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Research Paper

SYMBIONT BACTERIA IN USHERHOPPER, *Poekilocerus bufonius* (ORTHOPTERA: PYRGOMORPHIDAE) GUT

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Abstract

The microbiota within insect guts play important roles such as the prevention of resident bacteria and protect the insect from gut infection by fungal and bacterial pathogens. In this study, we determined the gut bacterial of usherhopper, *P. bufonius* which inhabits in Western region of Saudi Arabia throughout analysis of partial sequence of 16S ribosomal DNA. Sequencing analysis indicated that the intestinal symbiotic bacteria of *P. bufonius* had four types of phylotypes included *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella* sp. and *Streptococcus* sp. It could be to suggest that further investigations are required for detection of all symbiont bacteria in the usherhopper, *P. bufonius* through other molecular techniques such as denaturing gradient gel electrophoresis (DGGE).

Key words: Insect, Gut bacteria content, Symbiosis, Plant hopper,16S.

INTRODUCTION

Bacterial mutualists in insect guts play an important role in regulating the host's metabolism and promote efficient digestion for extraction of maximum energy from ingested foods (Kaufman and Klug, 1991). The microbiota within insect guts play important roles by engaging in beneficial and pathological interactions with these hosts (Yun et al., 2014). Also, Bacteria in the digestive tract of *Locusta migratoriodes* convert lignin to locustol (5-ethyl guanol), a pheromone involved in aggregation (Dillon and Dillon 2004). Most important functional role of gut microbiota in animals is the prevention of Resident bacteria and protect the locust from gut infection by fungal and bacterial pathogens. Production of a potent combination of plant derived selectively antimicrobial phenolic compounds (3,4 dihydroxybenzoic acid and others) Gut bacterial population of locusts appears to be derived from food plants. Diversity of bacteria in the gut is limited compared to some other insects (Dillon and Charnley, 2002).

The microbiota of the insect gut has been analyzed using both culture-dependent (Tholen et al., 1997; Arias-Cordero et al., 2012) and culture-independent (Chandler et al., 2011; Toju and Fukatsu, 2011; Wong et al., 2011) methods. Culture-independent molecular ecological approaches based on analysis of the 16S rRNA gene have yielded a better and more comprehensive picture of bacterial communities and have resulted in a dramatic improvement in our understanding of the microbes living within the guts of insects (Jones et al., 2013).

Yun et al. (2014) had 174374 sequence reads from 21 taxonomic orders, identifying 9301 bacterial operational taxonomic units (OTUs) at the 3% distance level from all samples, with an average of 84.3 OTUs per sample. The insect gut microbiota were dominated by *Proteobacteria* (62.1% of the total reads, including 14.1% *Wolbachia* sequences) and *Firmicutes* (20.7%). Ademolu and Idowu (2011) studied the alimentary canal of *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae) and reported that this insect gut is harbor a variety of microorganisms, mainly bacteria, fungi, and molds. The transmission of gut microbes can be either by (1) vertical transmission, that is, from mother to egg, or (2) horizontal, that is, uptake by the host via a food source.

The polyphagous Usherhopper, *Poekilocerus bufonius* (Orthoptera: Pyrgomorphidae) was recorded in different regions in Saudi Arabia with high presence in western region (Elsayed and Sayed, 2014 and Sayed, 2016). A comprehensive understanding of the biology of insects requires that they be studied in ecological context with microorganisms as an important component of the system (Steinhaus, 1960). Thus, the present study aimed to determine the gut bacterial of usherhopper, *P. bufonius* which inhabits in Western region of Saudi Arabia.

MATERIAL AND METHODS

Usherhopper collection

Twenty usherhopper female adults were collected from four different locations (five individuals from each location) at Taif Governorate, Saudi Arabia durng April, 2016. The insects were kept in plastic cages (2 Liters volume) and covered with tulle. They were starved for 24 hours to empty any food residue from the gut and then they were dissected.

Insect dissection

Before dissection, each insect was surface-sterilized by 70% ethanol. The body cavity was opened by a ventral longitudinal cut which exposed the alimentary canal system. The gut was separated from adjoining tissues like fat bodies and malpighian tubules. The dissected tissues were cut into small pieces, homogenized with liquid nitrogen, dissolved in high purity water at each gut/1 mL. Then, all twenty guts were mixed in one falcon tube (25 mL)and stored at 4°C until use.

Inoculation of bacteria

One mL of the previous mixture was serial diluted Six-fold. Aliquots of 1 mL of 4-6 fold dilutions were plated in duplicate by a pour-plate technique using the nutrient agar media and incubated at 37°C for 48 h. After 48 h, the colony forming units were determined by visual counting. Purified colonies were grouped according to their colony morphology. Then, Ten colonies were selected according to visual difference. Each one of these selected colonies were inoculated in I mL liquid nutrient agar media at 37°C for 48 h and centrifugated at 12000 rpm for 1 min. Each pellet was suspended in 0.5 mL autoclaved didistilled water. These suspensions were used as templates for DNA extraction.

DNA extraction from bacteria

DNA was extracted from 0.5 mL bacterial culture for each colony with QIAGEN spin-column kits according to the manufacturer's instruction (QIAamp DNA Micro Kit). Extracted DNA concentration and quality were determined spectrophotmetrically at 260/280 nm and was used for PCR.

Polymerase Chain Reaction (PCR) and sequencing.

PCR was conducted to amplify about 800 bp of 16S gene. PCR was conducted in a final volume of 50 µL containing 2 µL DNA template and 2 µL of 10 picomolar forward primer (27F: 5' AGAGTTTGATCMTGGCTCAG 3'), 2 µL of 10 picomolar reverse primer (805R: 5' GACTACCAGGGTATCTAATC 3') of and 25 µL PCR master mix (Promega Corporation, Madison, WI) and 19 µL autoclaved deionized distilled water. PCR was carried out using a PeX 0.5 thermal Cycler with the cycle sequence at 95°C for 7 min one cycle, followed by 35 cycles each of which consisted of denaturation at 95°C for one min, annealing at 51°C for one min and extension at 72°C for one min with a final strand elongation for one cycle at 72°C was done for an additional 10 min. The PCR products were analyzed in 1% agarose gel electrophoresis in TAE buffer with ethidium bromide staining. 100bp DNA ladder (Biolabs) was used as a molecular marker. Then PCR products bands were visualized under UV light and photographed. The PCR products were then excised from agarose gels and purified using spin column (BioFlux, Tokyo, Japan) according to the manufacturer's instructions and sequenced in Macrogen (Korea) using the same primers that used in PCR reaction. Consequently, the sequenced results were inserted in BLASTN programs search nucleotide databases using a nucleotide query, NCBI.

RESULTS AND DISCUSSION

The present study shows that bacteria are present in the gut of *P. bufonius*, and as a phytophagous insect, it has associations with microorganisms and thus it possesses an open system that is suitable for different kind of organisms (Campbell, 1990). Generally, the guts of insects offer many such niches where the insect is able to take advantage of the products of bacterial metabolism and the adaptability of prokaryotes (Dillon and Dillon 2004).

PCR products sequencing of partial 16S for four *Poekilocerus bufonius* gut symbiont bacteria indicated that the products length ranged from 795 to 805 bp. (Table 1). BLAST sequencing analysis determined that the intestinal symbiotic bacteria of *P. bufonius* had four types of phylotypes (Table 1). These phylotypes included *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella* sp. and *Streptococcus* sp. This result is coincides with the finding of Ademolu and Idowu (2011). They detected these four species of bacteria in the gut of *Zonocerus variegates* which is from the same family of *P. bufonius*.

The nucleotide sequences in the current study were deposited in the GenBank (Table 1) under Accession numbers of (KY630524 for *Bacillus subtilis*, KY630525 for *Staphylococcus aureus*, KY630526 for *Klebsiella* sp. and KY630527 for *Streptococcus* sp.)

Some of these species of bacteria those detected in the present study such as *Klebsiella* spp. and *Bacillus* spp. produce the volatile alkyl disulfides present in the fecal pellets of the leek moth, *Acrolepiopsis assectella* which serve as kairomones to attract the parasitoid *Diadromus pulchellus* to the moth host (Thibout et al., 1995). Generally, comprehensive analysis of the bacterial diversity within a host species is a prerequisite in both insect physiology and microbial ecology to allow a better understanding of the ecological roles of insect symbionts and interactions with their insect hosts (Yun et al.,

2014). Moreover, Increasing the diversity of gut bacteria increases protection against pathogens (Dillon and Charnley, 2002).

In conclusion, The Usherhopper, *P. bufonius* used in this study is feeding on usher plant, *Calotropis procera* which contains secondary plant compounds. Moreover, microbial products play subtle roles in the life of the insect, being involved in the digestion of refractory food and detoxification of secondary plant compounds (Dillon and Dillon 2004). Moreover, This insect is able to digest cellulose. The grasshopper exhibited high intestinal cellulolytic activity and contained diverse types of microorganisms that were able to secrete cellulase. However, further investigation are required for detection of all symbiont bacteria in the usherhopper, *P. bufonius* through other molecular techniques such as denaturing gradient gel electrophoresis (DGGE).

Table (1): Length of PCR products resulted from four *Poekilocerus bufonius* gut symbiont bacteria and their accession numbers.

Number	PCR Product length (bp)	Accession numbers
1	805	KY630524
2	805	KY630525
3	795	KY630526
4	801	KY630527

Table (2): BLAST sequencing analysis of *Poekilocerus bufonius* gut symbiont bacteria.

Number	Most similar strain in GenBank (accession No.)	Identities (%)
1	*Bacillus subtilis* strain CA105 (KT996128)	99
2	*Staphylococcus aureus* strain C102 (KU198419)	100
3	*Klebsiella* sp. F51-1-2 (DQ277701)	99
4	*Streptococcus* sp. clone GI-10-M15 (GQ130105)	99

REFERENCES

Ademolu, K. O. and Idowu, A. B. (2011). Occurrence and Distribution of Microflora in the Gut Regions of the Variegated Grasshopper *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae) during Development. Zoological Studies. 50(4): 409-415.

Arias-Cordero, E.; Ping, L.; Reichwald, K.; Delb, H.; Platzer, M. and Boland, W. (2012). Comparative evaluation of the gut microbiota associated with the below- and above-ground life stages (larvae and beetles) of the forest cockchafer, *Melolontha hippocastani*. PLoS One 7:e51557.

Campbell, B. C. (1990). On the role of microbial symbiotes in herbivorous insects. *In* EA Bernays, ed. Insect-plant interactions. Boca Raton, FL: CRC Press, pp. 1-45.

Chandler, J.A.; Lang, J. M.; Bhatnagar, S.; Eisen, J. A. and Kopp, A. (2011). Bacterial communities of diverse *Drosophila* species: ecological context of a hostmicrobe model system. PLoS Genet. 7:e1002272.

Dillon, R. J. and Dillon, V. M. (2004). The gut bacteria of insects: non pathogenic interactions. Annual Review of Entomology. 49: 71-92.

Dillon, R., Charnley, K. (2002). Mutualism between the desert locust, Schistocerca gregaria and its gut microbiota. Research in Microbiology. 153: 503 – 509.

Elsayed, G. and Sayed, S. M. (2014). A First Record of *Blaesoxipha rufipes* (Diptera: Sarcophagidae) Parasitizing *Poekilocerus bufonius* (Orthoptera: Pyrgomorphidae) in Saudi Arabia. Entomological News. 124(1):33-37.

Jones, R. T.; Sanchez, L.G. and Fierer, N. (2013). A cross-taxon analysis of insect associated bacterial diversity. PLoS One 8:e61218.

Kaufman, M. G. and Klug, M. J. (1991). The contribution of hindgut bacteria to dietary carbohydrate utilization by crickets (Orthoptera, Gryllidae). Comp. Biochem. Physiol. 98**:**117–123.

Sayed, S. M. (2016). The first molecular characterization of the parasitoid, *Blaesoxipha rufipes* (Diptera: Sarcophagidae) and its diversity in western Saudi Arabia. Entomological News.126 (2): 87-96.

Steinhaus E.A. (1960). Insect pathology: Challenge, achievement, and promise. Entomological Society of America Bulletin. 6: 9-16.

Thibout, E.; Guillot, J. F.; Ferary, S.; Limouzin, P. and Auger, J. (1995). Origin and identification of bacteria which produce kairomones in the frass of *Acrolepiopsis assectella* (Lep., Hyponomeutoidea). Experientia. 51:1073–1075.

Tholen, A.; Schink, B. and Brune, A. (1997). The gut microflora of *Reticulitermes flavipes*, its relation to oxygen, and evidence for oxygen dependent acetogenesis by the most abundant *Enterococcus* sp. FEMS Microbiol. Ecol. 24:137–149.

Toju, H. and Fukatsu, T. (2011). Diversity and infection prevalence of endosymbionts in natural populations of the chestnut weevil: relevance of local climate and host plants. Molecular Ecology. 20:853–868.

Wong, C. N; Ng, P. and Douglas, A. E. (2011). Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. Environmental Microbiology. 13:1889–1900.

Yun, J. H.; Roh, S. W.; Whon, T. W.; Jung, M. J.; Kim, M. S.; Park, D. S.; Yoon, C.; Nam ,Y. D.; Kim, Y. J.; Choi, J. H.; Kim, J. Y.; Shin, N. R.; Kim, S. H.; Lee, W. J. and Bae, J.W. (2014). Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. Applied and Environmental Microbiology. 80(17):5254-5264.