Research Article   Open Access

A Recent Evaluation of the Sandfly, Phlepotomus Papatasi Midgut Symbiotic Bacteria Effect on the Survivorship of Leshmania Major

Mostafa I Hassan[1*], Bahera M Al-Sawaf[1], Mohamad A Fouda[1], Shaaban Al-Hosry[2] and Kotb M Hammad[3]

[1]Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt

[2]Department of Entomology- Faculty of Science Ain-Shams University, Cairo, Egypt

[3]Department of Vector Biology, NMRU-3, Cairo, Egypt

[*]Corresponding author: Mostafa I Hassan, Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt, Tel: +201223840969; Fax: +222629356; E-mail: mostafa012@gmail.com

Abstract

Like most entomophagus insects, the sandfly, *Phlebotomus papatasi* harbor both Gram-negative and Gram-positive bacteria in her midgut. The biological interactions between these bacteria and *Leishmania* parasites they transmit are not fully understood. In an effort to declare these interactions, the present study has been carried out. A total of six bacterial species were identified from the midgut of the sandfly, *Phlebotomus papatasi*. These species were; *Alcaligenes faecalis, Haemophillus parainfluenzae, Shigella sonnei, Serratia liquefaciens* (Gram-negative bacteria); *Listeria seeligeri* and *Bacillus thuringiensis* (Gram-positive bacteria). The *in vitro* effect of each isolated midgut bacteria species on the survivorship of *L. major* (promastigotes) was investigated. Results indicated that the most effective bacterial species was *B. thuringiensis* followed by *H. parainfluenzae* (at all concentrations used), where they caused 100% mortality of *Leishmania promastigotes*. In addition, the present study dealt with the interactions between the midgut bacteria and *Leishmania* parasites in *P. papatasi*. The results indicated that the aposymbiotic sandflies (with midgut-free bacteria) were more susceptible to the infection with L. major (81.25 % vs. 23.3%) than symbiotic ones (with midgut bacteria). This result may indicate that midgut bacteria play a very important role in inhibiting the development of *Leishmania* parasites, thus preventing the sandfly, *Phlepotomus paptasi* from transmitting *Leishmania* major to her hosts.

Keywords: Midgut symbiotic bacteria; *Phelopotomus paptasi*; *Leishmania major*

Introduction

Sandflies are the principal vectors of several diseases affecting humans, including leishmaniasis. The life cycle of *Leishmania* parasites involves amastigotes in the mammalian macrophage and flagellated promastigotes in the sand fly midgut. In insect-parasite interactions, once the *Leishmania* parasite enter the midgut of the sandfly, it passes through many physiological modifications in order to survive the adverse conditions present, such as proteolytic enzymes secreted by the midgut epithelium [1], and the formation of a peritrophic matrix (PM) that surrounds the blood meal [2]. Thus, for successful transmission by the sandfly, *Leishmania* needs to excape from the PM. So it produces a chitinase to degrade the PM [3]. Bacteria exist naturally in the midgut of wild and laboratory-reared mosquitoes [4], triatomines and Phlebotomines [5,6].

Bacteria occurring in the gut of haematophagous insects may have an important role in epidemiology of human infectious diseases. Such bacteria may interfere with the development of medically important pathogens. For example, high midgut counts of Gram-negative bacteria are known to significantly reduce oocyst numbers in plasmodium-infected mosquitoes [7]. A high prevalence of microbial infection in the digestive tract of wild-caught *Phlebotomus papatasi* females was suggested to have a negative effect on *Leishmania* transmission in endemic areas [8,9]. Recently, it was shown that the midgut bacteria, *Serratia marcescens* is lytic for *Trypanosoma cruzi* and *T. rangeli*, two species of parasites that use triatomine insects as vectors [10] and lytic for *Leishmania chagasi* (*in vitro*) a parasite that use sandflies as vector [11].

The possible role of symbionts in some blood-sucking insects was discussed by Mahdy et al., Hassan et al., Hammad et al. and Hurwitz et al. [4,5,12,13]. They suggested that the gut bacteria provide their host with some nutritional elements required for development, growth and reproduction and may affect the development of immature stages. Based on few previous studies it is believed that the microorganisms existing naturally in the midgut of wild and laboratory-reared insects might have an important role as determinants of parasite survival and development in insect hosts. This possibility, with notable exceptions, has been largely overlooked owing, at least in part, to problems in producing germ-free insects for experimentation.

Generally, the information on the biological interactions between the gut bacteria or the endosymbiotic bacteria of *Phlebotomus papatasi* and *Leishmania* parasite they transmit; and between these bacteria and the sandfly itself are somewhat limited. The aim of the present study was to isolate, identify and examine the prevalence of bacteria in the midgut of *Phlebotomus papatasi*, and to demonstrate the interactions between the sandfly midgut bacteria, and the *Leishmania* parasites they transmit.

Materials and Methods

Isolation of bacteria:

Bacteria were isolated after 24 h. from guts of laboratory reared female *Phlebotomus papatasi* either fed on sugar or blood meal. Also the isolation of bacteria from laboratory reared pupae and larvae were carried out according to the method of Pionar et al. and Hassan et al. [14,15].

Phlebotomus papatasi was dissected under sterile conditions using a binocular microscope. Ten guts of adults (newly emerged, sugar fed and blood fed females) and ten larvae and pupae were pooled in 5 ml. of sterile quarter strength Ringer's-solution and homogenized in homogenizer (model heidolph, Germany) at 2000 rpm. Each sample was serially diluted in Ringer's solution down to 10^{-5}. Fifty μl of last dilution of each sample were spread onto plates of selective and non-selective media. The plates were incubated at 30.0 0.1°C for 48 h. Bacterial count, colony forming units (C.F.U.) were determined and referred per ml.

Media used for isolation

Nutrient agar medium (Oxoid), Nutrient blood medium: The pH was adjusted to 7.0. This was non selective medium, Starch nitrate agar medium, Azide blood agar medium (Difco), Staphylococcus agar medium MacConkey's agar medium (Oxoid): it is a selective medium, Salmonella-shigella, it is also a selective medium.

Purification of bacterial isolates

The best growing colonies and the most characteristic ones were picked up by sterile loop and subjected to purification in the same isolation medium. Agar streak method was used for purification process. A well separated colony from each isolate was picked up on nutrient agar slopes and incubated at 28.0 0.1°C for 24 hrs. Purity was checked by microscopic examination of the isolate using Gram stain. All cultures were maintained under aerobic conditions.

Identification of bacterial isolates

Morphological identification

Gram stain: Jensen's modified method was applied using crystal violet as a basic dye and safranine as counter stain [16].

Physiological and biochemical identification

Many biochemical reactions were preceded for identification of bacteria according to the keys of Sneath et al. and Holt et al. [17,18].

Maintenance of Leishmania strains in the laboratory

Leishmania strains were maintained in the laboratory by routine passage in culture medium and cryopreserved in liquid nitrogen. Two main types of culture media were used, solid and liquid media. The solid media (NNN) were prepared according to the method of Chance et al. [19].

Antibiotic sensitivity

Antibiotic assay discs impregnated with antibiotics (Oxoid, England) were placed on blood agar plates previously seeded with the selective bacterial isolates. Resistance and zone of inhibition were recorded after 24 hrs, and incubation at 30°C. The antibiotics used were: Penicillin G (10 i.u.), Nalidixic acid (30 μg/ ml), Tetacycline (30 μg/ml), Clindamycin (2 μg/ml), Piperacillin (100 μg/ml), Minocycline (30 μg/ml), Cefoperazone (75 μg/ml), Rifampicin (30 μg/ml), Fortum ceftazidine (30 μg/ml), Norfloxacin (10 μg/ml), Cephaloridine (30 μg/ml), Ofloxcin (5 μg/ml), Cefotaxin (30 μg/ml), and Sulfamethoxazole trimethoprim (25 μg/ml).

Elimination of bacteria

Thirty nine female sand flies were fed on sponge piece soaked in a feeding medium, which composed of 30 gm. sucrose and 0.014 gm of a wide spectrum antibiotic (Tarivid) dissolved in 100 ml. distilled water. The elimination of bacteria was carried out according to the method of Hassan et al. [15]. This group of insects was used for aposymbiotic test.

In-vitro effect of isolated bacteria on Leishmania major, promasitgotes

The experiments were carried out using *Leishmania promastigotes* grown in NNN medium according to the standard method described by Adler and Theodor and Schlein et al. [20,21]. Cultured parasites were washed in sterile saline, counted and transferred to a feeding medium which consisted of fresh, defibrinated rabbit blood that had been inactivated at 56°C for 30 minutes. After inactivation, the blood was allowed to cool at room temperature before adding the parasites.

The *Leishmania* were cultured at 25°C in the medium for 5 days to reach the stationary phase of growth. Then they were collected by centrifugation (at 1,400 x g for 5 min. at room temperature, washed in saline solution, and resuspended in fresh complete medium to a final concentration. The number of live organisms was determined by counting with a hemocytometer after vital staining with leishman blue. Bacteria species, Alcaligenes faecalis, *Haemophilus paraifluensis, Shigella sonnei, Listeria seeligeri, Bacillus thuringiensis and Serratia liquefaciens*, were cultured in brain heart infusion (BHI) at different concentrations ($10^2,10^4,10^6$). The effects of each bacterial species on promastigotes of L. major were assessed.

Promastigotes (a mean of 27 viable cell/ml) were incubated in sterile test tubes at 25°C in complete medium in the presence of each bacterial species at a concentrations 102, 104 and 10^6, at the same time, control were prepared in complete medium. Parasite survival was estimated by microscopic counting of the number of viable (showing motile behavior and/or lack of staining) promastigotes in the treated cultures for every 24h for 5 days.

Experimental infection of P. papatasi with Leishmania major

In this investigation, golden hamsters were inoculated into the hind-foot pads to produce leishmanial lesions, using approximately 25 μl (2×10^6 organisms) of *Leishmania* major (MHOM/EG/99/RTC-30), Sinai strain in NNN liquid medium [19].

Infected animals were killed with CO2 and the cutaneous lesions were extirpated and homogenized in a glass tissue grinder containing phosphate-buffered saline. The resultant suspension was mixed with an equal volume of inactivated defibrinated hamster blood for

membrane feeding. Two groups of 4-6 day-old females of *P. papatasi* (50 flies/group) were starved for 24h and then allowed to feed for 2h upon the amastigotes-blood suspension of *L. major*, through a chick-skin membrane. Females were randomly selected from each group on days 5-7 post blood meal. The alimentary tracts of both groups were then dissected.

Dissection of infected flies

The fly is first anaesthetized by chilling and removed from the vial with a pair of fine forceps. It is transferred to a drop of normal saline solution on a microscope slide for dissection. The wings and legs are removed using a pair of fine mounting needles and the body transferred to a fresh drop of normal saline solution. Next the head is removed and kept in another drop of normal saline solution. This was examined later for the presence of metacyclic promastigotes and other forms. At this point promastigotes emerge from the severed end of the gut, which is broken. To remove the gut from the body one needle is placed on the thorax and the other on the tip of the abdomen and gently pulled. The gut is examined by light microscope.

Results

Interaction between the symbiotic bacteria and *Leishmania major* in *P. papatasi*: *In vitro* effect of each isolated bacteria species on the survival of *Leishmania major*.

Effect of isolated midgut bacteria of *Phlebotomus papatasi* at concentration of 102 on *L. major* (promastigotes)

Data given in Table 1 showed the *in vitro* effect of each isolated midgut bacteria of *P. papatasi* at a concentration of 10^2 on the survivorship of *L. major* promastigotes for a period of 5 successive days.

The effect was arranged disendingly as follows: *B. thuringiensis* > *H. parainfluenzae* > *L. seeligeri*>*S. liquefaciens*>*A. faecalis*>*Sh. sonnei*, where the survived count of *L. major* promastigotes was 1.8+0.4, 4.8+1.4, 10.0+1.5, 18.2+1.7, 18.8+0.8 and 20.6+0.9 CFU/ml, respectively, compared to 39.2 for the control (*L. major* not treated). On the second day, the most effective bacterial species against *L. major* was *B. thuringiensis*, where the survived count of *L. major* promastigotes was nill followed by *H. parainfluenzae*, where the count of survived *Leishmania* recorded 1.4+1.1 while the survived count as induced by *A. faecalis, S. liquefaciens, L. seeligeri* and *Sh. sonnei* was 3.4+1.1, 7.4+1.1, 7.8+1.4 and 9.0+1.0; respectively. Generally, a remarkable decrease in the count of survived *L. major* promastigotes was induced by all isolated midgut bacterial species on the third day. The count of survived *L. major* recorded were 0.0+0.0, 0.6+0.5, 2.2+1.3, 2.2+1.3, 3.2+1.3 and 4.0+1.6 as induced by *B. thuringiensis, H. parainfluenzae, A. faecalis, S. liquefaciens, L. seeliger* and *Sh. sonnei*; respectively. On the fourth day after treatment no promastigotes were survived in the presence of *B. thuringiensis, L. seeligeri* and *H. parainfluenzae* bacteria, where the count of survived promastigotes was zero. Meanwhile *S. liquefaciens, A. faecalis* and *Sh. sonnei* induced only a survival count of 0.2+0.4, 0.2+0.4 and 0.4+0.5; respectively.

However, all isolated midgut bacterial species of *P. papatasi* caused 100% mortality of *L. major* promastigotes after 5 days from treatment. Also, it could be concluded that, the most effective bacterial species in *P. papatasi* against *L. major* promastigotes was *B. thuringiensis* followed by *H. parainfluenze*.

Bacteria species	Mean No. of *L. major* (promastigotes) + S.D.				
Used in the treatment of	Days post treatment				
Leishmania	1st day	2nd day	3rd day	4th day	5th day
Alcaligenes faecalis	18.8+0.8	3.4+1.1	2.2+1.3	0.2+0.4	0.0+0.0
Haemophilus parainfluenzae	4.8+1.4	1.4+1.1	0.6+0.5	0.0+0.0	0.0+0.0
Shigella sonnei	20.6+0.9	9.0+1.0	4.0+1.6	0.4+0.5	0.0+0.0
Listeria seeligeri	10.0+1.5	7.8+1.4	3.2+1.3	0.0+0.0	0.0+0.0
Bacillus thuringiensis	1.8+0.4	0.0+0.0	0.0+0.0	0.0+0.0	0.0+0.0
Serratia liquefaciens	18.2+1.7	7.4+1.1	2.2+1.3	0.2+0.4	0.0+0.0
Leishmania promastigotes (control)	39.2+1.6	42+1.5	51+2.4	64.4+3.2	76.2+2.3
Mean no. of promastigotes at the beginning of experiment = 27					

Table 1: *In vitro* effect of isolated bacteria of *Phlebotomus papatasi* at concentration of 10^2 on the survivorship of *Leishmania* major, *promastigote*s

Effect of isolated bacteria of *Phlebotomus papatasi* at concentration of 104 on *L. major*, promastigotes

Data presented in (Table 2) indicated the *in vitro* effect of different isolated bacterial species of *P. papatasi* at a concentration of 10^4 on the survivorship of *L. major* (promastigotes) during a period of 5 successive days after treatment.

As shown from (Table 2), *B. thuringiensis* caused the highest mortality percent in *L. major* (promastigotes), where the count of survived promastigotes recorded 0.8+0.4 and zero on the first day and second day of treatment compared to 39.2+1.6 and 42.0+1.5 for control; respectively. On the other hand, *H. parainfluenzae* induced complete mortality in promastigotes population beginning from the third day after treatment. Meanwhile, the bacterial species; *L. seeligeri, A. faecalis* and *S. liquefaciens* caused complete mortality after the fourth day from treatment. However, all isolated midgut bacteria caused complete mortality on the fifth day of treatment.

From the aforementioned results it could be concluded that the most effective bacteria species on the survivorship of *L. major* was *B. thuringiensis* followed by *H. parainfluenzae* and *L. seeligeri*.

Bacterial species	Mean No. of L. major (promastigotes) + S.D. used in treatment of Days post treatment				
Leishmania	1st day	2nd day	3rd day	4th day	5th day
Alcaligenes faecalis	10.0+1.5	3.2+0.8	0.8+0.4	0.0+0.0	0.0+0.0
Haemophilus parainfluenzae	2.8+1.3	1.0+0.7	0.0+0.0	0.0+0.0	0.0+0.0
Shigella sonnei	16.8+1.4	7.6+1.1	2.4+1.1	0.2+0.4	0.0+0.0

Listeria seeligeri	6.8+1.4	2.6+1.1	0.6+0.5	0.0+0.0	0.0+0.0
Bacillus thuringiensis	0.8+0.4	0.0+0.0	0.0+0.0	0.0+0.0	0.0+0.0
Serratia liquefaciens	11.4+1.1	5.2+1.3	1.8+1.3	0.0+0.0	0.0+0.0
Leishmania promastigotes (control)	39.2+1.6	42+1.5	51+2.4	64.4+3.2	76.2+2.3
Mean no. of promastigotes at the beginning of experiment=27					

Table 2: *In vitro* effect of isolated bacteria of *Phlebotomus papatasi* at concentration of 104 on the survivorship of *Leishmania major*, promastigotes

Effect of isolated bacteria of *Phlebotomus papatasi* at a concentration of 106 on *L. major*, promastigotes

As shown from the (Table 3), *B. thuringiensis* was the most effective bacterial species isolated from the different developmental stages of *P. papatasi* on the survivorship of promastigotes followed by *H. parainfluenzae*, where they caused complete mortality in *L. promastigotes* population after one day and two days from treatment, respectively. On the other hand, the treatment with *A. faecalis* bacteria induced complete mortality of promastigotes on the third day of treatment. The complete mortality of promastigotes was also attained after 4 days from the treatment by each of *L. seeligeri* and *S. liquefaciens* bacteria. However, the complete mortality of *L. major* promastigotes was caused by all isolated midgut bacterial species 5 days post-treatment.

Bacterial species used in treatment of Leishmania	Mean No. of L. major (promastigotes) + S.D. Days post treatment				
Alcaligenes faecalis	1st day	2nd day	3rd day	4th day	5th day
Haemophilus parainfluenzae	8.6+1.5	0.6+0.5	0.0+0.0	0.0+0.0	0.0+0.0
Haemophilus parainfluenzae	1.2+0.8	0.0+0.0	0.0+0.0	0.0+0.0	0.0+0.0

Shigella sonnei	8.6+1.1	3.6+1.1	0.4+0.5	0.0+0.0	0.0+0.0
Listeria seeligeri	3.2+1.3	1.2+0.8	0.2+0.4	0.0+0.0	0.0+0.0
Bacillus thuringiensis	0.0+0.0	0.0+0.0	0.0+0.0	0.0+0.0	0.0+0.0
Serratia liquefaciens	10.2+1.3	3.6+0.5	0.2+0.4	0.0+0.0	0.0+0.0
Leishmania promastigotes (control)	39.2+1.6	42+1.5	51+2.4	64.4+3.2	76.2+2.3
Mean no. of promastigotes at the beginning of experiment=27					

Table 3: *In vitro* effect of isolated bacteria of Phlebotomus papatasi at a concentration of 106 on the survivorship of Leishmania major, promastigotes

Susceptibility of symbiotic and aposymbiotic *Phlebotomus papatasi* to infection with *L. major*

In order to study the susceptibility of symbiotic (normal females with symbiotic midgut bacteria) and aposymbiotic (females free from the midgut bacteria) *P. papatasi* to the infection by *L. major*, two groups of laboratory reared females; the first group "symbiotic" and the second group named aposymbiotic (treated with antibiotic with the sugar meal to eliminate the symbionts from the gut) were challenged with *L. major* through membrane feeding of infected blood.

Data given in (Table 4) showed that out of the 50 females fed on infected blood with L. major, 42 flies (84 %) were blood engorged. Out of this number 5 flies were taken from the engorged females after 5 days from feeding, dissected and microscopically examined. They were found to be negatively infected with *L. major*. After 6 days of blood engorgement another 10 flies were dissected and microscopically examined. Only 2 flies were found to be positively infected. On the other hand, when 15 flies from the engorged females were dissected and examined 7 days post feeding, 5 flies were found to be positively infected. In general, out of the 30 dissected females throughout a period of 7 days after feeding, seven flies were found to be positively infected with a percentage of 23.3 %.

Condition	No. of females used	No. of fed females	No. of dead females	No. of dissected females	No. of infected females days post feeding			Total infection (%)
					5 days	6 days	7 days	
Symbiotic	50	42	12	30	0	2	5	7 (23.3)
Aposymbiotic	50	46	14	32	5	9	12	26 (81.25)

Table 4: Susceptibility of symbiotic and aposymbiotic *P. papatasi* to infection with *L. major*.

Table 4 showed the susceptibility of aposymbiotic bacterial *P. papatasi* to infection with *L. major*.

Out of 50 females which fed on blood infected with *L. major*, 46 flies (92%) were blood engorged. When the guts of 7 flies were dissected and examined 5 days post feeding, 5 flies (71.4%) were found to be positively infected with *L. major*. After 6 days from feeding, another 10 flies were dissected and examined. Nine flies (90.0%) were positively infected. On the other hand, out of the dissected 15 flies after 7 days from feeding, 12 flies were found to be positively infected.

In general, out of the 32 dissected guts, 26 were found to be positively infected by *L. major* with a percentage of 81.25. From the

aforementioned results, it could be concluded that the aposymbiotic *P. papatasi* was more susceptible to the infection by *L. major* than symbiotic ones.

Discussion

Interaction between endosymbiotic bacteria and *Leishmania major* in *Phlebotomus papatasi*.

Information on the biological interactions between midgut bacteria of sand flies and *Leishmania* parasites they transmit is somewhat limited. However, the differences in susceptibility to leishmanial infection based on geographical distribution of sandflies were studied by many authors [22,23]. At the molecular level, the results of Sacks et al. [3] indicated that the susceptibility of a sand fly species to a given species of *Leishmania* may be determined by binding sites for lipophosphoglycan (LPG) on the surface of the fly's midgut.

Bacteria isolated from midgut of Phlebotomus papatasi

It is important to consider the nature of the microorganisms from vector insects, the present study revealed that *P. papatasi* female harbored both Gram–negative and Gram–positive bacteria in her gut. Six bacterial species were isolated and identified. These species were: *Alcaligenes faecalis*, *Haemophillus parainfluenzae*, *Shigella sonnei*, *Serratia liquefaciens* (Gram–negative bacteria); *Listeria seeligeri* and *Bacillus thuringiensis* (Gram–positive bacteria). In addition to, the four species Moraxella (Bramhamella) *ovis*, *Neisseria cinerea*, *Escherichia coli* and *Acinetobacter calcoaceticus*, were recorded earlier by Hassan et al. and Wahba et al. [5,24] .

The most frequently isolated bacteria species from the different developmental stages of *P. papatasi* in the present study was *S. liquefaciens* (59×10^5 CFU/ml.) in the larval stage, followed by *L. seeligeri* (40×10^5 CFU/ml.) in the newly emerged adults and *B. thuringiensis* (25×10^5 CFU/ml.) in the blood fed females. Dillon et al. [25] reported that the predominant bacteria species in the gut of female *P. papatasi* caught in Nekhel and El Arish regions of Sinai, Egypt were *Enterobacter cloacae*, *E. sakazaki* and *Aeromonas sobria* (order Enterobacteriaceae). On the other hand, Hassan et al. [5] isolated 4 species of Gram–negative bacteria namely; *Acinetobacter calcoacetius*, *Staphylococcus aureus*, *Staphylococcus haemolyticus* and *Neisseria mucosa* from the gut of *P. papatasi* collected from North Sinai and *P. langeroni* collected from El Agamy (Alexandria).

The bacterial counts, changed dramatically during the different developmental stages of *P. papatasi*. This change was also dependent on adult–feeding status, where an increase in the bacterial counts after a blood meal was recorded. These results fit with other studies for *Culex pipiens* by Hammad [4]. Clements [26] suggested that the decrease in midgut bacteria in mosquitoes between the larval and adult stages is due to the mechanical shedding of the larval midgut and its contents, shortly after emergence.

Effect of symbiotic and aposymbiotic of *Phlebotomus papatasi* on *L. major*

The present authors discussed the interactions between the midgut bacteria and *Leishmania parasites* in *P. papatasi*. The results showed that the aposymbiotic *P. papatasi* were more susceptible to the infection by *L. major* (81.25%) than symbiotic ones (23.3%). In agreement with these results Curtis et al. [27] found that the

aposymbiotic mosquitoes were fully susceptible to the filarial infection, although this contrasts the result of Duhrkopf and Trpis [28] for Aedes scutellaris complex. According to Jadin et al. [29], *Klebseilla* (which is a gram–negative bacterium) can obstruct sporogonic development of *Plasmodium berghei* in *Anopheles stephensi* and cause mortality in the mosquitoes. Pumpuni et al. [3] concluded that inhibition of *Plasmodium falciparum* development by gram–negative bacteria in A. stephensi leads to speculate that if the midguts of indigenous vector populations are sufficiently colonized with gram–negative bacteria, the natural transmission of malaria may be interrupted or reduced. Alder and Theodor [20] were the first to suggest that the presence of other microorganisms might prevent the development of *Leishmania* spp. in the sand fly gut. Schlein et al. [7] recorded a high incidence of microbial contamination in the digestive tract of *P. papatasi* and suggested that gut contamination might interfere with *Leishmania* transmission. Dillon and Dillon [25] showed that the *Leishmania* parasites often grow poorly in competition with bacteria in *P. papatasi*, probably because of their relatively slow generation time. However, in experimentally infected sandflies, *Leishmania* parasites could survive in the presence of very low count of bacteria [24].

Dealing with the *in vitro* effect of each isolated midgut bacteria on the survivorship of *L. major* (promastigotes), the present study indicated that the most effective bacterial species was *B. thuringiensis* followed by *H. parainfluenzae* (at all concentrations used), where they caused 100% mortality of *Leishmania promastigotes*. This preliminary study indicated that the gut microbiota might well contribute to the modulation of insect vector competence but, the mechanisms involved are largely unknown.

References

1. Borovsky D, Schlein Y (1987) Trypsin and chymotrypsin-like enzymes of the sandfly Phlebotomus papatasi infected with Leishmania and their possible role in vector competence. Med Vet Entomol 1: 235-242.

2. Pimenta PF, Modi GB, Pereira ST, Shahabuddin M, Sacks DL (1997) A novel role for the peritrophic matrix in protecting Leishmania from the hydrolytic activities of the sand fly midgut. Parasitology 115 : 359-369.

3. Sacks DL, Saraiva EM, Rowton E, Turco SJ, Pimenta PF (1994) The role of the lipophosphoglycan of Leishmania in vector competence. Parasitology 108 Suppl: S55-S62.

4. Hammad KM (2001) Studies of certain factors (microbial flora) affecting on some biological aspects of mosquito, Culex pipiens L. (Diptera: Culicidae).

5. Hassan MI, Mahdy H, Lotfy NM (1998) Biodiversity of the microbial flora associated with two species of sandflies Phlebotomus papatasi and P.langeroni (Diptera: Psycho-didae). J Egypt Ger Soc Zool 26: 25-36.

6. Oliveira SM, Moraes BA, Gonçalves CA, Giordano-Dias CM, D'Almeida JM, et al. (2000) [Prevalence of microbiota in the digestive tract of wild females of Lutzomyia longipalpis Lutz & Neiva, 1912) (Diptera: Psychodidae)]. Rev Soc Bras Med Trop 33: 319-322.

7. Pumpuni CB, Demaio J, Kent M, Davis JR, Beier JC (1996) Bacterial population dynamics in three anopheline species: the impact on Plasmodium sporogonic development. Am J Trop Med Hyg 54: 214-218.

8. Schlein Y, Polacheck I, Yuval B (1985) Mycoses, bacterial infections and antibacterial activity in sandflies (Psychodidae) and their possible role in the transmission of leishmaniasis. Parasitology 90 : 57-66.

9. Gouveia C, Asensi MD, Zahner V, Rangel E, De Oliveira MP (2008) Study on the Bacterial Midgut Microbiota Associated to Different Brazilian Populations of Lutzomyia longipalpis (Lutz & Neiva) (Diptera: Psychodidae). Neotrop Entomol 37: 597-601.

10. Castro DP, Moraes CS, Garcia ES, Azambuja P (2007) Inhibitory effects of d-mannose on trypanosomatid lysis induced by Serratia marcescens. Exp Parasitol 115: 200-204.

11. Moraes CS, Seabra SH, Castro DP, Brazil RP, de Souza W, et al. (2008) Leishmania (Leishmania) chagasi interactions with Serratia marcescens: Ultrastructural studies, lysis and carbohydrates effects. Exp Parasitol 118: 561-568.

12. Mahdy HM (1997) Assessment of bacterial diversity associated with two genera of sandflies. Journal of the faculty of education 22: 647-658.

13. Hurwitz I, Hillesland H, Fieck A, Das P, Durvasula R (2011) The paratransgenic sand fly: a platform for control of Leishmania transmission. Parasit Vectors 4: 82.

14. Pionar OJR, Thomas MG (1978) Diagnostic manual for the identification of insect pathogens. Plenum press New York and London.

15. Hassan MI, Fouda MA, Al-Daly AG, Hammad KM (2001) Survivorship and development of Culex pipiens mosquitoes as influenced by their midgut bacterial association. Sc J Az Med Fac.

16. Cruickshank R, Duguid J, Marmion B, Swain R (1975) Medical Microbiology: the practice of medical microbiology, 12th ed., Vol. II. Chur-chill Livingstone, London.

17. Sneath PHA (1986) Bergey's Manual of Systematic Bacteriology. 2: 1325-1329.

18. Holt J, Krieg N, Sneath P, Staley J, Williams S (1994) Bergey's Manual of Determinative Bacteriology, 9th ed., Williams and Wilkins, Baltimore.

19. Chance ML, Peters W, Shchory L (1974) Biochemical taxonomy of Leishmania. I. Observations on DNA. Ann Trop Med Parasitol 68: 307-316.

20. Adler S, Theodor O (1927) The behaviour of cultures of Leishmania sp. In Phlebotomus papatasi. Nature 119: 565.

21. Schlein Y, Warburg A, Schnur LF, Shlomai J (1983) Vector compatibility of Phlebotomus papatasi dependent on differentially induced digestion. Acta Trop 40: 65-70.

22. Wu WK, Tesh RB (1990) Selection of Phlebotomus papatasi (Diptera: Psychodidae) lines susceptible and refractory to Leishmania major infection. Am J Trop Med Hyg 42: 320-328.

23. Hanafi HA, el Sawaf BM, Fryauff DJ, Beavers GM, Tetreault GE (1998) Susceptibility to Leishmania major of different populations of Phlebotomus papatasi (Diptera: Psychodidae) from endemic and non-endemic regions of Egypt. Ann Trop Med Parasitol 92: 57-64.

24. Wahba M, Hassan MI, El-Kordy E, Mahdy H (1999) Impact of bacteria infection of Phlebotomus papatasi on Leishmania major. Third International Symposium on Phlebotomine Sandflies (ISOPS), August, 23-27, Montpellier, France.

25. Dillon RJ, Dillon VM (2004) The gut bacteria of insects: nonpathogenic interactions. Annu Rev Entomol 49: 71-92.

26. Clements AN (1992) The biology of mosquitoes: Development, nutrition and reproduction, Volume 1. Chapman and Hall, New York.

27. Curtis CF, Ellis DS, Doyle PE, Hill N, Ramji BD, et al. (1983) Susceptibility of aposymbiotic Culex quinquefasciatus to Wuchereria bancrofti. J Invertebr Pathol 41: 214-223.

28. Duhrkopf RE, Trips M (1981) The effect of tetracycline treatment on filarial susceptibility in members of the Aedes scutellaris complex. Mosq News 41: 729-732.

29. Jadin J (1967) [Role of bacteria in the digestive tube of insects, vectors of plasmodidae and trypanosomidae]. Ann Soc Belges Med Trop Parasitol Mycol 47: 331-342.