Original Article

Aerobic Microbial Community of Insectary Population of *Phlebotomus papatasi*

Naseh Maleki-Ravasan [1], *Mohammad Ali Oshaghi [1], Sara Hajikhani [2], Zahra Saeidi [1], Amir Ahmad Akhavan [1], Mohsen Gerami-Shoar [3], Mohammad Hasan Shirazi [2], Bagher Yakhchali [4], Yavar Rassi [1], Davoud Afshar [2]

[1]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

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Abstract

Background: Microbes particularly bacteria presenting in the gut of haematophagous insects may have an important role in the epidemiology of human infectious disease.
Methods: The microbial flora of gut and surrounding environmental of a laboratory strain of *Phlebotomus papatasi*, the main vector of Zoonotic Cutaneous Leishmaniasis (ZCL) in the old world, was investigated. Biochemical reactions and 16s rDNA sequencing of the isolated bacteria against 24 sugars and amino acids were used for bacteria species identification. Common mycological media used for fungi identification as well.
Results: Most isolates belonged to the Enterobacteriaceae, a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. Enterobacteriaceae groups included *Edwardsiella*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Kluyvera*, *Leminorella*, *Pantoea*, *Proteus*, *Providencia*, *Rahnella*, *Serratia*, *Shigella*, *Tatumella*, and *Yersinia* and non Enterobacteriaceae groups included *Bacillus*, *Staphylococcus* and *Pseudomonas*. The most prevalent isolates were *Proteus mirabilis* and *P. vulgaris*. These saprophytic and swarming motile bacteria were isolated from all immature, pupae, and mature fed or unfed male or female sand flies as well as from larval and adult food sources. Five fungi species were also isolated from sand flies, their food sources and colonization materials where *Candida* sp. was common in all mentioned sources.
Conclusion: Midgut microbiota are increasingly seen as an important factor for modulating vector competence in insect vectors so their possible effects of the mirobiota on the biology of *P. papatasi* and their roles in the sandfly-*Leishmania* interaction are discussed.

Keywords: Symbiont, Microflora, Bacteria, Fungi, *Phlebotomus papatasi*, Leishmaniasis

Introduction

Symbiont microorganisms of vector insects are involved in many aspects of the host life including nutrition, reproduction, tolerance to environmental perturbations, maintenance and/or enhancement of host immune system homeostasis, defense, speciation, mucosal barrier fortification, xenobiotic metabolism, and pathogen transmission ability (O'Neill et al. 1997, Baumann et al. 2000, Volf 2002, Bourtzis and Miller 2003, Rio et al. 2004, Dillon and Dillon 2004, Weiss and Aksoy 2011). The gut of sand flies is a site where most *Leishmania*'s entire life cycle takes place, thereby resident gut bacteria could possibly

***Corresponding author:** Prof Mohammad Ali Oshaghi, E-mail: moshaghi@sina.tums.ac.ir

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have a role in modulating the parasite development, either enhancing or inhibiting it. In nature, sand flies feed on different kinds of sugars, so having the chance to acquire contaminating microorganisms, such as bacteria and fungi that can eliminate the leptomonad infection or even kill the sand fly (Schlein et al. 1985, Killick-Kendrick and Killick-Kendrick 1987, Schlein and Yuval 1987, Cameron et al. 1995).

Yeasts and bacteria could reduce the infection rate of *L. major* in *Phlebotomus papatasi* (Schlein et al. 1985). *P. papatasi* is of particular interest as a vector of Zoonotic Cutaneous Leishmaniasis (ZCL) to humans in the old world. The conventional vector control measures such as application of insecticide has got undesirable effects on the environment, human health, and the emergence of insecticide resistance in sand flies (Alexander and Maroli 2003). The larvae of sand flies live in contaminated soils of domesticated animal shelters, termite mounds and rodent burrows and are exposed to a variety of different soil microbes that could be ingested alongside of food (Feliciangeli 2004, Mukhopadhyay et al. 2012). Moreover, gravid *P. papatasi* attracts to the oviposition sites contaminated by frass and certain soil bacteria (Radjame et al. 1997, Wasserberg and Rowton 2011). Therefore it is expected that the gut of sand flies will support a range of microorganisms. These microbes could be genetically manipulated to express an antiparasitic molecule and then be reintroduced back into the sand fly gut through larval breeding places. This is a new vector borne disease control method called paratransgenesis that leads to reduce pathogen transmission by an insect vector (Chavshin et al. 2013). This method has been successfully applied for a few insect vectors such as *Rhodnius prolixus*, vector of *Trypanosoma cruzi* the causative agent of the Chagas disease in Central America (Beard et al. 2001), *Glossina morsitans,* the vector of african sleeping sickness (Aksoy et al. 2008, Pontes and Dale 2011), and *Anopheles stephensi*, a vector of malaria in Asia (Wang et al. 2012).

Boulanger et al. (2004) demonstrated that bacteria present in the digestive tract of *P. duboscqi* induced the secretion of antimicrobial peptides with a significant antiparasitic activity. Dillon et al. (1996) and Volf et al. (2002) demonstrated that the maximum prevalence of bacteria in *P. papatasi* and *P. duboscqi* is recorded two days after blood feeding. High diversity in the bacterial gut microbiota, associated with different populations of *P. argentipes* and *Lutzomyia longipalpis*, was registered using culture-dependent methods (Gouveia et al. 2008, Hillesland et al. 2008).

Notwithstanding these studies, there are a few reports available on the micro flora of *P. papatasi* (Dillon et al. 1996, Guernaoui et al. 2011, Mukhopadhyay et al. 2012). Gut flora is depended upon the environment they live. Male and female sand flies feed on natural sugars, such as nectar, sap, and aphid and coccid secretions (Young et al. 1980, Killick-Kendrick and Killick-Kendrick 1987). These sugars are the main sources of carbohydrates for adults sand flies. Furthermore, females also feed on blood. Sand fly larvae are terrestrial and feed on soil organic matter (Feliciangeli 2004). It is thus supposed that the diversity in their feeding behavior, as well as conditions that pre-imaginal stages encounter during their development, affect gut microbiota and could impact their overall capacity to sustain *Leishmania* development.

Sand flies gut bacterial flora has been investigated on the isolated or pooled guts via culture of bacterial gut content and were identified by the use of classical bacteriology, cloning, and ribotyping (16S rDNA sequencing) (Rajendran and Modi 1982, Hillesland et al. 2008, Gouveia et al. 2008, Chavshin et al. 2012). Fungi (yeasts) have been used as larva nutrient (e.g. Wermelinger and Zanuncio 2001) or entomopathogen (Warburg 1991) of

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sand flies. However, there is a little information about fungi as part of gut flora of sand flies (Schlein et al. 1985).

The aim of the present study was to examine different aerobic microbial community (bacteria and fungi) in the gut, food sources and colonization materials of a laboratory-reared colony of *P. papatasi* (Isfahan strain) in the School of Public Health, Tehran University of Medical Sciences (SPH-TUMS). Results of this study may lead to identify appropriate candidate/s for paratransgenesis approach.

Materials and Methods

Sand fly insectary

Sand flies of Badrood strain originally collected from Badrood (33° 44´ N, 52° 2́ E), Natanz district, Isfahan Province, central Iran, were reared under laboratory condition at 24–27 °C, 80% RH and 14:10 (L: D) photoperiod in the sand fly Insectary of SPH-TUMS, following the method of Modi and Tesh for mass rearing of *P. papatasi* with some modifications (Modi and Tesh 1983). We have examined microbial infestation of a variety of sand fly biological specimens as well as non-biological materials used in the rearing of sand flies in the laboratory. The biological specimens included sand fly eggs, 1–4 instar larvae, pupae, unfed males, honey fed males, unfed females, honey fed females, blood and honey fed females, white mouse auricle, and white mouse muzzles. Tested non-biological materials were larval chow as immature sand fly food sources, saturated sucrose and honey solution 50% as mature sand fly food sources, and sea sand (Merck), plaster (Chalk), and soil as colonization materials (Table 1).

Isolation of bacteria

Isolation of sand fly guts was conducted in a sterile environment on a sterile glass slide. Before dissection, each fly was surface sterilized for 1min in 70% ethanol. Bacterial microflora was isolated from each sand fly by one of the two following methods. For adults, the gut from each sand fly was microdissected and homogenized in test tubes containing 5cc brain heart infusions (BHI) broth. For immature sand flies, the whole body of each individual larva homogenized in BHI broth.

Sterile cotton swabs (presenting in a sterile test tube) were used to swab the area around the auricle or nose of white mouse where sand flies choose to take blood meal. The infested swabs then were merged in BHI broth. For larval chow, sucrose, honey solution, sea sand, plaster and soil weights equal to 0.7 micro tubes volume were poured in the medium. BHI broth was chosen as a non-selective medium to promote growth a diverse range of microbes including nutritionally fastidious bacteria and even fungi. The complete transparent test tubes were incubated aerobically at 37 °C overnight. After 24 hours, opaque test tubes considered as positive and sub cultured in BHI agar medium overnight at the same condition. The colonies with different phenotype were sub cultured sequentially to obtain single colony of the microbes. Pure cultures for each microbe were used for further identification procedure. A test tube containing BHI broth open near the dissection area constituted our sterility control during the dissection process.

Bacterial identification procedure

All of the isolates were differentiated by standard gram staining and morphotypes. MacConkeys Agar, a special selective medium for gram negative bacteria which can differentiate those bacteria capable to ferment lactose was used for further confirmation. Biochemical properties of the gram negative rods belonging to the Entrobacteriaceae were investigated by “DS-DIF-ENTRO-24” kit. This kit is produced for the identification

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and differentiation of 81 species and subspecies corresponded to 25 genera of the family Entrobacteriaceae within 24 hours. This ready to use kit has been designed for the performance of 24 biochemical tests including simmons citrate, malonate, esculine, lysine, arginine, ornithine, hydrogen sulphide, phenylalanine, voges-proskauer, indole, glucose, β-galactosidase, lactose, mannitole, sucrose, inositole, sorbitol, arabinose, maltose, adonitol, trehalose, ramnose, dulsitol and urease. The interpretation of the results was based on numerical probabilistic identification using EasyBac software package and the distance method.

Gram-positive isolates that were not able to grow on the MacConkeys Agar medium, tested with manual laboratory examinations such as oxidase, catalase, coagulase, novobiocin susceptibility tests and mannitol medium.

To test the accuracy of the DS-DIF-ENTRO-24 kit, three bacterial isolates from adult (FU), larvae (34L1) and white mouse muzzle (Mn2Moz) were selected randomly and subjected for molecular ribotyping following PCR amplification and PCR-direct sequencing of a subset of about 1500bp of 16S rRNA gene according to Weisburg et al. (1991) protocol. Homologies with the available sequence data in GenBank was checked by using basic local alignment search tool (BLAST) analysis software (www.ncbi.nlm.nih.gov/BLAST).

Isolation of fungi

For initial isolation of fungi we followed overnight BHI broth test tubes. In the subsequent subcultures we used Subaru Dextrose Agar with chloramphenicole medium and samples were incubated at 25–27 °C for a week. Positive samples were examined macroscopically and then microscopic slides were prepared using the lactophenol cotton blue wet mount. Chrome Agar Candida, a chromogenic differential culture medium, used for isolation and identification of the yeast species in incubation conditions of 35 °C for 2 days. Corn Meal Agar medium plus Tween 80 were used for discrimination of *Candida albicans* from non-albicans species.

Results

Biochemical identification of bacteria

Totally 25 bacterial species were isolated comprising 20 species of Entrobacteriaceae, one species of Pseudomonadaceae, one species of Bacillaceae, two species of Staphylococcaceae, and one non-identified species in the Phelebtominae insectary of SPH-TUMS. Details of the bacterial isolates from different stages of sand flies (egg to adults), food sources, and colonization materials are listed in Table 1.

Molecular identification of the bacteria

Three isolates of 34L1, Mn2Moz and FU, that were biochemically identified as *Proteus vulgaris*, *P. vulgaris* and *Bacillus* sp. respectively, were identified accordingly as *P. vulgaris*, *P. penneri* and *B. flexus* based on partial sequences of 16S rRNA. Their sequences with length of 882, 995 and 1279 bp were deposited in GenBank with accession numbers of JQ928898-JQ928900 respectively.

Isolation of fungi

Five fungal species were identified including three molds species of *Cladosporium* sp., *Penicillium* sp., and *Aspergillus flavus* that were isolated from a honey and blood fed female, *Rhizopus* sp. from saturated sucrose, and one yeast species, *Candida* sp., from different sources including honey and blood fed females, unfed male, second instar larvae, sea sand, soil, larval chow, saturated sucrose, and honey (Table 1). Although *C. albicans* formed chlamydospores on Corn Meal Agar medium however mycelia have been observed on the medium. So we encountered *Candida* sp. other than *C. albicans*.

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Table 1. Summary of the isolated bacteria from the sand fly insectary and their sources identified by DS-DIF-ENTRO-24 kit (*based on 16srRNA ribotyping)

Sources / Species	Egg	Larvae 1	Larvae 2	Larvae 3,4	Pupae	Unfed Male	Honey fed Male	Unfed Female	Honey and Blood Fed Female	Sand	Larval Chow	Sucrose	Honey	Auricle of White Mouse	Muzzle of White Mouse	Chalk	Soil	Found in sand flies, insects or other sources [Notes]
***Bacillus* sp (**B. flexus*)**								X									X	*P. papatasi* (Mukhopadhyay et al. 2012), *Macrotermes carbonarius* (Tay et al. 2010) Plants (Sanchez-Gonzalez et al. 2011), Seaweed (Singh et al. 2011)
Staphylococcus aureus						X										X		*P. argentipes* (Hillesland et al. 2008), Cockroaches (Al-bayati et al. 2011), Human skin, somewhen systemic infections (Foster 2005)
Staphylococcus saprophyticus												X						*P. papatasi* (Mukhopadhyay et al. 2012), *P. argentipes* (Hillesland et al. 2008), *Musca domestica* (Butler et al. 2010)
Not identified bacterium							X											*****
Pseudomonas aeruginosa	X																	*P. papatasi* (Mukhopadhyay et al. 2012), *P. argentipes* (Hillesland et al. 2008), [Pathogen on human, insects and plants (Silby et al. 2011)]
Shigella dysenteriae		X												X				Cockroaches (Salehzadeh et al. 2007, Al-bayati et al. 2011), *Musca domestica* (Bolaños-Herrera 1959)
Edwardsiella ictaluri		X																[Pathogen on fish (Santander et al. 2010)]
Kluyvera ascorbata		X																*Ips typographus* (Muratoğlu et al. 2011)
Proteus vulgaris			X	X	X			X							X			Cockroaches (Al-bayati et al. 2011), *Ips typographus* (Muratoğlu et al. 2011)
Klebsiella oxytoca			X												X			*Ips typographus* (Muratoğlu et al. 2011) *Bactrocera cacuminata* (Thaochan et al. 2010)
Klebsiella planticola			X															*Ips typographus* (Muratoğlu et al. 2011)
Providencia rettgeri				X														[Pathogens on humans and *Drosophila melanogaster* (Galac and Lazzaro 2011)] *Bactrocera cacuminata* (Thaochan et al. 2010)
Klebsiella pneumonia ozaenae				X	X													Cockroaches (Al-bayati et al. 2011), *Bactrocera cacuminata* (Thaochan et al. 2010)
Leminorella grimontii				X														*Anopheles stephensi* (Rani et al. 2009)
Enterobacter amnigenus					X							X	X					*Anopheles dirus* (Khampang et al. 2001)
Proteus mirablis					X				X		X	X	X	X	X			Blow fly maggots (Erdmann 1987, Fleischmann et al. 2004), *Lucilia sericata* (Ma et al. 2012), *Calliphora vicina* (Greenberg et al. 1970), *Cochliomya hominivorax* (Erdmann and Khalil 1986), *Lucilia cuprina* (Mohd Masri et al. 2005), *Musca domestica* (Greenberg 1959), Parasitoide wasps, *Itoplectis* (Bucher 1963), Cockroaches (Al-bayati et al. 2011)
Escherichia coli						X												*Anopheles gambiae* (Dong et al. 2009) Cockroaches (Al-bayati et al. 2011)
Enterobacter gergoviae									X				X					*Pectinophora gossypiella* (Kuzina et al., 2002)
Pantoea agglomerans									X				X		X			*Anopheles gambiae* (Dong et al. 2009)
Escherichia blattae									X			X						Cockroaches (Burgess et al. 1973)
Tatumella ptyseos										X			X					[Pathogen on Human and Pineapple (Marín-Cevada et al. 2010, Hollis et al. 1981)]
Serratia plymuthica											X							*Manduca sexta* (Toth-Prestia and Hirshfield 1988)
Yersinia pseudotuberculosis											X							[Ubiquitous bacteria affecting humans, animals and fleas (Hinnebusch 2005)]
Rahnella aquatilis													X	X				Pacific Coast Wireworm,(Lacey et al. 2007), Southern Pine Beetle (Vasanthakumar et al. 2006), *Toxoptera aurantii* (Sevim et al. 2012)
Providencia alcalifaciens															X			[Pathogens on humans (Galac and Lazzaro 2011)]

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Discussion

We could isolate and identify 25 bacteria and five fungi species from the sand fly strain and its rearing materials. Majority of these microbes including 20 of the bacteria and four of the fungi species, were isolated from sand fly guts. Many species of *Candida* could be found in the gut of a variety of organisms including *C. albicans* in mammalian and insect as endosymbionts (Nguyen 2007, Suh 2008). Although bacterial micro flora of some laboratory reared sand flies have been pointed out in a few studies but the source of this flora is the first time that marked and studied in details. In this study we tested microbial infestation of all fields and materials used in sand fly colonization procedure.

In this study the correctness of the biochemical kit were examined against 16S-rRNA ribotyping. On general there was a great concordance between the results of two methods: both methods identified 34L1 and Mn2Moz isolates as *Proteus vulgaris*. It is noteworthy that *P. penneri* is formerly known as *P. vulgaris* biogroup 1. However, the molecular method could identify the FU isolate up to species level (*B. flexus)* whereas the biochemical method identified it up to genus level (*Bacillus* sp) concluding that the ribotyping is more specified than biochemical one.

Although we used a nonselective medium (BHI broth) to promote growth a wide range of bacteria and fungi, however, due to not using various media and culture conditions (e.g. anaerobic condition) the medium generally favored in growth of gram negative Enterobacteriaceae. Similarly, almost all of the studies analyzing the sand fly guts for bacterial communities have also relied on culture dependent techniques in their analyses where Enterobacteriaceae constituted the majority of their findings (Dillon et al. 1996, Hillesland et al. 2008, Mukhopadhyay et al. 2012). Even the studies that have implemented molecular tools used these tools only in the identification and analysis of isolated pure colonies from plate culture, not in the initial isolation of bacteria from the guts (Gouveia et al. 2008, Hillesland et al. 2008). The molecular tools used in both studies were implemented in the identification of bacterial colonies obtained by culturing, thereby limiting the findings to the small proportion of cultivable microbes. Taking into account the limitations of culture dependent techniques makes these findings incomplete.

A correlation between the type of microbial gut flora detected and the area inhabited by the sand fly has been addressed by Hillesland et al. 2008, where flies collected from the same region harbored almost the same kinds of bacteria. Therefore, it was suggested that gut flora diversity more or less is a reflection of the environment where the sand fly resides (Hillesland et al. 2008). For example, *Bacillus megaterium* that is present in biofertilizers widely used in the state of Bihar, India, was isolated from the guts of a number of sand flies inhabiting that area. Another example was that of *Brevibacterium linens*, the bacterium used in cheese ripening industry that was also isolated from the gut of sand flies collected from regions known to be involved in dairy preparations (Hillesland et al. 2008). Both these bacteria were proposed as candidates for use in a paratransgenesis model, being already employed in biotechnological operations without concerns about their safety (Hillesland et al. 2008). Mukhopadhyay et al. 2012 carried out a survey to study the abundance of different natural gut flora of *P. papatasi* in different habitats (Sheep shed, Rabbit hole, Chick/sheep shed, Human dwellings and Lab colony) of Tunisia, Turkey, India and Egypt. They found variation in the species and abundance of gut flora in sand flies collected from different habitats. How-

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ever *B. flexus* and *B. pumilus* were common in most of the sites examined. *B. flexus* is capable to grow under highly alkaline conditions that are the case for phlebotomine sand flies (Panizzi and Parra 1991) as alkaline amylase producers was found in our study from midgut of unfed female as well.

In our study we found *Staphylococcus saprophyticus* from the saturated sucrose used as adult's phytophagic meal. This bacterium which has already isolated from *P. argentipes* (Hillesland et al. 2008), *Musca domestica* (Butler 2010) and *P. papatasi* (Mukhopadhyay 2012) is a very strong oviposition inducer for gravid *P. papatasi* (Radjame 1997). Mukhopadhyay et al. 2012 could success in introducing of *Bacillus flexus*, *B. pumilus*, *B. licheniformis*, *B. megaterium* and *B. subtilis* as candidate species for paratransgenesis due to these bacteria ability in induction of sand fly oviposition behavior and real function of those as symbionts and not merely as environmental contaminants.

The presence of *P. mirabilis* and *P. vulgaris* nearly in all our analyzed samples raises questions about the nature of the interaction between this microorganism and sand flies. *Proteus* species are found in the digestive tracts of many animals including insects (Guentzel 1991, Rozalski 1997). A field survey of bacteria from the digestive tracts of newly emerged house flies breeding in horse manure showed the predominant flora to consist of *P. vulgaris*, *P. mirabilis*, *Aerobacter aerogenes*, and *Citrobacter freundii*. A similar census from flies bred in the laboratory recovered *P. vulgaris*, *P. mirabilis*, *P. morganii*, *P. rettgeri*, *E. coli*, and *Aerobacter aerogenes* (Greenberg 1959). Interestingly, in maggots, the bacterium *P. mirabilis* secrete antibacterial toxins (including phenylactic acid and phenylactaldehyde) that kill other microbes but do not harm the maggots (Erdmann 1987, Fleischmann et al. 2004). *P. mirabilis* is also highly resistant to the action of antimicrobial peptides, such as polymyxin B (PM), protegrin, and the synthetic protegrin analogI B-367 (McCoy 2001). Ma et al. 2012 found that *P. mirabilis* interkingdom swarming signals attract blow flies. They obtained *P. mirabilis* from the salivary glands of the blow fly *Lucilia sericata*, this strain swarmed significantly and produced a strong odor that attracts blow flies. Greenberg et al. (1970) demonstrated that microorganisms ingested by the blowfly *Calliphora vicina* are rapidly eliminated if *P. mirabilis* is also present in the ingested material. Several pathogenic microorganisms have been killed by brief exposure to 15 days old *P. mirabilis* culture broth (Erdmann and Khalil 1986). Greenberg (1968) referred to the active anti-bacteria constituents as "mirabilicide." It was also reported that the symbiotic relationship between *P. mirabilis* and the screwworm could protect the larvae from other harmful bacteria by bactericidal agents secreted by *P. mirabilis* (Erdmann and Khalil 1986). Interestingly, complete sterility of *Lucilia cuprina* maggots for the purpose of debriding intractable wounds was achieved in all cases, except that *P. mirabilis* was consistently found. However, the presence of this microorganism was considered beneficial (Mohd-Masri et al. 2005).

To what extent the bacterium could play a similar role during sand fly larval development remains unknown and has to be investigated. A few studies have examined the impact of the gut microbiota on the establishment of human pathogens and parasites in their insect vectors. The possibility that colonization resistance is involved in suppressing medically important parasites such as *Plasmodium* and *Leishmania* in their dipteran vectors has been discussed (Dillon et al. 1996, Pumpuni et al. 1996). Although a recent study suggesting a dose dependent inhibitory effect of gut bacteria on *Leishmania* promastigotes (Muniaraj et al. 2008), however, more investigation need to find the most effective bacteria or bacterium which can be used as bio-agent for combating *Leishmania*

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parasites. An indigenous biota (not transient one) is present in all individuals of the species and maintains stable climax communities (Dillon and Dillon 2004) also in some cases an indigenous species may colonize only in the presence of other members of the microbial community (Dillon and Charnley 2002). Indigenous bacteria modulate expression of genes involved in several important intestinal functions including nutrient absorption, mucosal barrier fortification, and xenobiotic metabolism (Dillon and Dillon 2004). Regarding to these criteria, herein we can nominate *Proteus* spp. as indigenous isolates of *P. papatasi.* However, whether or not, the resident gut microbiota as a micro ecological factor can regulate the prevalence of sand flies with transmissible infections need to be investigated.

Koch and Schmid-Hempel (2011) showed that the presence microbiota protects bee hosts against a widespread and highly virulent natural parasite, *Crithidia bombi*. Their results emphasize the importance of considering the host microbiota as an "extended immune phenotype" in addition to the host immune system itself and provide a unique perspective to understanding bees in health and disease.

Metacyclic promastigotes are highly adapted for transmission and early survival in the vertebrate host (Kamhawi 2006). Messy condition of sandfly midgut and many interactions will determine the triumphant. Peritrophic matrix, enzymes, temperature, pH, oxygen pressure, insect immune system, vertebrate host immune system, sugar meals, blood meals, *Leishmania*, bacteria, fungi and many putative unknown internal/external factors are determinants in these relationships (Young et al. 1980, Killick-Kendrick and Killick-Kendrick 1987, Kamhawi 2006, Bates 2007, Bates 2008, Oshaghi et al. 2009, Weiss and Aksoy 2011). As major contestant, microbiota and *Leishmania* parasites interactions make it possible to introduce a sand fly as a vector or non-vector rather than other intrinsic and extrinsic criteria. Although midgut bacteria are increasingly seen as an important factor determining vector competence in mosquitoes and there are some recently examples against or assist this point (Mourya et al. 2002, Xi et al. 2008, Meister et al. 2005, Cirimotich et al. 2011, Rodrigues et al. 2011). Recent studies have shown the capacity of endogenous bacteria to decrease viral and parasitic infections in mosquito and tsetse fly vectors by activating their immune responses or directly inhibiting pathogen development (Cirimotich et al. 2011). This could be happen in the sand fly gut and this in turn may lead to a reduction in *Leishmania* infection within the sand fly host.

Conclusion

Midgut microbiota are increasingly seen as an important factor for modulating vector competence in insect vectors so the possible effects of the mirobiota on the biology of *P. papatasi* and their roles in the sandfly-*Leishmania* interaction were discussed. However a more in depth research on the interactions between sandfly and their midgut residing bacteria and *Leishmania* is required.

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