and 10L: 14D photoperiod) in the insectary of the Department of Entomology, Faculty of Science, Cairo University, Giza, Egypt up till now. This colony was used for supplying immature stages (eggs, larvae and pupae) during this study. Briefly, eggs were set up to hatch, 1st instar larvae were seeded into plastic cups 25x35x7 cm^3 containing water at a constant density of 300 individuals per cup. Larvae were provided with activated yeast or tetramin every 2 days until pupation. Water was changed on feeding days to avoid bacterial growth on the water surface. On pupation, cups were placed inside an emergence cage (27x40x35 cm^3) and provided with a source of 10% sugar solution for the emerged adults.

2.2. Bacterial isolation

2.2.1.Bacterial isolation from the insect

Twenty individuals of *Cx. pipiens*(egg rafts, fourth instar larvae, pupae, adult male and adult female) were surface sterilized with 70% ethanol for 5 min followed by twice washing in phosphate buffered saline (PBS) to remove possible contamination. The surface sterilized samples were homogenized in Ringer's solution using a glass grinder. 0.1 ml suspension was spread on 20 cm diameter plates containing Nutrient agar, MacConkey agar, Mannitol salt agar, Brilliant green agar and *Salmonella-Shigella* agar (S.S.) agar media separately using a sterilized scalpel inside laminar air flow hood (Thiery and Frachon, 1997).These plates were incubated at 30 ºC for 1-2 days and then investigated for bacterial isolation and identification.

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2.2.2. Bacterial isolation from the insect environment
0.1 ml was collected from breeding water (immature breeding environment) as well as sucrose water and air (mature breeding environment), then spread separately on20 cm diameter plates containing Nutrient agar, MacConkey agar, Mannitol salt agar, Brilliant green agar and S.S. agar media separately using a sterilized scalpel inside laminar flow hood (Thiery and Frachon, 1997).These plates were incubated at 30 ºC for 1-2 days and then investigated for bacterial isolation and identification. In the case of isolation from the air, the plates were exposed to air inside the cage for 30 minutes, incubated at 30 ºC for 1-2 days and then investigated for bacterial isolation and identification.

2.3 Characterization of the Bacterial Isolates

2.3.1. Phenotypic characterization
Phenotypic characterization of all isolates studied were performed and compared to phenotypic data of known organisms described in the Bergey's Manual of systematic Bacteriology (Noel ,1985) as well as Gram's staining according to the standard gram staining protocol (Gram, 1884).

2.3.2. Biochemical characterization
Indole test, Methyl red test (MR), Voges-Proskaeur's test (VP), Citrate test, Urease test, Catalase test, Oxidase test, Sugar fermentation test (glucose, lactose and sucrose), Triple Sugar Iron (TSI) and Hydrogen sulfide production (H_2S)tests were employed in the identification system.

2.3.3. Differential media characterization
MacConkey Agar, Brilliant Green Agar, Mannitol salt agar and S.S. agar media were inoculated separately with bacterial isolates at 30 °C for 1-2 days.

2.4 Antagonistic activity between bacterial isolates

For testing antagonistic activity, agar medium was used. Aftersolidification, agar surface was inoculated with 0.5 ml suspension of the bacterial strain. Agar diffusion method was used; 0.5 mm paper disks were inoculated with bacterialsuspension. Cultures were examined for the presence of clear inhibition zone around discs. These clear inhibition zones were measured and compared to control. Each experiment was repeated thrice.

3. Results

3.1. Bacterial isolates

A total of 32 bacterial isolates were identified during this study. These isolates were isolated from different mosquito developmental stages and their breeding environment as well.

3.1.1. Bacterial isolates from mosquito developmental stages
Out of 28 bacterial isolates were isolated from different developmental stages of the laboratory bred mosquito, *Cx. pipiens*. Out of these 28 isolates, 3, 4, 3, 9 and 9 isolates were isolated from egg, larval, pupal and adult male and female stages respectively.

Results of bacterial identification revealed that we got 11 different bacterial strains (Table 1). From these 11 bacterial strains only *E. coli* was the most persistent one that appeared in all mosquito developmental stages (egg, larva, pupa and adult female and male). *E. coli* was followed by *Shigellaflexneri* which appeared in all developmental stages except larval stage, then *Salmonella typhimurium* which lost in egg and pupal stages and appeared in other stages. Meanwhile,*Chryseobacteriumindologenes* was the least persistent one and isolated only from mosquito larval stage.it is worthy mentioned that all bacterial isolates were isolated from the adult mosquitoes, female and male except *Proteus mirabilis* and*Chryseobacteriumindologenes* (Table 1).

3.1.2. Bacterial isolates from mosquito environment
Out of the 32 bacterial isolates, 4 isolates were identified from the breeding environment of mosquito (3 from air and water and 1 from sucrose). Bacterial identification results revealed that no additional strains were recognized. *E. coli*, *Shigellaflexneri* and *Salmonella typhimurium* were isolated from both air and breeding water, as well. Meanwhile, *Pseudomonas aeurginosa* was the only strain isolated from sucrose water (Table 1). All successfully identified bacterial strains were propagated and characterized phenotypically, biochemically and by using differential media, as well.

3.2. Characterization of bacterial strain

The eleven bacterial strains isolated from internal organs of *Cx. pipiens* developmental stages and from the breeding environment were definitely characterized.

3.2.1. Phenotypic characterization
3.2.1.1. Characteristics of the colony
Bacterial strains were characterized by shape, size, elevation, opacity and margins shown in (Table 2). It was observed that all bacterial colonies were circular in shape except*Shigellaflexneri*, *Proteus Mirabilis* and *Bacillus subtilis* ones which were irregular in shape. One large, 7 medium and 3 small–sized colonies were noticed. Four convex, 4 raised, 2 falt and one umbonate–elevated colonies were reported. All colonies were seen opaque except *E. coli*, *Shigellaflexneri* and*Enterobacteraggelomorans*which were seen translucent. In addition, one undulate, 4 lobate and 6 entire-margined colonies were reported (Table 2).

3.2.1.2. Gram characteristics of the isolates
Gram staining and bacterial cell morphology of 11 bacterial isolates were summarized in (Table 3). It was noticed that all bacterial cells were gram-stained negative except *Staphylococcus epidermidis* and *Bacillus subtilis*. Cell morphology examination revealed that all cells were rod-shaped except *Staphylococcus epidermidis* and *Pseudomonas aeurginosa*which were cocci and coccobacilli in shape (Table 3).

3.2.2. Biochemical characterization of bacterial strains
Specific biochemical assays were carried out to assess the economic and commercial viabilities of the isolates. It was observed that all bacterial isolates could secrete catalase enzyme, only 4 isolates (*Chryseobacteriumindologenes, Bacillus subtilis, Klebsiellaozonae* and *Pseudomonas aeurginosa*) could secrete oxidase and 6 isolates

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(*Enterobacteraggelomorans, Bacillus subtilis, Chryseobacteriumindologenes, Proteus mirabilis* and *Staphylococcus epidermides*) could secrete urease (Table 3). These enzymes can be commercially harnessed and marketed.

IMViC tests indicated that only 3 bacterial strains (*E. coli*, *Shigellaflexneri* and*Chryseobacteriumindologenes*) could secret tryptophanase enzyme and indole, as well; 6 bacterial strains (*E. coli*, *Shigellaflexneri*, *Salmonella typhimurium*,*Chryseobacteriumindologenes, Proteus mirabilis* and *Klebsiellaozonae*) were glucose-acidic-fermenters; 4 bacterial strains (*Bacillus subtilis, Pseudomonas aeurginosa*,*Enterobacteraggelomorans* and *Serratiamarcescens*) were glucose-alkaline-fermenters. Sugar fermentation tests revealed that 8 bacterial strains (*E. coli*, *Shigellaflexneri*, *Salmonella typhimurium*, *Proteus mirabilis*,*Chryseobacteriumindologenes*, *Klebsiellaozonae*, *Pseudomonas aeurginosa* and *Enterobacteraggelomorans*) were non-sucrose-fermenters and 8 bacterial strains (*Shigellaflexneri*, *Salmonella typhimurium*, *Proteus mirabilis*, *Chryseobacteriumindologenes*,*Bacillus subtilis*,*Klebsiellaozonae*, *Pseudomonas aeurginosa* and *Serratiamarcescens*) were non-lactose-fermenters.

In addition, TSI and H_2S tests revealed that 4 bacterial strains (*Salmonella typhimurium*, *Proteus mirabilis*, *Chryseobacteriumindologenes*and*Klebsiellaozonae*) were trisugar-acidic-fermenters producing H_2S gas with CO_2 production; 4 bacterial strains (*Shigellaflexneri*,*Bacillus subtilis*, *Enterobacteraggelomorans*and *Serratiamarcescens*) were trisugar-acidic-fermenters lacking both CO_2 and H_2S gas production; *E. coli* was trisugar-acidic-fermenter producing CO_2 and lacking H_2S gas production; *Staphylococcus epidermides*was trisugar-acidic-fermenter producing H_2S and lacking CO_2 gas production; and *Pseudomonas aeurginosa* was trisugar-non fermenter.

3.2.3. Differential media characterization:
Six differential media were employed to characterize and differentiate between the obtained bacterial isolates. Bacterial growth and characteristic colors of bacterial colonies were summarized in Table (5). Seven growths with two characteristic colors were observed with MacConkey agar media, 4 growths with 4 characteristic colors with nutrient agar media, 3 growths with 2 characteristic colors were observed with S.S. agar media, 2 growths with 2 characteristic colors were observed with BGA media and one growth with a characteristic color was observed with both MSA and MHA media (Table 5). These results revealed that the more number of differential media was used, the best differentiation was obtained.
Characterization by using differential media showed insufficient differentiation between all bacterial strains and it need further differentiation.

3.3. Antagonistic activity between bacterial strains

Combination of 2 or more microorganisms in a single culture medium may indicate synergism if all of them can grow on the medium and it may indicate antagonism when only single specie could grow on the medium. Our results revealed that *Bacillus subtilis* was antagonistic to *E. coli*,*Shigellaflexneri*was antagonistic to *Salmonella typhimurium*. It was reported that both *Bacillus subtilis* and*Staphylococcus epidermides* were antagonistic to the multi-drug resistant *Staphylococcus aureus* bacteria (Table 6).

4. Discussion

Studies on mosquito-microbiota interaction are growing subject in many laboratories. Understanding the role of symbiotic bacteria in insect internal organs and their interactions with other pathogens is very important. Thesemicrobiota may interact with the host development, as well. In consequence, these naturally acquired microbial flora can modulate the mosquitoes' vectorial capacity(Kumar, 2013).

In the present study, eleven bacterial strains namely; *E. coli, Staphylococcus epidermides, Shigellaflexneri, Salmonella typhimurium, Proteus mirabilis, Chryseobacteriumindologenes, Bacillus subtilis, Klebsiellaozonae, Pseudomonas aeurginosa, Enterobacteraggelomorans* and *Serratiamarcescens* were isolated from the internal organs of *Cx. pipiens* and 4 of them were isolated from their environmental sources, as well. Symbiotic bacteria were ubiquitously located in insect guts with other symbioses ranging from pathogenic to mutualistic and from facultative to obligaterelationship (Wegensteiner, 2007).Many authors studied the association between bacteria and mosquitoes **(Merritt** *etal*., 1992**; Demaio***etal*., 1996**; Fouda***et al*., 2001;**Akman***et al*., 2002;**Mourya***et al*.,2002and **Kumar** *et al*., 2013). **Fouda***et al*. (2001) isolated *Wolbachia* from the gut of *Cx. pipiens* mosquito and**Favia***et al.* (2007) studied thebacteria of the genus *Asaia* and found that it was stably associated with *Anopheles stephensi*, an Asian malarial mosquito vector. Many bacterial strains such as *Acinetobacter*, *Serratia*, *Pseudomonas*, *Stenotrophomonas*, *Flavimonas*and *Enterobacter*, were isolated from environmental sources. The same bacterial strains were found to be associated with the guts of insects living in the same environment (Oliveira *etal.,* 1998; Campbell *et al*., 2004; La Scola&Raoult, 2004 and Zayed& Bream, 2004).These findings suggested bacteria to be part of thenatural or transient flora of these insects. Generally, these observations support the idea that field studies are necessary to get an integrated view of mosquito-associated microbiota. Combining our results with the previous studies, it can be concluded that colonization of bacteria in mosquitoes occurs early during their development. It is reasonable to assume that infection of mosquitoes occurs by acquisition of different bacterial species from the environment.

Horizontal transmission is also possible when mosquitoes pick up bacteria from microhabitats when emerging and/or depositing eggs. In adults, symbiotic bacteria determine their fitness and efficiency at which they can transmit pathogens. Indeed, Hoffmann *et al.* (2011) and Walker *et al.* (2011) demonstrated that the ability of *Aedesaegypti*to transmit dengue fever virus greatly reduces when infected with certain strains of *Wolbachia*bacteria. This may explain why some species are more efficient vectors than others. Lastly, as is the case in most hematophagous insects, symbiotic

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bacteria in mosquito provide hemolytic enzymes required for blood digestion to aid in the development of eggs (Minard*et al.*, 2013a; and Gaio*et al.*, 2011).
Transstadial transmission mechanisms could be the reason behind the observed similarity in bacterial composition between habitats, larvae and adults. This is due to the ingested microhabitat bacteria may evade gut sterilization mechanisms during metamorphosis; and in the process get transferred to later stages of development where they may be required as symbionts(Brione*set al.*, 2008; Lindh*et al.*, 2008a). These symbionts benefit the hosts by breaking down complex food substances for easy assimilation through provision of degradative enzymes or essential vitamins to the hosts (Minard*et al.*, 2013a). Apart from transstadial transmission, adults may horizontally acquire the bacteria from aquatic microhabitats when laying eggs or during adult emergence. Minard*et al.* (2013b) demonstrated that *Asaia*sp bacteria found in adult *Ae. albopictus*had a strong positive correlation with *Asaia*sp in the mosquitoes' sampling sites pointing towards either of these mechanisms as the primary form of acquisition.

Bacterial symbionts associated with mosquito vectors have recently been found to interact with pathogens they transmit, modifying the outcome of the multipartite interactions. For instance, it was shown that removing bacterial communities from *Anopheles gambiae* increased its susceptibility to *Plasmodium falciparum* infection (Dong *et al*., 2009). On the contrary, Boissière*et al.* (2012) demonstrated that the presence of some bacteria could favor parasite infection, as they found a positive correlation between the abundance of members of the Enterobacteriaceae family in the mosquito midgut and the *Plasmodium* infection status. Conversely, Zouache*et al*. (2012)demonstrated that chikungunya virus infection could modify the diversity of symbiotic bacteria in*Ae. albopictus*.

The eleven bacterial strains isolated from internal organs of *Cx. pipiens* developmental stages and from the breeding environment were definitely characterized phenotypically, biochmeically and by using differential media. These charactering methods were used and sufficiently differentiated and identified our bacterial strains to the species level.Insect-associated microorganisms, particularly endosymbionts, were known to produce bioactive compounds that protect the host against adverse environmental conditions, predators or competitors;thus, they have been suggested as suitable for biotechnological applications (Chaves*et al.*, 2009). Studies on microbial diversity can contribute to the discovery of new substances that can be used in the pharmaceutical and food industries (Chaves*et al.*, 2009).Our bacterial strains were proved to have the ability to produce enzymes and other products that can be harnessed and commercially marketed. In addition, many strains were proved to be sugar-fermenters. The fermentation process was proved to be very important in many biotechnological applications.

Combination of 2 or more microorganisms in a single culture medium revealed that *Bacillus subtilis*, *Shigellaflexneri* and *Staphylococcus epidermides* could inhibit growths of some other pathogenic bacterial strains. More studies is necessary to throw the light on the true causes of this antagonistic activity.

In mosquitoes, the origin of commensal bacteria has not yet been fully resolved. Usually bacteria can be acquired in two ways, either by vertical inheritance through generations or through continual acquisition from the environment. Moreover, the mosquito gender is also an important factor that affects bacterial microbiota composition, as already demonstrated . This difference is mainly due to the fact that male and female mosquitoes exhibit different ecological behaviors in terms of nutritional capabilities.

Conclusively, the present study described the isolation and characterization of 11 bacterial strains from the internal organs of different developmental stages of *Cx. pipiens* mosquito. Out of these 11 strains, only 4 were isolated from mosquito and their environment. The mosquito was then incriminated in transmission of the other 7 bacterial strains to human and animals. In addition, isolation from 3 bacterial strains from more than one developmental stage of the mosquito increased interest to study the possibility of vertical, trans-stadial and venereal transmission of these bacteria. Moreover, combination and integration of the characterization methods used in this study showed that they could differentiate and characterize the 11 bacterial strains to species level. Consequently, many of the obtained bacterial strains were proved to have economical and commercial importance in many applications. Finally, the phenomenon of antagonistic activity is worth to be sufficiently studied in a separate investigation.

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Tables

Table 1: Internal bacterial isolates from eggs, larvae, pupae, adult maleandadult female, and from their environment.

Stages / Bacteria	Eggs	Larvae	Pupae	Males	Females	Environment		
						Breeding water	sucrose water	Air
E.coli	√	√	√	√	√	√		√
Proteus mirabilis	√	√						
Shigellaflexneri	√		√	√	√	√		√
Salmonella typhimurium		√		√	√	√		√
Chryseobacteriumindologenes		√						
Enterobacteraggelomorans				√	√			
Staphylococcus epidermidis				√	√			
Bacillus subtilis				√	√			
Klebsiellaozonae				√	√			
Pseudomonas aeurginosa				√	√		√	
Serratiamarcescens				√	√			

√ : appearance

Table 2: Colony characteristics of the bacterial isolates

Colony characteristics / Bacterial colony	Shape	Size	Elevation	Opacity	Margin
E. coli	Circular	Small	slightly raised	Translucent	Entire
S.epidermidis	Circular pinhead colonies	Small	Convex	Opaque	Entire
Shigellaflexneri	Irregular	Medium	Convex	Translucent	Entire
Salmonella typhimurium	Circular	Medium	Convex	Opaque	Entire
Proteus mirabilis	Irregular	Medium	Raised	Opaque	Lobate
C.indologenes	Circular	Small	Convex	Opaque	Entire
B.subtilis	Irregular	Large	slightly raised	Opaque	Lobate
K.ozonae	Circular	Medium	Flat	Opaque	Entire
P.aeurginosa	Circular	Medium	Raised	Opaque	Undulate
E.aggelomorans	Circular	Medium	Flat	translucent	Entire
Serratiamarcescens	Circular	Medium	Umbonate	Opaque	Entire

Table 3: Gram characteristics of the obtained bacterial isolates

Cell parameters / Bacteria	Cell Gram Character	Cell Morphology
E. coli	-ve	Rod
Staphylococcus epidermides	+ve	Cocci
Shigellaflexneri	-ve	Rod
Salmonella typhimurium	-ve	Rod
Proteus mirabilis	-ve	Rod
Chryseobacteriumindologenes	-ve	Rod
Bacillus subtilis	+ve	Rod
Klebsiellaozonae	-ve	Rod
Pseudomonas aeurginosa	-ve	coccobacillus
Enterobacteraggelomorans	-ve	Rod
Serratiamarcescens	-ve	Rod

+ve : positive gram stain, -ve: negative gram stain

Table 4: Biochemical characterization of the obtained bacterial isolates

	Ind	MR	Vp	C	Ur	Ox	Cat	Glucose	Sucrose	Lactose	TSI	H2S
E. coli	+	+	-	-	-	-	+	+Gas	-No gas	+ Gas	A/A G	-
Staphylococcus epidermides	-	-	+	-	+	-	+	+No gas	+No gas	+Gas	A/A	+
Shigellaflexneri	+	+	-	-	-	-	+	+No gas	-No gas	-No gas	K/A	-
Salmonella typhimurium	-	+	-	+	-	-	+	+Gas	-No gas	-No gas	K/A G	+
Proteus mirabilis	-	+	+	+	+	-	+	+Gas	-No gas	-No gas	K/A G	+
Chryseobacteriumindologenes	+	-	-	-	+	+	+	+No gas	-No gas	-No gas	A/A G	+
Bacillus subtilis	-	-	+	+	+	+	+	-No gas	+No gas	-No gas	K/A	-

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Klebsiellaozonae	-	+	-	+	-	+	+	+Gas	-No gas	-No gas	A/A G	+
Pseudomonas aeurginosa	-	-	-	+	-	+	+	-No gas	-No gas	-No gas	K/K	-
Enterobacteraggelomorans	-	-	+	+	+	-	+	+Gas	-No gas	+No	A/A	-
Serratiamarcescens	-	-	+	+	-	-	+	+No gas	+No gas	-No gas	K/A	-

(+) positive result, (-) negative result, (Ind) indole, (MR) methyl red, (VP) Voges-Proskaeur, (C) citrate, (U) urease, (Ox) oxidase, (Cat) catalase, (+) fermentation (-) non fermentation, (K) alkaline, (A) acid, (G) gas.

Table 5: Differential media characterization of the obtained bacterial isolates.

Media	Bacteria	Color
Macconkey agar	*E.coli,Enterobacteraggelomorans*and*Klebsiellaozonae* *Shigellaflexneri, Salmonella typhimurium,Proteus mirabilis* and *Pseudomonas aeurginosa*	pink colonies colorless colonies with dark centers
MSA	*Staphylococcus epidermidis*	red colonies
SS agar	*E. coli* *Shigellaflexneri* *Salmonella typhimurium*	pink colonies colorless colonies
BGA	*E. coli* *Salmonella typhimurium*	green colonies pink colonies
NA	*Staphylococcus epidermidis* *Bacillus subtilis* *Pseudomonas aeurginosa* *Serratiamarcescens*	white colonies cream color colonies or brown greenish color colonies red color colonies
MHA	*Chryseobacteriumindologenes*	Yellow color colonies

Table 6: Antagonistic activity of the obtained bacterial strains

Bacterial combination	*Strain antagonistic activity*	*Growths*
E. coli + individual other strains	-ve	*E. coli* growth was inhibited by *B. subtilis*
Staphylococcus epidermides+ individual other strains	-ve	No inhibition of growth was observed
S. epidermides+ *S. aureus*	+ve	*S. aureus* growth was inhibited by *S. epidermides*
Shigellaflexneri+ *Salmonella typhimurium*	+ve	*Salmonella typhimurium*growth was inhibited by *Shigellaflexneri*
Shigellaflexneri+ individual other strains	-ve	No inhibition of growth was observed
Salmonella typhimurium+ individual other strains	-ve	*Salmonella typhimurium*growth was inhibited by *Shigellaflexneri*
Proteus mirabilis + individual other strains	-ve	No inhibition of growth was observed
Chryseobacteriumindologenes+ individual other strains	-ve	No inhibition of growth was observed
Bacillus subtilis + *E. coli*	+ve	*E. coli* growth was inhibited by *B. subtilis*
Bacillus subtilis+ individual other strains	-ve	No inhibition of growth was observed
Bacillus subtilis + *S. aureus*	+ve	*S. aureus* growth was inhibited by *B. subtilis*
Klebsiellaozonae+ individual other strains	-ve	No inhibition of growth was observed
Pseudomonas aeurginosa+ individual other strains	-ve	No inhibition of growth was observed
Enterobacteraggelomorans+ individual other strains	-ve	No inhibition of growth was observed
Serratiamarcescens+ individual other strains	-ve	No inhibition of growth was observed

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