Original Article

Aerobic Bacterial Community of American Cockroach *Periplaneta americana*, a Step toward Finding Suitable Paratransgenesis Candidates

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Abstract

Background: Cockroaches mechanically spread pathogenic agents, however, little is known about their gut microbiota. Identification of midgut microbial community helps targeting novel biological control strategies such as paratransgenesis. Here the bacterial microbiota of *Periplaneta americana* midgut, were identified and evaluated for finding proper paratransgenesis candidate.

Methods: Midgut of specimens were dissected and cultivated in different media. The bacterial isolates were then identified using the phenotypic and 16S-rRNA sequencing methods.

Results: The analytical profile index (API) kit showed presence of 11 bacterial species including: *Escherichia coli*, *Shigella flexineri*, *Citrobacter freundii*, *E. vulneris*, *Enterobacter cloacae*, *Yersinia pseudotuberculosis*, *Y. intermedia*, *Leclericia adecarboxylata*, *Klebsiella oxytoca*, *K. planticola*, and *Rahnella aquatilis* in the cockroach midguts. The first three species are potentially symbiotic whereas others are transient. The conventional plating method revealed presence of only four isolates of *Salmonella*, *E. coli*, and *Proteus* which in three cases mismatched with API and 16S-rRNA genotyping. The API correctly identified the four isolates as *Shigella flexneri*, *Citrobacter freundii*, and *E. coli* (n= 2). 16S-rRNA sequence analysis confirmed the API results; however the *C. freundii* sequence was identical with *C. murliniae* indicating lack of genetic variation in the gene between these two closely related species.

Conclusion: A low number of potentially symbiotic bacteria were found in the American cockroach midguts. Among them *Enterobacter cloacae* is a potential candidate for paratransgenesis approach whereas other bacteria are pathogens and are not useful for the approach. Data analysis showed that identification levels increase from the conventional to API and to genotyping respectively.

Keywords: *Periplaneta americana*, Midgut bacteria, *Enterobacter cloacae*, 16s rRNA, Analytical profile index (API) kit

Introduction

Cockroaches are one of the most important insects in medicine. They inhabit in the dirty environment such as home sewage and could contaminate human foods with pathogenic agents. They can spread germs mechanically as they move freely from areas that may harbor pathogenic organisms: for example, from sewers to food or food preparation surfaces. A number of cockroaches have become pests and live in or around homes where they are omnivorous scavengers. The two most significant pest cockroaches worldwide are the German cockroach *Blattella germanica* and the American

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cockroach *Periplaneta americana*. German cockroach is more common inside homes particularly in kitchens while the later is found around the home close to water pipes and drainage systems (Bell et al. 2007, Hashemi-Aghdam and Oshaghi 2014). The presence of the American cockroach, in human dwellings causes damage and distress worldwide. Cockroach body has bad smell secretions, which are one of the sources of allergens responsible for asthma (Arruda et al. 2001, Litonjua et al. 2001, Arlian 2002).

A numerous pathogens counting 32 species of bacteria (including *Salmonella* and *Shigella* species), 15 species of fungi and moulds, 7 helminths (intestinal parasites), 2 protozoans, and 1 virus which are all harmful to humans being found in or on cockroaches or in the faeces (Mille and Peters 2004, Pai et al. 2005, Zarchi and Vatani 2009, Allotey et al. 2009). Intestinal microbial communities of some omnivorous cockroaches such as *P. americana*, and *B. orientalis* (Blattidae), *B. germanica* (Ectobiidae), and *Eublaberus posticus* (Blaberidae) have been examined using cultivation-based studies (Burgess et al. 1973, Cruden and Markovetz 1984, 1987, Tachbele et al. 2006, Vahabi et al. 2007, Akbari et al 2014). However, a comprehensive analysis of the midgut microbiota of the American cockroach is so far lacking. The only cockroach whose gut microbiota has been characterized with cultivation-independent molecular methods is Turkistan cockroach *Shelfordella lateralis* (Schauer et al. 2012).

Traditionally, control of cockroaches relies on application of different classes of insecticides which is often associated with environmental toxicity, adverse effects on human health and the emergence of insect resistance (Limoee et al. 2006, Enayati and Motevalli-Haghi 2007). A new control strategy named paratransgenic, symbiotic or commensally microbes of host insects are transformed to express gene products that reduce fitness of insect or interfere with pathogen transmission (Hurwitz et al. 2011, Chavshin et al. 2012, Wang and Jacobs-Lorena 2013). These genetically modified microbes are reintroduced back to the insect where expression of the engineered molecules decreases the host's fitness or ability to transmit the pathogen. Recently, Jiang et al. (2007) successfully utilized this strategy to develop an engineered densovirus (PfDNV) to control the smoky-brown cockroach (*Periplaneta fuliginosa*) nymphs.

Identification of a causative pathogen is essential for the choice of treatment as well as evaluation of the presence of virulence factors and antibiotic resistance determinants for most infectious diseases. Methods for the identification and discrimination of bacterial isolates can be separated into traditional and molecular groups (Nazarowec-White and Farber 1999). The traditional microbiological techniques (phenotyping) are based on secondary characteristics of bacteria including staining, cultures, biochemical reactions, antibiograms, serotyping, and bacteriophage typing. Phenotypic methodologies still play a significant role in identifying, verifying, and providing antibiotic susceptibility testing for many microbial pathogens. However, the application of molecular techniques to microbiology has led to the development of new and rapid methods for the detection, identification and characterization of many microorganisms including bacteria. These applications are stepwise replacing or complementing phenotypic assays in microbiology laboratories (Weile and Knabbe 2009). The most common molecular typing methods used in microbiology include chromosomal DNA restriction analysis, plasmid typing, ribotyping, pulsed-field gel electrophoresis (PFGE) and PCR-based methods such as randomly amplified polymorphic DNA (RAPD) typing, restriction fragment length polymorphisms (PCR-RFLP), and 16S-rRNA sequencing (Eisenstein 1990, Grant and Kroll

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1993, Maslow et al. 1993, Farber 1996, Krishna and Cunnion 2012, Chavshin et al. 2012). Other molecular techniques such as whole or partial genome sequencing, real-time PCR, microarrays are also being used (Weile and Knabbe 2009, Krishna and Cunnion 2012).

The rDNA gene sequences are highly conserved within living organisms of the same species, but that they differ between organisms of other species. 16S-rRNA is commonly used for taxonomic studies in bacteria because it is present in almost all bacteria, its function over time has not changed, and is large enough for informatics purposes (Barney et al. 2001, Harmsen and Karch 2004, Janda and Abbott 2007, Woo et al. 2008).

In this study we analyzed the bacterial flora of American cockroach midgut and evaluated their possible usefulness as proper candidate for paratransgenesis approach. To identify the potential symbiont bacteria two routinely used commercial phenotypic methods namely the traditional methods (including staining, cultures, and biochemical reactions) and analytical profile index (API) biochemical fingerprinting kit compared to genotyping (16S-rRNA sequencing) as a reference method.

Materials and Methods

Isolation and purification of cockroaches' midgut bacteria

Adult American cockroach specimens were collected alive from underground of households and confectionary premises in center of Tehran, Iran. Specimens were trapped using small box or food-baited traps and transferred to the laboratory of School of Public Health, Tehran University of Medical Sciences (SPH-TUMS), Iran. The specimens were kept in freezer about 5 min until they became immobilized. Before dissection, the specimens were surface sterilized for 2 min in 70% ethanol. Bacterial isolation was conducted in a sterile environment on a sterile Petri-dish. The legs were then removed, and the alimentary canal was exposed by making a ventral incision extending from the terminal sternum to the prothorax. The intact midgut from each specimen was dissected and transferred to sterile tubes separately. To separate transient bacteria from potential symbiotic bacteria, the midgut specimens were divided randomly into three following groups before homogenization: 1) intact midgut with its contents 2) removing midgut contents without washing, and 3) removing midgut contents with three times washing by PBS and distilled water.

Phenotypic Identification

Traditional method

The midguts were homogenized with glass pestles in 200 µl PBS buffer. Homogenized midguts from *P. americana* adults were poured in a 1.7 ml micro tube containing BHI broth and incubated at 37 °C for 24 h. The media was serially diluted in sterile PBS, and 0.1 to 0.5 ml of each dilution was spread on plates of four different media including Brain Heart Infusion broth (BHI broth), BHI agar, MC Conkey agar and Blood agar (Merck, Germany) media. Spread plates were placed in incubator until colonies developed (24 h). The colonies with different phenotype were selected for further characterization and were streaked for purification onto fresh plates of the same medium from which they had been picked. Colonies grown on the media were purified by several sub-cultures and then were stored at 4 ºC for further analysis. A test tube containing BHI agar open near the dissection area constituted our sterility control during the dissection process. Besides, we used a laboratory strain of *Escherichia coli* as reference bacterium in the study.

Characteristics of the bacteria were evaluated by routine microbiological methods. Cell morphology such as size, pigment, smoothness of colony's surface, having reg-

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ular or irregular edges and hemolysis was observed by photonic microscopy after Gram staining. Identification of bacteria to genus level was performed by using Biochemical tests (motility, utilization of citrate, indole formation, lysine degradation, SH2 production and fermentation carbohydrate) according to methods described by Sneath (1984). Results of biochemical tests were compared with the test results with dichotomous keys.

API biochemical fingerprinting kit

This phenotypic identification was carried out using the API test kit of DS-DIF-ENTRO-24 (Microgene) which is commercially available in Iran as described by Maleki-Ravasan et al. (2013). Prior to testing, isolates were fresh cultured overnight at 37 °C on blood agar. Suspension was prepared from the colonies with the turbidity equivalent to 2 McFarland and injected to the wells, incubated for 24 h at 37 °C and reagents was added. Positive and negative results using created color changes were determined based on API kit protocol. Tests were carried out according to manufacturers' instructions and results were interpreted using the appropriate laboratory computer software or reference indices recommended by the manufacturer. Bacteria can be identified to species level and sometimes to subspecies level.

Genotypic identification

Genomic DNA from fresh colonies incubated overnight in liquid cultures in nutrient broth was extracted using boiling method. For genotyping, a 1500bp of 16s rRNA gene was amplified with specific primers according to Weisburg et al. (1991) protocol. Amplification was carried out on the isolates with the following PCR cycling conditions: an initial denaturation at 94 °C for 10 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 56.5 °C for 40 s, and extension at 72 °C for 40 s, and final extension at 72 °C for 10 min (Karimian et al. 2011).

DNA sequencing

The PCR products of the isolates were sequenced by Seqlab, Germany using the amplification primers. Consensus sequences obtained from forward and reverse sequences and their homologies with the available sequence data in GenBank were tested by using the basic local alignment search tool (BLASTn) alignment program and the NCBI nucleotide database NCBI (www.ncbi.nlm.nih.gov/BLAST).

Results

Phenotypic identification

Totally 45 adult American cockroaches were collected from the premises during the May-August 2011. The mid-guts of the specimens were homogenized individually and cultured in different media as described in M and M section. Totally 20 purified bacterial colonies in different media cultures were isolated from the midgut of the adult American cockroaches.

These colonies were identified as *Escherichia coli* (n= 11), *Salmonella* (n= 7), and *Proteus* (n= 2) using the traditional methods. The classical method also identified the *E. coli* reference strain correctly. Details of biochemical characters used for classical identification of the isolates are shown in Table 1. *Escherichia coli* and *Salmonella* were the most prevalent isolates. When the isolates were tested using API kit, the number of genus and species greatly raised and the specimens were categorized in eight genera of *Escherichia*, *Citrobacter*, *Shigella*, *Yersinia*, *Klebsiella*, *Rahnella*, *Enterobacter*, and *Leclercia* (Table 2). The API biochemical analysis revealed presence of 11 bacterial species in the midgut of the American cockroaches, however, the bacterial community

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was varied in the number and composition when the midgut tested with or without its contents and or before and after washing with PBS. The number of species was seven, five, and three when the midgut tested with its contents, without its contents and no washing, and without its contents plus washing with PBS respectively (Table 2). *Escherichia coli*, *Shigella flexneri*, and *Citrobacter freundii* were present before and after washing the midgut contents. The isolates that were present in the midgut after washing seem to be potentially symbiotic since the transient bacteria normally would wash out and the remaining presumably are symbiotic.

Genotypic identification and its congruence with phenotypic

As previously indicated the classical bacteriological method identified the midgut bacteria as *Salmonella*, *Proteus* and *E. coli*. The classical method also identified the *E. coli* reference strain correctly (Table 1). We selected a single colony of *Proteus*, *Salmonella* (positive to citrate), *Salmonella* (negative to citrate), and *E. coli* for further API biochemical fingerprinting and genotyping.

The API kit using 24 biochemical tests (Table 3) identified the four isolates as *S. flexneri*, *E. coli* (n= 2), *C. freundii*, and the *E. coli* reference strain. This result was not in accordance with the traditional method in three out of four cases. The two *Salmonella* isolates were identified as *S. flexneri* and *C. freundii*, and the *Proteus* isolate identified as *E. coli* (Table 4). Then the four isolates plus the reference strain of *E. coli* were analyzed by genotyping using 16S rRNA PCR-direct sequencing. DNA extractions from the isolates contained enough bacterial DNA for PCR amplification. All samples except negative control produced visible PCR products of about 1500 bp whereas the negative controls were blank. The four isolates that were biochemically (API kit) identified as *S. flexneri*, *C. freundii* and *E. coli* (n= 2) respectively, were identified accordingly as *S. flexneri*, *C. murliniae/ C. freundii*, and *E. coli* (n= 2) based on partial sequences of 16srRNA (Table 4). Their sequences with length of 766, 766, 764, and 768 bp were deposited in GenBank with accession numbers of KC 017349, KC017346, KC017348, and KC017 347 respectively. The correspondences of the phenotypic and genotypic methods as well as details of the homologous species and their homology percentage of the species identified in this study with the ones available in GenBank are shown in Table 4.

Table 1. Details of biochemical characters used for classical identification of bacteria in the American cockroach midgut

Species	Biochemical characters					
	SIM		KIA and gas production	Lysine	H2S	Citrate
	Indole	Motility				
Salmonella	+	-	R/Y +	-	+	+
E. coli	+	+	Y/Y +	+	-	-
Proteus	+	-	R/Y +	-	-	-
Salmonella	+	-	R/Y +	+	+	-
E. coli	+	+	Y/Y +	+	-	-
Reference strain						

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Table 2. AIP Phenotypic identification of bacterial community of American cockroach midgut before and after washing and removing contents

	Intact	Removing contents without Washing	Removing contents with three times PBS Washing
Bacterial species	*Escherichia coli* *Shigella flexineri* *Citrobacter freundii* *Yersinia pseudotuberculosis* *Yersinia intermedia* *Klebsiella oxytoca* *Leclericia adecarboxylata*	*Escherichia coli* *Eischercia vulneris* *Klebsiella planticola* *Enterobacter cloacae* *Rahnella aquatilis*	*Escherichia coli* *Shigella flexineri* *Citrobacter freundii*

Table 3. Details of reaction tests used in API kit for species identification of four bacteria isolated from midgut of the American cockroaches

No.	Test reaction	Results of reaction			
1	Indole	-	-	+	+
2	VP	-	+	+	+
3	Citrate	-	+	-	-
4	SH2	-	-	-	-
5	Urease	+	+	+	+
6	Phenylalanine	+	-	-	-
7	Laysine	-	-	+	+
8	Argentine	-	-	+	+
9	Ornitine	-	-	-	-
10	Malonate	-	-	+	+
11	Glocuse	-	-	-	-
12	Adonitole	-	-	-	-
13	Arabinose	-	-	-	-
14	Dolicitole	+	+	-	-
15	Inositole	-	-	-	-
16	Lactose	+	+	+	+
17	Maltose	+	+	-	-
18	Mannitol	+	+	+	+
19	Ramnose	+	+	+	+
20	Sucrose	-	-	-	-
21	Surbitole	+	+	+	+
22	Trehalose	+	+	+	+
23	Eskoline	-	-	+	+
24	ONPG	-	-	+	+
Identified species		Shigella flexneri	Citrobacter freundii	E.coli	E.coli

Table 4. Details of congruence between phenotypic and genotypic methods in diagnosis of bacteria isolated from the American cockroach midgut. AN: Accession number

Classic	API	16S rRNA sequencing (A.N in GenBank)	Homolog species (A.N in GenBank)	Homology Rate %
Salmonella	*Shigella flexneri*	*S. flexneri* (KC017349)	*S. flexneri* (HQ701686)	100
			S. flexneri (CP001383)	100
			S. flexneri (CP001386)	100
Proteus	*Escherichia coli*	*E. coli* (KC017348)	*E. coli* (CP003034)	99
			E. coli (JN578644)	99
			S. sonnei (NR074894)	99
E. coli	*E. coli*	*E. coli* (KC017347)	*E. coli* (JQ609683)	100
			E. coli (CP001925)	100
			E. coli (CP002967)	100
			E. coli (CP002970)	100
			E. coli (CP002291)	100
Salmonella	*Citrobacter freundii*	*C. murliniae* (KC017346)	*C. murliniae* (JN092600)	100
			C. freundii (JX860618)	100

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Discussion

The results of this study using API kit revealed that the intact midgut of *P. americana* harbors fairly a diverse community of gram negative aerobic bacteria of Enterobacteriaceae. However, most of these bacteria were transient and acquired from the environment and or the food sources the cockroaches live and feed on as previously described by Kane and Breznak (1991). Therefore the non-transient bacteria comprise small part of the midgut community. The low number of non-transient (possibly symbiotic) microbial community of the American cockroaches found in this study concord to earlier perceptions of the cockroach midgut microbiota, which were based on cultivation-based studies that yielded mostly isolates of the genera *Enterobacter*, *Klebsiella*, and *Citrobacter* (Cruden and Markovetz 1987). It is worth mentioning that since the bacterial community obtained by culture media is limited by the selectivity of the media employed, the species (number and diversity) estimated in this study are not definitely estimates of the total real community. Initial using of nonselective medium (BHI broth) to promote growth of bacteria generally favored the growth of gram negative Enterobacteriaceae in the media. However, it is the case for almost all of the studies analyzing the insect gut bacterial communities (Hillesland et al. 2008, Mukhopadhyay et al. 2012, Chavshin 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.

The microbial community of different compartments of intestinal tract of cockroaches including crop, midgut, rectum and colon of *P. americana* and *Shelfordella lateralis* (Turkistan cockroach) previously have been compared using culture media or molecular tools (Bignell 1977, Bracke et al. 1979, Schauer et al. 2012). These studies showed that each compartment harbor diverse community of bacteria, where the anterior colon of the cockroaches contained the highest abundance of microorganisms. The diversity in different compartments of cockroach gut is related to the microbial activities, such as the accumulation of hydrogen and the other microbial products, and the physiochemical characteristics of each part of the gut, such as pH and redox potential (Schauer et al. 2012 and references herein). Previous studies of cockroaches have reported a decrease in redox potential along the gut, with oxidizing conditions in crop and midgut and reducing conditions in the hindgut. The low redox potential in the hindgut lumen is consistent with the accumulation of hydrogen and the presence of a large and diverse community of Clostridiales (Bignell 1977, Vinokurov et al. 2007, Schauer et al. 2012). Besides, it is shown that in *P. americana,* the foregut is a site of considerable lactate production owing to the abundance of lactic acid bacteria (Kane and Breznak 1991). In this study we focused only on the midgut of the American cockroach because the midgut has an endodermic origin which does not destroy in molting (ecdysis) and hence its microbial community remains intact or is less prone to diminish during multiple molting of cockroach life span. This fact is important for selection of a

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proper candidate bacterium for paratransgenesis approach.

In the present study *Enterobacter cloacae* was found among the transient bacteria after removing midgut content but did not remained after washing with PBS. However, this bacterium could be a potential candidate for paratransgesis approach because it is found as the normal gut flora of many humans and is not usually a primary pathogen (Keller et al. 1998). *Enterobacter cloacae* have already been used for paratransgenesis and successfully could deliver, express, and spread foreign genes in termite colonies (Husseneder and Grace 2005) and sand fly *Phlebotomus papatasi* (Maleki-Ravasan et al. 2014). This species was genetically transformed with Defensin (small cysteine-rich cationic proteins found in both vertebrates and invertebrates) to reduce *Leishmania* parasites in vitro conditions (Maleki-Ravasan et al. 2014). *Enterobacter cloacae* also has been transformed with an ice nucleation gene to reduce the mulberry pyralid moth, *Glyphodes pyloalis* (Watanabe et al. 2000). These documents show that the species is amenable for transformation with foreign genes. In this study we also found that *E. coli* is possibly a symbiotic bacterium in the midgut of American cockroaches. This bacterium was genetically manipulated and tested for paratransgenic approach (Riehle et al. 2007, Chavshin et al. 2013). However *E. coli*, and other two potential symbiotic bacteria *S. flexneri* and *C. flexineri* are not appropriate candidate for paratransgenesis approach due to their pathogenic effect that can cause diarrhea or meningitis in humans (Badger et al. 1999, Niyogi 2005). Further investigation need to test the utility of *E. cloacae* or to find another appropriate symbiont for genetic manipulation and delivering effector molecules to control and diminish cockroach pest populations.

The results of this study showed the importance of choosing the correct identification method for exact speciation of bacterial species. Correct identification impacts directly on treatment outcomes and on the epidemiological analysis of emerging bacterial infections in arthropod borne diseases. The present study revealed that 75% of classical test systems including staining, cultures, and biochemical reactions, yielded wrong speciation results when compared to API kit and genotyping (Table 3). For example, classical method identified two isolates as *Salmonella*, however these two isolates were subsequently identified by API and genotyping as *S. flexneri* and *C. murliniae* which could mislead the diagnostician and subsequent treatment methods. On the other hand, species classification based on phenotypic features is often time-consuming and is not always easy to carry out (Springer et al. 1995, Nagy et al. 2006, Erme et al. 2009). The classical methods cannot detect the heterogeneity in species, are not reproducible, and challenge with limited database for phenotypic characteristics for common species. Common strains are easily identified with charts or keys but when we are facing to rare or intermediate strains, identification is difficult with these tools (Barkeley et al. 1984). Inability of classical method in recognition of some bacterial species is result of phenotypic variation, phenotypic homogeneity without enough differential characteristics and tendency of traditional method toward established taxa. Also some species are complex (phenospecies) and have more than one DNA group then classic method cannot separate them phenotypically (Janda and Abbot 2007). Besides, some bacteria particularly anaerobic ones are extremely slow growing or not cultivatable at all. One of the arguments for using classical tests is that they are less costly than API and genotyping, however, the potential penalty of misidentification must be considered.

On general there was a great congruence among the results of API and 16S rRNA

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genotyping: both methods identified the four selected isolates as *S. flexneri*, *E. coli* (n= 2), and *C. freundii*. However, the 16S rRNA sequencing revealed possible mismatching of *C. freundii* with *C. murliniae* because both species had identical sequences in the region sequenced. Studies of Geraghty et al. (2013) on the efficacy of routinely used phenotypic methods compared to genotypic approaches for the identification of staphylococcal species showed that the API Staph 32 kit correctly identified all *S. aureus* isolates (11/11), but 83% (10/12) of the SIG species, and 66% (19/29) of the coagulase negative species. They concluded that although the API Staph 32 test performed with the highest degree of accuracy for the coagulase positive Staphylococci, however they are inadequate for the correct identification of both coagulase negative and coagulase positive staphylococcal species.

The API kits are produced for the human diagnostics market and are interpreted against databases with reference strains of human origin. Therefore it is suggested that the reproducibility and reliability of these kits are uncertain when applied to none human origins. The API test is based on the evaluation of expression of genetically encoded characteristics and erroneous identification may be due to variable expression of biochemical traits within species (Blaiotta et al. 2010). Other disadvantages of API kits included long incubation period (18 h), lack of the required turbidity in suspension produced, bubbles in kit's well, incorrect incubation period which leads to error while reading the results of the color changes. Also it is necessary that the databases identification kits updated by their manufactures to include new genus and species (Laclaire and Facklam 2000). However, the biochemical API kit is less expensive than the molecular method and is more suits for most small clinical laboratories where the implementation of advanced molecular techniques is not feasible owing to the high cost of instrumentation and reagents.

Nowadays, DNA based methods, particularly PCR techniques, for the detection and characterization of microorganisms have revolutionized diagnostic microbiology and are now part of routine specimen processing. These methods have now progressed beyond identification to detect antimicrobial resistance genes and provide public health information such as strain characterization by genotyping (Mellmann et al. 2003, Woo et al. 2003, 2008, Cloud et al. 2004, Speers 2006, Sibley et al. 2012). Literature show that 16S rDNA sequencing is essential and key tool for bacterial identification, particularly important in the case of bacteria with unusual phenotypic profiles, rare bacteria, slow growing bacteria, uncultivable bacteria and culture-negative infections (Clarridge 2004, Woo et al. 2008). There are many conserved primers to amplify a region of a gene, such as the 16S rRNA bacterial gene, and the amplified product is usually sequenced and compared to about 4 million 16s RNA sequences from different bacteria in Internet databases such as GenBank (www.ncbi. nlm. nih.gov/Genbank), EMBL Data Library (www. ebi.ac.uk/embl), and the DNA Data Bank of Japan (www.ddbj.nig.ac.jp) with daily data exchange between them, and more specialized high quality databases such as RIDOM (www.ridom-rdna.de/) for bacterial rDNA sequences used for mycobacterial speciation (Speers 2006). In spite of above mentioned advantages, experience showed that 16s rRNA gene sequences is able to identify most cases (>90%) at genus level but in species level decrease to 65 to 83% and 1 to 14 % of isolates remains unidentified (Mignard and Flandrois 2006). Some other limitations are 1) identifying of new taxa is impossible, 2) limited number of sequences or partial sequences existence in the nucleotide database, 3) similarity of 16s rRNA gene sequence in some species, 4) error in nomenclature species

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and 5) use of sequences with gap to comparison (Hall et al. 2003, Bosshard et al. 2006).

Conclusion

This study suggests that midgut of American cockroaches harbor few symbiotic bacterial community at least in case of aerobic gram negative species. Except for *E. cloacae*, the other symbiotic isolates are not proper candidate for paratransgenesis and further studies need to find other proper candidates. Secondly traditional methods are not proper to identifying the genus and species level but 16S rRNA sequencing is the best method for identification at species level, although the API kit is a reasonably reliable phenotypic alternative.

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