Galal *et al. J Pure Appl Microbiol,* **13**(**3**), 1619-1628 | September 2019
Article 5813 | https://doi.org/10.22207/JPAM.13.3.35

Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Isolation, Characterization and Antagonistic Activity of the External Microflora of the House fly, *Musca domestica* (Diptera: Muscidae)

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Abstract

Experiments were designed to isolate, characterize and study the interaction between external microbiota (bacteria and fungi) carried by adult *M. domestica* after dipping, then removal of the flies from distilled water, sugar solution and saline solution. *M. domestica* was collected from Sakaka city, Northwestern Saudi Arabia. Three groups of adult *M. domestica* were completely dipped in and then removed from each of the above-mentioned solutions separately. Bacteria and fungi were isolated using corresponding media, characterized using macro and microscopic examinations, and then tested for antagonistic activity. Three bacterial species; *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* and three fungi; *Candida albicans*, *Rhizopus stolonifer* and *Aspergillus niger* have been isolated, characterized and tested for antagonism. Biochemical tests of bacterial strains confirmed the ability to secrete economically important materials. Different efficiencies to ferment sugars and produce gases have been confirmed, too. Antagonistic tests between microorganisms have revealed that both *E. coli* and *P. aeruginosa* bacteria are antagonists to both *A. niger* and *C. albicans* fungi. However, *R. stolonifer* fungus is antagonist to both *E. coli* and *P. aeruginosa* bacteria. *B. subtilis* bacterium is antagonist to the 3 fungi and to the other 2 bacteria. The antagonistic activity of our bacterial strains could be attributed to the secretion of antimicrobial materials. Further study on the mechanism of antimicrobial activity of *B. subtilis* strain is recommended. It was concluded that this strain could be useful in controlling some bacterial and fungal infections.

Keywords: *M. domestica*, microbiota, bioactive materials, bacterial fungal antagonism.

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(Received: 02 August 2019; accepted: 05 September 2019)

Citation: Fatma H. Galal, Taghreed ElShammari and AlaaEddeen M. Seufi, Isolation, Characterization and Antagonistic Activity of the External Microflora of the House fly, *Musca domestica* (Diptera: Muscidae), *J Pure Appl Microbiol*., 2019; **13**(**3**): 1619-1628. https://doi.org/10.22207/JPAM.13.3.35

INTRODUCTION

The house fly, *Musca domestica*, is one of the most common health pests worldwide. *M. domestica* possesses morphological and behavioral characteristics which make it not only annoying, but a mechanical vector of more than 100 pathogens[1-10]. *M. domestica* is closely related to human activities and it breeds on decaying organic matter such as animal manure, human wastes, open toilets, garbage, foods, vegetables and plants. All of mentioned breading media are full of diverse and active microbial communities[3,4,11]. Many researchers have studied the microbes associated with the wings of some fly species[12-14]. But only one article studied the effect of natural fall and dipping of *M. domestica* on microbial contamination of distilled water and milk[15].

The present study is based on interaction between the external microbiota (bacteria and fungi) carried by adult *M. domestica* after dipping, then removal of the flies from distilled water, sugar solution and saline solution. Consequently, the antagonism between the isolated strains was investigated.

MATERIALS AND METHODS

Collecting flies

The house fly, *M. domestica*, were collected from the Sakaka city, AlJouf, Northwestern Saudi Arabia. Collected flies were transported to the laboratory in sterile cups and then they were morphologically identified. *M. domestica* was reared and maintained in the insectary under controlled conditions (27±2 °C and 70±5% Relative humidity (RH) and 14/10 light/dark photoperiod cycle), according to[16]. These flies were used as a stock for the experimental work.

Solutions used

The experimental solutions were chosen to represent the normal drinks and foods of the human beings. Distilled water represents the normal drinking water of human. The 10% sterile sugar solution represents juices and other sugary drinks consumed by human. The 10% sterile saline solution represents the balanced salting of all types of salads and cooked foods with sauces. All solutions were sterilized using bacterial filters and all tools were autoclaved.

Experimental design

Three groups of adult *M. domestica* (10 flies/ group) were completely dipped in and then removed from each of the following solutions separately: 200 ml of sterilized distilled water (DW), 200 ml of 10% sterile sugar solution (SU), and 200 ml of 10% sterile saline solution (SA). Immediately after dipping and removal of flies, bacterial and fungal flora were cultured from the three solutions, separately (DW, SU and SA). One hour later after dipping and removal of flies, bacterial and fungal flora were cultured from the three solutions, separately (DW1, SU1 and SA1).

Bacterial isolation using differential media

A fixed volume (100 µl) of each of the solutions DW, DW1, SU, SU1, SA and SA1 was spread by sterilized scalpel on 20 cm diameter plates containing Nutrient agar (NA), Mannitol salt agar (MSA), MacConkey agar, Brilliant green agar (BGA) and Salmonella-Shigella agar (SSA) media, separately. Plates were sealed tightly with parafilm, placed upside down and incubated at 30 °C for 24- 48 h. Plates were then investigated, bacteria were isolated, identified and stored until used in subsequent experiments. Procedure was carried out inside laminar air flow hood[17,18].

Characterization of the Bacterial Isolates

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[19] as well as Gram's staining according to the standard gram staining protocol[20].

Antagonistic activity between bacterial isolates

Antagonistic activity was tested according to[21]. Briefly, 0.5 ml of a bacterial suspension was spread on the surface of solidified nutrient agar and paper-disc diffusion method[22] was used for the other bacterial strains. Clear inhibition zones were measured and compared to positive and negative controls. Each experiment was repeated thrice.

Fungal isolation

A fixed volume (100 µl) of the solutions DW, DW1, SU, SU1, SA and SA1 was spread onto 20 cm diameter plates containing Czapek-Dox's agar medium and Potato Dextrose Agar (PDA) medium, separately. Chloramphenicol (25.0 mg/ L) or Chlortetracycline (40.0 mg/ L) was added to the media to inhibit bacterial growth. Plates were

sealed tightly with parafilm, placed upside down and incubated at 28 °C for 7-15 days[23].

Identification of fungal isolates

Purification of the colonies was carried out by transferring each single colony to a sterile PDA plate and incubating plates at 28 °C for 7-15 days. The propagated colonies were mounted on slides and stained with lactophenol cotton blue to be examined under light microscope. Macroscopic morphology of mycelium and conidia was observed and used for fungal identification[24,25].

Antagonism between fungi and associated bacteria

Antagonistic activity was tested according to (26). Briefly, one ml of each fungus was spread onto the surface of solidified Czapek-Dox's agar media. A paper-disc diffusion method was used as described above[22]. Three replicates were incubated at 30 °C for 15 days, and inhibition zones were measured and compared to a reference chart.

RESULTS

Characterization of bacterial strains

A total of 18 bacterial isolates were identified during this study from all samples. These isolates were isolated from DW, DW1, SU, SU1, SA and SA1. Isolates were definitely characterized as three species; *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (Table 1).

Table 1. Isolation of bacterial species from different solutions after dipping and removal of *M. domestica* immediately and one hour later

Solution	DW	SU	SA	DW1	SU1	SA1
Bacterial Species						
E. coli	√	√	√	√	√	√
P. aeruginosa	√	√	√	√	√	√
B. subtilis	√	√	√	√	√	√

Morphological characterization of bacterial colonies

Shapes, sizes, elevation, opacity and margins of the bacterial colonies are summarized in Table (2). All colonies were elevated and opaque except the translucent colony of *E. coli*. Circular colonies of *E. coli* and *P. aeruginosa* and irregular *B. subtilis* colony were observed, too. In addition, small-sized with entire margin colonies of *E. coli*, medium-sized with undulate margin colonies of *P. aeruginosa* and large-sized with lobate margin colonies of *B. subtilis* were noticed (Table 2).

Gram characteristics of the bacterial species

Table (3) summarizes Gram's staining and cell morphology of the bacterial species. All bacterial cells were Gram-negative except *B. subtilis* which was Gram-positive. Meanwile, all cells were rod-shaped except *P. aeruginosa* which were coccobacilli.

Biochemical characterization of bacterial species

Specific biochemical assays were carried out to evaluate economic and commercial values of the species. All bacterial species secrete catalase, *B. subtilis* and *P. aeruginosa* secrete oxidase and only *B. subtilis* secretes urease (Table 4). These enzymes can be commercially harnessed and marketed.

IMViC tests indicated that only *E. coli* secretes tryptophanase enzyme and indole. Additionally, *E. coli* is glucose-acidic-fermenter.

Table 2. Colony characteristics of the isolated bacterial species

Colony Characteristic	Shape	Size	Elevation	Opacity	Margin
Bacterial Species					
E. coli	Circular	Small	Raised	Translucent	Entire
P. aeruginosa	Circular	Medium	Raised	Opaque	Undulate
B. subtilis	Irregular	Large	Raised	Opaque	Lobate

Table 3. Gram's characteristics and cell morphology of the isolated bacterial species

Cell parameters	Cell Gram Character	Cell Morphology
Bacterial species		
E. coli	-ve	Rod shaped
P. aeruginosa	-ve	Coccobacilli
B. subtilis	+ve	Rod shaped

Table 4. Biochemical characteristics of the isolated bacterial species

Bacteria	*E. coli*	*P. aeruginosa*	*B. subtilis*
Biochemical test			
Catalase	+ve	+ve	+ve
Oxidase	-ve	+ve	+ve
Urease	-ve	-ve	+ve
Tryptophanase	+ve	-ve	-ve
Indole	+ve	-ve	-ve
Glucose fermentation	+ve Acidic	+ve Alkaline	+ve Alkaline
Sucrose fermentation	-ve	-ve	+ve Alkaline
Lactose fermentation	+ve Acidic	-ve	-ve
TSI- test	+ve Acidic	-ve	+ve Acidic
CO_2 production	+ve	-ve	-ve
H_2S production	+ve	-ve	-ve

However, both *B. subtilis* and *P. aeruginosa* are glucose-alkaline-fermenters. Sugar fermentation tests revealed that *E. coli* and *P. aeruginosa* are non-sucrose-fermenters. Both *B. subtilis* and *P. aeruginosa* are non-lactose-fermenters (Table 4).

In addition, TSI and H_2S tests revealed that *B. subtilis* is trisugar-acidic-fermenter lacking both CO_2 and H_2S gas production. *E. coli* is trisugar-acidic-fermenter producing CO_2 and lacking H_2S gas production. Whilst, *P. aeruginosa* is non-trisugar-fermenter (Table 4).

Characterization by differential media

In order to differentiate between the obtained bacterial species, 5 differential media were employed. Bacterial growth and characteristic colors of bacterial colonies were summarized in Table (5). Three growths with two characteristic colors were observed with MacConkey agar, two growths with two characteristic colors with NA media, only one growth with a characteristic color was observed with SSA, BGA and MSA media (Table 5). Insufficient characterization has been observed when using differential media.

Table 5. Colony characterization by using differential media

Media	Bacteria	Color
MacConkey agar	Tow growths;	
	E. coli	Pink colonies.
	P. aeruginosa	Colorless colonies with dark centers.
MSA	*E. coli*	Pink colonies.
SSA	*E. coli*	Pink colonies.
BGA	*E. coli*	Greenish colonies.
NA	Tow growths;	
	B. subtilis	Creamy or brown color colonies.
	P. aeruginosa	Greenish color colonies.

Antagonistic activity between bacterial species

Growth of two or more microorganisms in a single culture medium may indicate synergistic activity. However, growth of a single species on the medium may indicate antagonistic activity of the growing species. Our results revealed that *B. subtilis* is antagonistic to both *E. coli* and *P. aeruginosa* (Table 6).

Table 6. Antagonistic activity of the isolated bacterial species

Bacterial combination	Antagonistism	Growths
E. coli + P. aeruginosa	-ve	Two growths and no inhibition
E. coli + B. subtilis	+ve	Growth of *B. subtilis* only
P. aeruginosa + B. subtilis	+ve	Growth of *B. subtilis* only
E. coli+ P. aeruginosa+ B. subtilis	+ve	Growth of *B. subtilis* only

Fungal isolation

A total of ten fungal isolates were isolated during the current work. Only one isolate from DW and DW1, two isolates from SU and SU1, two isolates from SA and SA1 were isolated. Fungal isolates were identified as *Candida albicans*, *Rhizopus stolonifer* and *Aspergillus niger* (Table 7). *C. albicans* was persistent in all solutions, *R. stolonifer* appeared in sugar solutions and *A. niger* grew in salt solutions (Table 7).

Characterization of fungal isolates

Macroscopic and microscopic characterization

Table (7) clarified that all fungal isolates were identified to three different species; *C. albicans* was isolated from all solutions (6 isolates), *R. stolonifer* was isolated from sugar solutions (2 isolates) and *A. niger* was isolated from salt solutions (2 isolates). Table (8) summarizes the macroscopic and microscopic characteristics of the isolated fungi. *C. albicans* appeared as white non-

Table 7. Isolation of fungal species from different solutions after dipping and removal of *M. domestica* immediately and one hour later

Solution	DW	SU	SA	DW1	SU1	SA1
Fungal Species						
C. albicans	√	√	√	√	√	√
R. stolonifer	—	√	—	—	√	—
A. niger	—	—	√	—	—	√

branching globular structures with pseudohyphae. *R. stolonifer* appeared as dense, cottony structures which fill culture plate. Branched aerial mycelia with filamentous non-septate hyphae were observed. Sporangia with many spores are carried by sporangiophores. *A. niger* was reported as dichotomous branched mycelia with septate hyphae. Numerous black spores are carried by long, smooth and hyaline conidiophores (Table 8).

Antagonistic activity

E. coli and *P. aeruginosa* bacteria prohibited growths of both *A. niger* and *C. albicans*, whatever bacteria have applied individually or in combination. However, *R. stolonifer* prohibited growths of *E. coli* and *P. aeruginosa* whatever applied to the fungus individually or mixed with each other. Interestingly, *B. subtilis* bacteria prohibited the growths of all fungi whatever it has applied individually or in combination with other bacteria (Table 9).

DISCUSSION

The current study presents 3 bacterial and 3 fungal colonies with distinct morphological characters were identified. Two Gram negative Proteobacteria; *E. coli* (Enterobacteriales, Enterobacteriaceae) and *P. aeruginosa* (Pseudomonadales, Pseudomonadaceae) and one Gram positive Firmicutes bacteria; *B. subtilis* (Bacillales, Bacillaceae) were isolated. In addition, 2

Table 8. Macroscopic and microscopic characterization of the isolated fungi

Fungi	*A. niger*	*R. stolonifer*	*C. albicans*
On agar plate	Powdery structures with numerous black dots.	Dense, cottony, aerial mycelia fill the plate. It appears white then became grey.	White colony.
Branching	Dichotomous branching.	Branched.	Non-branching.
Hyphae	Septate and hyaline.	Non-septate. Stolons connecting fungal bodies.	Pseudohyphae.
Conidiophores	Conidiophores are long, smooth, hyaline and darker at the apex.	Noticeable sporangiophores.	Absent.
Spores	Numerous and black.	Globose sporangia with many spores, and flattened base. Grayish black and powdery in appearance.	Reproduction by budding.

Table 9. Antagonistic activity between fungi and bacteria

Bacteria	Fungi	Antagonism	Growths
E. coli	*A. niger*	+ve	Growth of *E. coli*
P. aeruginosa		+ve	Growth of *P. aeruginosa*
B. subtilis		+ve	Growth of *B. subtilis*
E. coli + P. aeruginosa		+ve	Growth of *E. coli + P. aeruginosa*
E. coli + B. subtilis		+ve	Growth of *B. subtilis*
P. aeruginosa + B. subtilis		+ve	Growth of *B. subtilis*
E. coli + P. aeruginosa + B. subtilis		+ve	Growth of *B. subtilis*
E. coli	*R. stolonifer*	+ve	Growth of *R. stolonifer*
P. aeruginosa		+ve	Growth of *R. stolonifer*
B. subtilis		+ve	Growth of *B. subtilis*
E. coli + P. aeruginosa		+ve	Growth of *R. stolonifer*
E. coli + B. subtilis		+ve	Growth of *B. subtilis*
P. aeruginosa + B. subtilis		+ve	Growth of *B. subtilis*
E. coli + P. aeruginosa + B. subtilis		+ve	Growth of *B. subtilis*
E. coli	*C. albicans*	+ve	Growth of *E. coli*
P. aeruginosa		+ve	Growth of *P. aeruginosa*
B. subtilis		+ve	Growth of *B. subtilis*
E. coli + P. aeruginosa		+ve	Growth of *E. coli + P. aeruginosa*
E. coli + B. subtilis		+ve	Growth of *B. subtilis*
P. aeruginosa + B. subtilis		+ve	Growth of *B. subtilis*
E. coli + P. aeruginosa + B. subtilis		+ve	Growth of *B. subtilis*

Ascomycotic fungi; *C. albicans* (Saccharomycetales, Saccharomycetaceae), *A. niger* (Eurotiales, Trichocomaceae) and one Zygomycotic fungus; *R. stolonifer* (Mucorales, Mucoraceae). Bacterial association with flies is attracting subject to authors from 1912 up till now. Due its accessibility to humane living, special attention to house fly was markedly noticeable. Several authors have isolated more than 32 bacterial genera including our species from the house fly; *M. demestica*. The

reported 32 genera belong to 3 phyla, 12 orders and 21 families within bacterial kingdom (*e.g.* 10, 27-42). In parallel, more than 21 fungal genera including our species have been isolated from the house fly; *M. demestica*. The reported 21 genera belong to 4 phyla, 13 orders and 12 families within fungal kingdom (*e.g.* 33, 43-49). More than 100 species of parasites and microorganisms have been isolated from the house fly[36,37]. Authors have paid attention to the bacterial communities of other flies[50-52].

The antagonistic activity of our bacterial strains could be interpreted by the ability of bacteria to secrete enzymes and other economic materials as shown in biochemical characterization. Antagonistic tests between microorganisms have revealed that both *E. coli* and *P. aeruginosa* bacteria are antagonists to *A. niger* and *C. albicans* fungi. Agreeable results have been presented by[51] who revealed that *E. coli* secretes a fungicide that kills *C. albicans*. Also *P. aeruginosa* was reported as antagonist to *A. niger*[53]. Other studies have reported that *P. aeruginosa* is antagonist to *Aspergillus fumigatus* in planktonic growth[54] and in bio lm, too[55-58]. Contrary to our results, no antagonism between *E. coli* and *C. albicans* has been found[26]. Interestingly, *P. aeruginosa* and *A. fumigatus* have been reported to possess mutual antagonism at different stages of bio lm development[59]. Recently, the complexity beyond the simple antagonistic interaction between *P. aeruginosa* and *C. albicans* has been intensively reviewed[60]. *E. coli*, *Pseudomonas sp.* and *Bacillus sp.* have been reported as antagonists to *A. niger* and could be used in biocontrol of the fungus[61]. *E. coli* has exhibited antagonistic activity to pathogenic *Aspergillus spp.*[62]. However, *R. stolonifer* fungus is antagonist to *E. coli* and *P. aeruginosa* bacteria. A previous study has presented that *R. stolonifer* fungus showed antagonistic effect to *A. niger* and *C. albicans* fungi and to *P. aeruginosa* and *E. coli* bacteria. This activity was attributed to toxic secondary metabolites secreted by the fungus[63]. *B. subtilis* bacterium is antagonist to the 3 fungi and to the other 2 bacteria. In antagonistic study, *B. subtilis* has proved to produce a biosurfactant that prohibited the growth of *Salmonella*, *Shigella* and *Staphylococcus* bacteria[64]. Antifungal activity of *Bacillus* isolates against phytopathogenic fungi may be attributed to the cyclic lipopeptide; fungycin which plays important role in this process[65-68]. Recently, the antimicrobial compounds of *B. subtilis* have been intensively reviewed[69]. No microbial competition between bacteria and fungi was recorded in the present study. However, microbial competition after natural falling and dipping of house fly in water and milk has been reported[15]. The total number of microbes has decreased within one hour after dipping in the case of water. Meanwhile, immediate decrease in total number of microbes in the case of milk has been reported[15]. Further research on the effect of falling and dipping of *M. domestica* using electron microscopy and molecular techniques is recommended.

Overall, the current work presents isolation, characterization and antagonistic activity of six microorganisms isolated from external surface of the house fly; *M. domestica* after dipping in DW, SU and SA solutions. Our results revealed that our bacterial strains secrete many economically important materials which could be harnessed and marketed. Different efficiencies of sugar fermentation and gas production have been observed, too. In addition the antagonistic activity, especially the ability of *B. subtilis* bacterium to prohibit growth of all bacterial and fungal strains could be interpreted in the light of its production of bioactive materials. Further study on the mechanism of antimicrobial activity of *B. subtilis* strain is recommended. We concluded that this strain could be useful in controlling some bacterial and fungal infections.

ACKNOWLEDGEMENTS

We thank Dr. Shaymaa Nabil for her technical support.

CONFLICTS OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Conceived and designed the experiments: FHG, AMS. Performed the experiments: FHG, AMS, TES. Analyzed the data: FHG, AMS. Wrote the paper: AMS, FHG. All authors have approved the final manuscript.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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