Circadian changes in the hindgut bacterial composition of the American cockroaches, *Periplanata americana* (Dictyoptera, Blattodea)

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Summary: The gut of American cockroaches, *Periplanata americana* hosts numerous symbionts that help in digestion and synthesis of many substances. The hindgut of cockroaches is unique in that it harbors higher number of microbes than other parts of the insect's gut. Unlike gut enzymes activities, little is known about the daily pattern of the gut microbiota. Newly emerged adult American cockroaches collected from student hostel were used for this study. The hindgut microbial composition was examined at four periods of the day (6am, 12pm, 6pm and 12am) using Pour Plate Methods. The results showed that highest colony forming units (cfu) of the hindgut were recorded at 6:00pm (19.28×10^6 cfu/ml) followed by 6:00am (13.85×10^6 cfu/ml) while 12:00pm (3.02×10^6 cfu/ml) had the least. Also, a total of five (5) different bacteria (*Klebsiella* sp., *Staphylococcus aureus, Pseudomonas aeruginosa, Citrobacter* sp. and *Escherichia coli*) were isolated from the hindgut of the cockroaches. The most predominant of these bacterial isolates from the hindgut were *Klebsiella* sp. and *Staphylococcus aureus* while the least isolated organism was *Citrobacter* sp. Hence, there was circadian variation in the microbial composition of the hindgut of *Periplaneta americana*.

Key words: *Periplaneta americana*, circadian, hindgut, microbiota.

Introduction

The American cockroach, *Periplaneta americana* (L.) (Blattidae), is the largest of the cockroaches measuring an average 4 cm in length. Adults are reddish-brown in appearance with a pale-brown or yellow band around the edge of the pronotum. It is found mainly in basements, sewers, steam tunnels and drainage systems (Rust *et al*. 1991) making it difficult to control. *P. americana* is a voracious omnivore that feasts on almost anything such as paper, boots, hair, bread, fish, fruit, peanuts, old rice, the soft part on the inside of animal hides, dead insects and cloth, thereby causing economic loss (Bell and Adiyodi 1981).

The cockroach is divided into three sections; the body is flattened and broadly oval, with a shield-like pronotum covering its head. A protonum is a plate-like structure that covers all or part of the dorsal surface of the thorax of certain insects. They also have chewing mouth parts, long, segmented antennae, and leathery fore wings with delicate hind wings. The third section of the cockroach is the abdomen (Bell *et al*. 2007). The insect can travel quickly, often darting out of sight when a threat is perceived, and can fit into small cracks and under doors despite its fairly large size. It is considered one of the fastest running insects (Bell *et al*. 2007).

P. americana makes their ways to houses from drainages pipes via the plumbing, and from trees and shrubs located alongside buildings or with branches overhanging roofs. During the day, the *P. americana*, which response negatively to light, rests in harborages close to water pipes, sinks, baths, and toilets where the microclimate is suitable for survival (Bell and Adiyodi 1981). They feed on a variety of foods, with a preference for starchy and sugary material, also on book bindings, sizing, inner linings of shoe soles and dead insects.

The long and somewhat coiled digestive tube in *P. americana* could be divided into three regions: the foregut, the midgut and the hindgut. The occurrence of a multitude of digestive enzymes in the gut of cockroaches is consistent with their omnivory and feeding adaptability. Digestive tract of *P. americana* harbours xylanase, laminaribiase, cellobiase, maltase, sucrase, α- and β-glucosidase, α- and β-glycosidase, β-fucosidase, chitinase and N-acetyl-β-glucosaminidase that attack various polysaccharides including those in the plant and fungal cell walls (Genta *et al*. 2003). Recently, Iyeh *et al.* (2021) reported activities of lipase, proteinase, amylase and glucosidase in the gut homogenates of adult *P. americana.* Furthermore, highest enzyme activities were recorded in the female hindgut at night hour.

Out of the three gut sections of cockroaches, the hindgut has the highest bacterial density and diversity (Cruden and Markovetz 1987). This hind gut microbial community breaks down recalcitrant dietary components from food that has passed through the fore- and midgut, supplying the cockroach with volatile fatty acids such as acetate (Cruden and Markovetz 1987). Related work also suggested that the hindgut microbiota is responsible for producing

pheromones, including volatile fatty acids, which promote social behavior among cockroaches (Wada-Katsumata *et al*. 2015). While cockroach gut microbes are most closely related to microbes found in termites and other insects, they share many clades with those found in mammals, including humans (Dietrich 2014, Schauer *et al*. 2012). Despite these reported crucial roles of gut microbes to the physiology of cockroaches, little has been reported on their composition and diversity on a daily basis. The aim of this study is to examine the circadian changes in the bacterial composition of American cockroaches (*P. americana*) found in Abeokuta, Nigeria.

Materials and methods

Samples Collection

Forty newly emerged adult male American cockroaches, *P. americana*, were collected at night from female student's hostel of Federal University of Agriculture, Abeokuta Nigeria. The cockroaches were brought to the Insectary of the Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Nigeria, where they were acclimatized in perforated plastic containers for 7 days. They were maintained on bread, biscuits and water before the gut analysis.

Experimental Procedures

The forty live male American cockroaches were dissected as described by Iyeh *et al.* (2021) at different time intervals (6am, 12pm, 6pm and 12am), ten insects at each intervals. Prior to dissection, each cockroach was surface-sterilized by swabbing with iodine followed by 70% ethanol. The hindgut contents were emptied into labeled Petri dishes and using a sterile mortar and pestle, each hindgut section was homogenized in 1 ml of sterile distilled water. The homogenate was decanted into labeled bottles containing 9 ml of sterilized water; 1 ml of a sample was homogenized in 9 ml of sterile diluted water, and 6-fold serial dilutions were made. Aliquots of 1 ml of 4-6 fold dilutions were plated in duplicate by a pour-plate technique using nutrient agar (NA) for the bacteria enumeration. NA was incubated at 37°C for 48 h. After 48 h, the colony forming units (cfu) were determined by visual counting. Purified colonies were grouped according to their colony morphology and cell characteristics. Further identification was carried out by patterns of sugar fermentation (glucose, galactose, maltose and lactose). Bacterial isolates were identified using Bergey's Manual of Systematic Bacteriology (Sneath *et al*. 1986) and methods of Hugh and Leifson (1963) and Harrigan and MacCance (1970).

Statistical Analysis

Data collected on the bacterial counts of the hindgut region across the four periods of the day were analyzed by One way analysis of variance and separation of means done by Student Newmans-Kuel test.

Results

The colony forming units/ bacteria counts of the hindgut region of *P. americana* during four periods of the day are presented in Table 1. The highest count was recorded at 6:00pm followed by 6:00am, while the least was recorded by 12pm.

Table 1: The colony forming unit derived from the hindgut of *Periplaneta americana* during 24 hours.

Period of the day	Average Mean Count (cfu/ml) × 10^6
6:00 am	13.85^b
12 pm	3.02^c
6:00 pm	19.28^a
12:00 am	3.33^c

Table 2 shows the colonial characteristics of the bacterial isolates from the hindgut of *P. americana* at different times of the day. Isolates were creamy white in color, circular in shape and moist, smooth in appearance. Bacterial isolates at 12pm and 12am were predominantly circular in shape while isolates of 6am and 6pm were circular and irregular.

List of isolated bacterial species from the hindgut at different periods of the day is presented in Table 3. *Klebsiella* sp. and *Staphylococcus aureus* were isolated at all the four periods of the day followed by *Escherichia coli* which was only absence at 6:00am. *Pseudomonas aeruginosa* and *Citrobacter* sp., were isolated at only two periods of the day (6am/6pm and 12pm/6pm respectively).

Discussions

There was a circadian variation in the bacterial count of hindgut of American cockroaches, *P. americana*. Highest colony forming units (cfu) were recorded at the dark hours (6:00pm and 6:00am), while least units were recorded during day. This bacteria count pattern runs parallel to what was observed in enzyme activities of gut regions of *P. americana*. Iyeh *et al.* (2021) reported that highest gut enzymes activities were reported at night hours of 12 am and 6am. This similarity might not be unconnected with the enzymes synthesis role played by the bacteria. Bacterial isolates from cockroaches and other insects secrete enzymes that degrade linamarin and cellulose (Idowu *et al*. 2009).

Phytophagus insects acquire their gut microbiota mostly non-vertically but through their feeds (Ademolu *et al*. 2011 and Carrasco *et al*. 2014). The higher cfu at night periods suggests the nocturnal activities of the insect. American cockroaches performs most activities (feeding, mating and movement) at night (Bell *et al*. 2007), thus higher gut cfu followed the feeding exercise.

Table 2: Colonial characteristics of isolates from the hindgut of *Periplaneta americana* during 24 hours.

Period of the day	Color on media	Shape	Edge	Elevation	Opacity	Surface appearance
6:00 am	Creamy white, creamy with a yellow tinge, creamy with a green tinge and fruity odour	Circular and irregular	Entire and irregular	Raised	Translucent and opaque	Moist, smooth and glistering
12 pm	Creamy white, creamy with a yellow tinge	Circular	Entire	Raised	Some are opaque, few are translucent	Smooth, some are moist and glistering
6:00 pm	Creamy white, creamy with a yellow tinge,creamy with a green tinge and fruity odour	Many are circular, few are irregular	Entire and irregular	Raised	Opaque and translucent	Smooth, moist and glistering
12:00 am	Creamy white, large creamy, creamy with a yellow tinge	Circular	Entire	Raised	Many are opaque, few are translucent	Smooth, moist and glistering

Table 3: Bacterial isolates from the hindgut of *Periplaneta americana* during 24 hours.

Period of the day	Bacterial isolates
6:00 am	*Klebsiella spp, Pseudomonas aeruginosa, Staphylococcus aureus*
12 pm	*Klebsiella spp, Escherichia coli, Citrobacter spp, Staphylococcus aureus*
6:00 pm	*Staphylococcus aureus, Escherichia coli, Citrobacter spp , Pseudomonas aeruginosa, Klebsiella spp*
12:00 am	*Klebsiella spp, Escherichia coli, Staphylococcus aureus*

Klebsiella sp. and *S. aureus* were present in hindgut region at the four period of the day. *Klebsiella* sp. is everywhere in nature (soil, water, insects, humans) due to distinct sublineages developing specific niche adaptations with associated biochemical adaptations which makes them better suited to a particular environment. Similarly, *Klebsiella* does not have special growth requirements like other members of enterobacteriaceae (Ryan and Ray 2004).

Similar organisms were isolated from the gut regions of *Zonocerus* (Ademolu *et al*. 2011). This might be due to similar environment of the insects. In a recent study, Ademolu *et al.* (2020) observed that locations affected the gut microbial content of *P. americana*. However, *Clostridium* sp. which was commonly found in the gut regions of cockroaches (Schaver *et al*. 2012) was absent in this study. This might be due to the diet eaten by the roaches. In this study, *P. americana* was maintained on biscuits and bread, while in other study they were fed chicken feeds.

There were variations in the cfu and diversity of bacterial isolates across the four periods of the day. This might be caused by different physiological properties (pH, temperature, humidity, oxygen concentration) of the hindgut at different times of the day and the characteristics/behavior of the microbes. Habitat (gut regions) with similar conditions tends to select and support similar microbial community (Costello *et al*. 2012). Similarly, Curtis and Sloan (2004) observed that similar microbial communities were isolated when gut reservoir was low. Changes of gut microbial communities in insects affect insect physiology. Gut microbial communities are import for insect physiology, including pesticide degradation (Reeson *et al.* 2003). This present study showed that there was a circadian pattern in the microbial composition of *P. americana* which coincides with its feeding time and the need for hydrolyzing enzymes required for digestion.

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