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Bacterial communities of big-headed ants (*Pheidole rugaticeps*) and American cockroaches (*Periplaneta americana*) revealed pathogens of public health importance

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ABSTRACT

Aims: Several cockroach and ant species have been revealed to infest households with inadequate insect control and food storage practices. These household insects harbor countless bacteria species of public health, agricultural and industrial importance. Many studies have reported disease-causing bacteria from both cockroaches and ant's species collected from hospitals and residential areas. The aim of this study was to characterize the culturable bacterial communities of two common household insects, big headed ants (*Pheidole rugaticeps*) and American cockroaches (*Periplaneta americana*) using 16S rRNA genes sequencing.
Methodology and results: A total of 64 bacterial sequences were obtained from *P. rugaticeps* (48.44%) and *P. americana* (51.56%) and Firmicutes was the most dominant phylum from both insect species. *Bacillus* was the most dominant bacterial genus from both cockroach and ant samples. Other important genera isolated were *Pseudomonas* and *Stenotrophomonas* which have previously been suggested to have members that are of biotechnological importance. Food poisoning bacterial species, *B. cereus* and other bacterial strains such as *B. subtilis*, *Acinetobacter baumannii*, *Burkholderia cepacia*, *P. aeruginosa*, *Staphylococcus epidermidis*, *Serratia marcescens* and *S. pseudintermedius* with the history of human infections were isolated from some of the insect's specimens.
Conclusion, significance and impact of study: Thus, these household insect pests harbor bacterial species known to cause diseases of serious public health importance that needs serious attentions. Similarly, the insects harbor other bacteria species that may provide opportunities for biotechnological exploration.

Keywords: 16S rRNA genes, *Bacillus*, *P. americana*, *P. rugaticeps*, public health

INTRODUCTION

Insects are the most diverse group of organisms with a tremendous impact on public health, agriculture and food production. Several insects are significant vectors of many pathogens and they can facilitate the transmission of various microbes like viruses, bacteria, fungi and nematodes (Sarwar, 2015; Dieng *et al*., 2017). Cockroaches and ants are among the mostly observed insects around human and are associated diseases and allergies (Rust *et al.*, 1991; Gore and Schal, 2005; 2007). Majority of the cockroaches are home-infesting insects (Graczyk *et al.*, 2005; Mpuchane *et al.*, 2005) and their infestation trend have lately increased (Nasirian, 2017). *Periplaneta americana* is one of the most observed insects in toilets, bathrooms and kitchens (Graczyk *et al.*, 2005; Mpuchane *et al.*, 2005; Dehghani *et al.*, 2014). *Periplaneta americana* are reddish-brown roaches with an oval body shape that is dorsoventrally flattened and have a pronotum that shields the thorax (Barbara, 2008). They are virtually cosmopolitan in distribution because of the human activity such as the global commerce (Bell and Adiyodi, 1981; Smith and Whitman, 1992). Also, their existence especially around human habitats can be a serious source of health problem (Rust *et al.*, 1991). Some of the diseases potentially harbored by cockroaches are cholera, diarrhea, dysentery, leprosy, plague, typhoid, poliomyelitis, and other allergic reactions, itching, swelling of the eyelids, dermatitis and even serious respiratory infections (Stankus *et al.*, 1990).

Pheidole rugaticeps like other ants are eusocial insects that live in large groups and sometimes enter structures with deficient control and food storage practices (Zarzuela *et al.*, 2002; dos Santos *et al.*, 2009; Ashigar and Ab Majid, 2020a). Their workers are easily recognized by their complete dimorphism. The major

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workers have disproportionately large heads which give them the name big-headed ants. Both major and minor workers of this ant group perfume activities such as foraging, food processing, storage and colony defense (Wilson, 2003; Ashigar and Ab Majid, 2020a). These ant species search for food, nesting sites and other biotic interactions in human residence (Benson and Harada, 1988; Hölldobler and Wilson, 1990; Reyes-Lopez *et al.*, 2003; Ashigar and Ab Majid, 2020a). Diverse behavioral features like polygyny, colony fragmentation and various feeding preference aids their dispersal and adaptation in human communities (Bueno and Campos-Farinha, 1998; Hedges, 1998; Man and Lee, 2012). Until now, *Pheidole* has been the most dominant ants in tropics and around human habitation according to many studies (Silva *et al.*, 2014; Ashigar and Ab Majid, 2020a). Many pathogenic bacteria have been cultivated from other *Pheidole* spp. (Garcia and Lise, 2013; Lima *et al.*, 2013; Silva *et al.*, 2014; Oliveira *et al.*, 2017). Furthermore, a recent microbiome study of *P. rugaticeps* using Illumina MiSeq high-throughput sequencing of the bacterial 16S ribosomal DNA gene revealed many bacterial genera *Acinetobacter* (including *Acinetobacter baumannii*), *Pseudomonas*, *Escherichia-Shigella* (including *Escherichia coli*) and *Shimwellia* among others (Ashigar and Ab Majid, 2020b).

Cultivation-dependent method is a vital approach that facilitates the genomic, metabolomic, proteomic and transcriptomic analyses of the bacteria isolates making direct biotechnological exploitation of important species possible (Guzman and Vilcinska, 2020). For instance, biotechnological applications of insect-derived bacteria groups such as Enterobacteriaceae for their toxicity against pest insects (Zhang *et al.*, 2010), as source of plant-stimulation (Pan *et al.*, 2019), as antimicrobial metabolites (Vivero *et al.*, 2019), as well as in insect-rearing (Augustinos *et al.*, 2015; Azis *et al.*, 2019) were all reported previously. In cockroach, the bacteria species, *Shimwellia blattae* originally isolated from *Blatta orientalis* (Burgess *et al.*, 1973) and other cockroaches can interestingly synthesize cobalamin de novo and used as a biotechnological source of vitamin B12 (Andres *et al.*, 2004; Brzuszkiewicz *et al.*, 2012).

The bacterial genera *Acinetobacter* and *Pseudomonas* were commonly cultured from cockroaches and ants. Culture-based investigations have also demonstrated that these bacterial groups are highly abundant in the crop of Surinam cockroach (Lampert *et al.*, 2019). *Pseudomonas* species are vital in biotechnology due their production of bioactive metabolites (Gross and Loper, 2009), their use in bioremediation (Wasi *et al.*, 2013) and as a source of potent lytic enzymes (proteases, lipases) for industrial processes. Bacterial infections of human due *A. baumanii* or *Pseudomonas aeruginosa* are challenging to cure because of their antibiotic resistance. Several studies have cultured *P. aeruginosa* strains from cockroaches and ants. *Stenotrophomonas maltophilia* is another strain that been cultivated from several cockroach species (Le Guyader *et al.*, 1989; Elgderi *et al.*, 2006; Mpuchane *et al.*, 2006; Ozdal *et al.*, 2016). One of the isolates from *Blatta orientalis* degrade organochlorinated pesticide like endosulfan and transform it into lesser toxic metabolites (Ozdal *et al.*, 2017).

Bacterial communities of *P. americana* and *Pheidole rugaticeps* were cultivated and established using 16S rRNA gene sequencing in this study. This approach is said to be extremely sensitive and precise (Frank, 2014). According to Wilson (1995), 16S rDNA gene sequencing is certainly the most familiar method presently used in the identification of bacteria providing an excellent performance (Drancourt *et al.*, 2000). However, most studies on the bacteria pathogens associated with ants collected in hospitals and households were done through traditional phonetic methods (Garcia and Lise, 2013; Lima *et al.*, 2013; Silva *et al.*, 2014; Oliveira *et al.*, 2017; Alharbi *et al.*, 2019). Therefore, this study was aimed at determining the culturable bacterial communities of big-headed ants (*Pheidole rugaticeps*) and American cockroaches (*Periplaneta americana*) using 16S rRNA genes sequencing.

MATERIALS AND METHODS

Collection and identification of insect samples

The insect samples (240 workers of ants and 16 adult cockroaches) were collected from rural and urban neighborhoods of Nasarawa State, Nigeria. The rural areas include Akunza (AKZ), Akunzan Sama (AKS), Gwandara (KDR) and Kurikyo (KRK), while the urban areas were Akwanga (a Primary Healthcare, PHC and a Low-cost Housing Estate, LHE), Lafia (Dalhatu Arab Specialist Hospital, DASH) and Keffi (Government Residential Area, GRA) as shown in Table 1. During the insect sampling, toilets, kitchens and other cockroach harborage were sprayed with an aerosol insecticide (Knockdown, Guangzhou Konnor Daily Necessities Co., Ltd.). After 30 min of insecticide application, the area is observed for *P. americana* as well as ants were aseptically collected and identified using the standard taxonomic keys (Bell, 1981; Taylor, 2012; Antweb, 2020). After sorting and identification of the ant specimens, *P. rugaticeps* Emery was found to be the most dominant ant scavengers around dead American cockroaches. The adults of *P. americana* and workers of *P. rugaticeps* were used for the isolation of the bacterial communities of these two insects.

Isolation of bacteria

The bacteria isolation method employed by Alharbi *et al.* (2019) was performed with slight modification. During laboratory examination, the insect specimens were selected randomly from the storage containers using sterile forceps and transferred into dilution tubes containing 1 mL of phosphate-buffered saline (1× PBS) solution. The insect specimens were crushed by means of sterile pestle and mortar before transferring to the 1× PBS solution container. A total of 9 mL of sterile water was

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Table 1: Bacterial strains (%) isolated from *Pheidole rugaticeps* and *Periplaneta americana* in each location.

Communities	Locations	Coordinates	*P. rugaticeps* (%)	*P. americana* (%)	Total %
Urban	Primary Health Care (PHC), Akwanga	08˚54'50.41" N 08˚24'51.86" E	12.90	9.09	10.94
	Low-cost Housing, Akwanga (LHE)	08˚55'38.29" N 08˚24'46.91" E	6.45	9.09	7.81
	Dalhatu Specialist Hospital (DASH)	08˚30'08.95" N 08˚31'21.95" E	0.00	15.15	7.81
	Gov't Residential Area (GRA) Keffi	08˚50'53.25" N 07˚53'08.48" E	9.68	3.03	6.25
Mean ± SEM			2.25 ± 0.85	5.25 ± 0.75	
Rural	Kurikyo (KRK)	08˚31'32.09" N 08˚35'51.59" E	16.13	12.12	14.06
	Gwandara (KDR)	08˚34.11.95" N 08˚29'50.59" E	16.13	21.21	18.75
	Akunzan Sama (AKS)	08˚28'07.87" N 08˚36'04.02" E	19.35	18.18	18.75
	Akunza (AKZ)	08˚28'11.69" N 08˚35'24.03" E	19.35	12.12	15.63
Mean ± SEM			5.50 ± 0.28	3.00 ± 0.81	
Total					100
ANOVA			(F(13.00) = 21.125, p = 0.011)	(F(4.119) = 10.125, p = 0.089)	

then added to each test tube containing 30 workers of *P. rugaticeps* and 2 adult American cockroaches. The test tubes containing the mixtures were thoroughly shaken for at least 2 min. A ten-fold serial dilution (10^1 to 10^{10}) of the solutions containing the specimens were made. Amount of 0.1 mL aliquots were pipetted onto the surface of prepared nutrient broth agar plates and incubated at 30 °C for 24–48 h. During and after the incubation period, the plates were checked for the presence of the bacterial growth. Colonies that are morphologically distinct were purified by re-streaking. Freshly purified re-streaked pure colonies were then used for colony PCR reaction (Damnjanovic *et al.*, 2019). Sterile toothpicks were used to picked colonies and then resuspended into PCR tubes containing 10 µL of dH_2O. These were then heated at 94 °C for 5 min prior to the PCR.

PCR technique (Colony PCR)

The PCR reaction of the DNA samples from the ants and the cockroaches were performed using the universal primers RW01 (5′-AAC TGG AGG AAG GTG GGG AT-3′) and DG74 (5′-AGG AGG TGA TCC AAC CGC A-3′) that amplify 370-base pair sequences (Matar *et al.*, 1998). The 16S rRNA gene were amplified in a 25 µL PCR reaction containing final concentration of 12.5 µL of Master Mix (EconoTaq PLUS GREEN 2× Master Mix, Lucigen) and 6.5 µL of ddH_2O, 2.5 µL (8 pmol) of each primer and 1 µL of the bacteria colony solution. Before this, pure colonies obtained from re-streaked colonies were picked and transferred into 10 µL sterile distilled water using sterile toothpick and heated at 94 °C for 5 min. PCR reaction was done with a G-Storm Dual Block Thermal Cycler PCR. The thermal cycling conditions were 95 °C for 3 min; 30 cycles of 95 °C for 30 sec; 55 °C for 30 sec; 72 °C for 1 min and end with 72 °C for 5 min. Subsequently, 5 µL of each PCR product were used for gel electrophoresis (1% agarose gel). The remaining 20 µL of each PCR product that produced clear and distinctive bands in the gel electrophoresis were purified using MEGAquick-spin™ Total Fragment DNA Purification Kit (iNtRON Biotechnology, Korea) and subsequently used for sequencing.

16S rRNA gene sequencing and phylogenetic tree

The purified PCR products were sequenced via Sanger sequencing at Apical Scientific Sdn. Bhd., Malaysia. The sequence data obtained were extracted to FASTA format using FinchTV 1.4 (www.geospiza.com). The sequences (forward and reverse) were then aligned using T-Coffee (Notredame *et al.*, 2000) and low-quality base pairs were removed. The edited FASTA format were then matched with 16S rRNA gene sequences available in NCBI database through the BLASTN programs search nucleotide databases (https://blast.ncbi.nlm.nih.gov), only bacterial sequences with 99—100% similarities match were considered as bacterial species in this study. The nucleotide sequences were submitted to NCBI database (https://www.ncbi.nlm.nih.gov/WebSub/) and the sequence accession numbers have been obtained and presented in the supplementary Table S1. Phylogenetic tree analyses of the bacteria sequences and alignments were done using the CLUSTAL X of the MEGA7 (Kumar *et al.*, 2016) and shown in the Figure 1.

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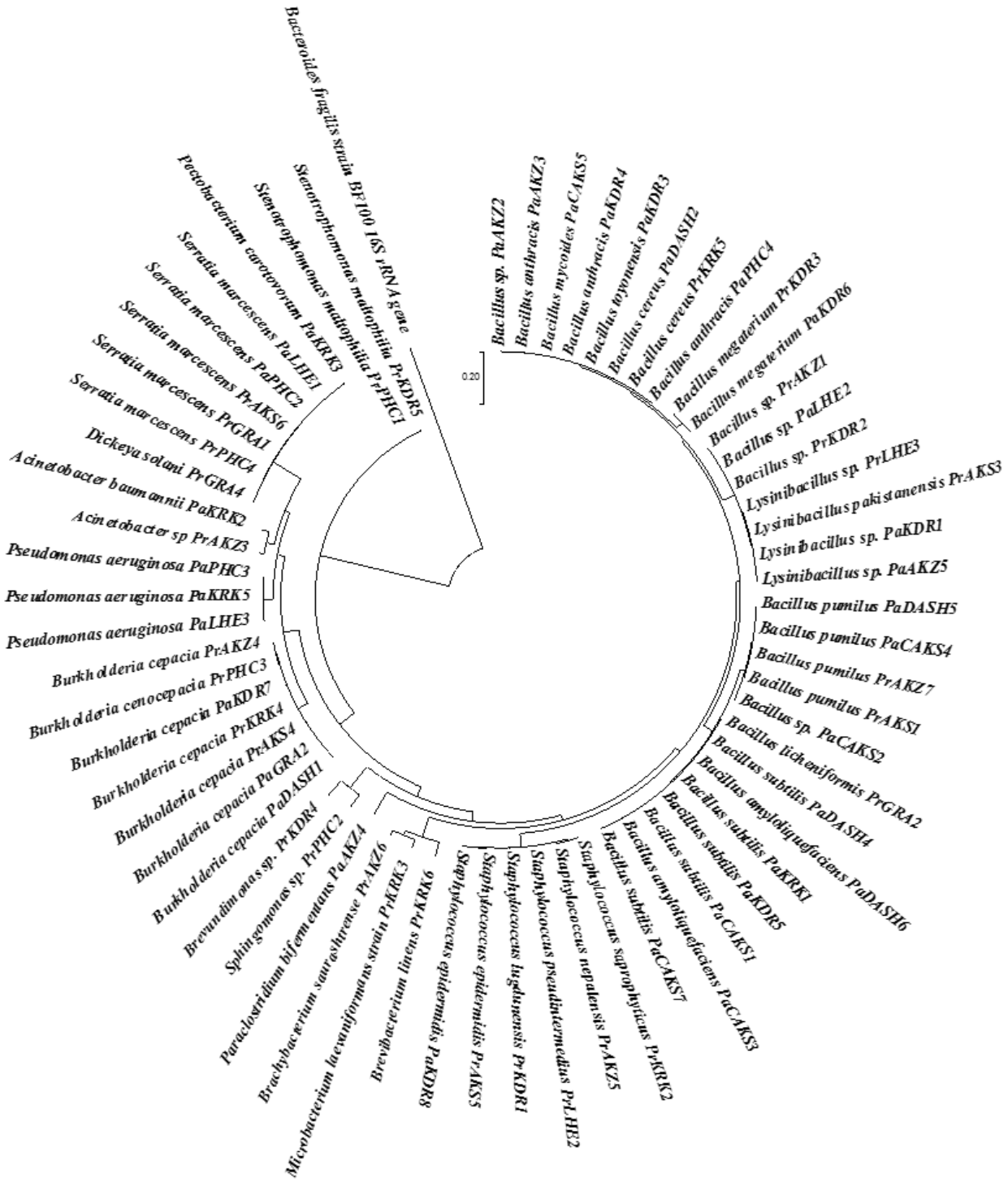

Figure 1: Molecular phylogenetic analysis of the bacteria isolated from *P. rugaticeps* and *P. americana* collected from eight different locations. The phylogenetic tree was constructed by Maximum Likelihood method based on the 16S rRNA partial gene sequences. *Bacteroides fragilis* strain BF100 16S rRNA gene was used as the outgroup.

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Statistical analysis

The number of bacterial isolates obtained from *P. rugaticeps* and *P. americana* between rural and urban communities were compared using one-way ANOVA. Means and standard error of means were used for comparing the results obtained. *P* value was used to determine the test of significance. This analysis was done using the IBM SPSS Statistics version 20. The percentage of bacterial isolates from *P. rugaticeps* and *P. americana* were presented in Table 1.

RESULTS

Bacteria composition of *P. rugaticeps* and *P. americana* in various locations

This study was carried out to isolate and identify bacteria harbored by the major and minor workers of *Pheidole rugaticeps* and adult *P. americana* collected from rural and urban residents in Nasarawa, Nigeria. The bacterial species frequency (%) of *P. rugaticeps* and *P. americana* from four rural and four urban neighborhoods were presented in Table 1. Out of the total 64 bacterial strains isolated, 48.10% were from *P. rugaticeps* and 51.90% were from *P. americana*.

From the one-way ANOVA analysis performed, *P. rugaticeps* has a mean (± standard error of the mean) of the bacteria isolates of 2.25 ± 0.85 from urban and from rural 5.50 ± 0.28. Apparently, this result revealed a statistically significant difference ($F(13.00) = 21.125$, $p = 0.011$) in the bacteriological compositions of *Pheidole* collected from the urban and rural communities. However, the one-way ANOVA result also showed that bacterial compositions of *P. americana* collected from rural and urban areas were 5.25 ± 0.75 and 3.00 ± 0.81, respectively. This result revealed that there is no statistically significance difference ($F(4.119) = 10.125$, $p = 0.089$) in the bacterial composition of *P. americana* collected from rural and urban.

Generally, high abundance of bacteria isolates was obtained from the insect samples collected from rural community (67.19%) than from the urban communities (32.81%). As shown in Table 1, AKZ community (19.35%) and AKS (19.35%) have the highest percentage of bacteria isolates from the ants samples whereas DASH (0.00%) recorded lowest percentage. As for bacteria isolates from the cockroach samples, KDR (21.21%) has the highest percentage whereas GRA (3.03%) has the lowest percentage (Table 1). As shown above, DASH location shows (0.00%) because the bacterial sequences obtained in this location had similarity threshold of less than 99% against the GenBank sequences.

Taxonomic group of the bacterial isolates

All the 64 sequenced bacterial isolates from the two insect groups were classified into 3 phyla, 16 genera and 32 species. The three phyla include Firmicutes, Proteobacteria and Actinobacteria which were all recorded from *P. rugaticeps* and only Firmicutes and Proteobacteria were recorded from *P. americana.* Firmicutes was the predominant phyla in both *P. rugaticeps* with 46.88% and *P. americana* with 68.75%. Proteobacteria was also isolated in large frequency in both *P. rugaticeps* (40.63%) and *P. americana* (31.25%). However, the phylum Actinobacteria was only isolated from *P. rugaticeps* (12.50%).

At genus level, 16 bacterial genera were identified from the two insect groups and 6 genera that include *Bacillus* (40.98%), *Burkholderia* (9.84%), *Lysinibacillus* (4.92%), *Serratia* (8.20%), *Staphylococcus* (9.84%) and *Acinetobacter* (3.28%) were all isolated from the two insect species. Furthermore, 7 genera such as *Stenotrophomonas* (3.28%), *Sphingomonas* sp. (1.64%), *Dickeya* (1.64%), *Brevundimonas* (1.64%), *Microbacterium* (1.64%), *Brevibacterium* (1.64%) and *Brachybacterium* (3.28%) were only isolated from *P. rugaticeps* and the remaining 3 genera including *Pectobacterium* (1.64%), *Pseudomonas* (4.92%) and *Paraclostridium* (1.64%) were only isolated from *P americana*. The genus, *Bacillus* was the predominant bacterial group in both *P. rugaticeps* (59.49%) and *P americana* (22.59%). The percentage frequencies of the bacterial genera identified from *P. rugaticeps* and *P americana* were presented in Figure 2.

A total of 32 bacterial species were identified from the two insect species and 8 bacterial species such as *Bacillus* sp. (ants, 6.45%; cockroach, 9.38%), *Bacillus cereus* (ants, 3.23%; cockroach, 3.13%), *Bacillus pumilus* (ants, 6.45%; cockroach, 6.25%), *Bacillus megaterium* (ants, 3.23%; cockroach, 3.13%), *Burkholderia cepacia* (ants, 9.68%; cockroach, 9.38%), *Lysinibacillus* sp. (ants, 6.45%; cockroach, 3.13%), *Serratia marcescens* (ants, 9.68%; cockroach, 6.25%) and *Staphylococcus epidermidis* (ants, 3.23%; cockroach, 3.13%) were isolated from both *P. rugaticeps* and *P. americana*. However, there are other bacterial species that were unique to each insect group as present in Table 2. From the *P. rugaticeps* samples, *B. cepacia* (9.68%) was the predominant bacteria, whereas *Bacillus subtilis* (15.63%) was the most dominant bacteria isolated from *P. americana*.

Interestingly, from the bacteria species isolated from these two household insects, a number of bacteria that have been associated with diseases were present as shown in Table 3. Bacterial species such as *Acinetobacter baumannii*, *Bacillus anthracis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus pseudintermedius*, *Staphylococcus saprophyticus* and *Stenotrophomonas maltophilia* were identified from study and identified as the species that cause diseases in human, animal as well as plants. Other bacterial species such as *Dickeya solani* and *Pectobacterium carotovorum* associated with diseases of agricultural crop have also identified from both ant and cockroach samples in the study. These bacterial species and their associated pathogenicity were presented in Table 3.

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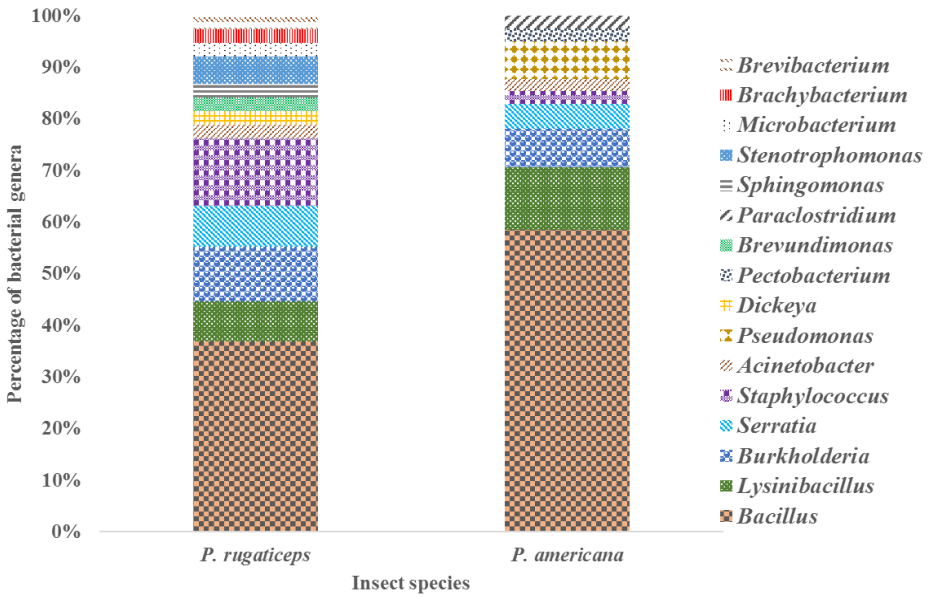

Figure 2: Percentage occurrence of bacterial genera from *Pheidole rugaticeps* and *Periplaneta americana* identified using PCR technique.

Table 2: Presents of the bacterial species isolated from *Pheidole rugaticeps* and *Periplaneta americana*.

Bacterial species	*P. rugaticeps* (%)	*P. americana* (%)
Burkholderia cepacia	9.68	9.38
Serratia marcescens	9.68	6.25
Bacillus sp.	6.45	9.38
Bacillus pumilus	6.45	6.25
Lysinibacillus sp.	6.45	3.13
Bacillus cereus	3.23	3.13
Bacillus megaterium	3.23	3.13
Staphylococcus epidermidis	3.23	3.13
Bacillus subtilis	-	15.63
Bacillus anthracis	-	9.38
Bacillus amyloliquefaciens	-	6.25
Stenotrophomonas maltophilia	6.45	-
Acinetobacter sp.	3.23	-
Acinetobacter baumannii	-	3.13
Bacillus licheniformis	3.23	-
Bacillus mycoides	-	3.13
Bacillus toyonensis	-	3.13
Brachybacterium saurashtrense	3.23	-
Brevibacterium linens	3.23	-
Brevundimonas sp.	3.23	-
Burkholderia cenocepacia	3.23	-
Dickeya solani	3.23	-
Lysinibacillus pakistanensis	3.23	-
Microbacterium laevaniformans	3.23	-
Paraclostridium bifermentans	-	3.13
Pectobacterium carotovorum	-	3.13
Pseudomonas aeruginosa	-	9.38
Sphingomonas sp.	3.23	-
Staphylococcus pseudintermedius	3.23	-
Staphylococcus saprophyticus	3.23	-
Staphylococcus lugdunensis	3.23	-
Staphylococcus nepalensis	3.23	-

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Table 3: Bacterial pathogens isolated from *Pheidole rugaticeps* and *Periplaneta americana*.

Organism	*P. rugaticeps* (%)	*P. americana* (%)	Related infections	Reference
Acinetobacter baumannii		3.13	Bacteremia and nosocomial infections	(Wong *et al.*, 2017; Alharbi *et al.*, 2019)
Bacillus cereus	3.23	3.13	Food poisoning	(Granum and Lund, 1997)
Bacillus anthracis		9.38	Anthrax disease of human beings and animals, potential role of insects	(Fasanella *et al.*, 2010)
Bacillus megaterium	3.23	3.13	Keratitis skin (cutaneous), brain abscess, pleuritis	(Ramos-Esteban *et al.*, 2006; Duncan and Smith, 2011; Guo *et al.*, 2015; Crisafulli *et al.*, 2019)
Bacillus pumilus	6.45	6.25	Bacteremia, central venous catheter infection, skin (cutaneous) infection, neonatal sepsis, septic arthritis	(Bentur *et al.*, 2007; Tena *et al.*, 2007; Kimouli *et al.*, 2012; Shivamurthy *et al.*, 2016)
Brevundimonas sp.	3.23		Emerging global opportunistic pathogens	(Ryan and Pembroke, 2018)
Burkholderia cepacia	9.68	9.38	Infect a range of hosts, including insects, human, animals and plants	(Uehlinger *et al.*, 2009; Sousa *et al.*, 2011; Lukasik *et al.*, 2013; Nikoh *et al.*, 2014)
Burkholderia cenocepacia	3.23		Opportunistic pathogens causing lung infections	(Holden *et al.*, 2009)
Dickeya solani	3.23		Pathogenic bacterium causing loss in potato yield	(Toth *et al.*, 2011; Kutsuna *et al.*, 2018; Rossmann *et al.*, 2018)
Paraclostridium bifermentans		3.13	Involved in metastatic osteomyelitis, necrotizing pneumonia and bacteremia	(Scanlan *et al.*, 1994)
Pectobacterium carotovorum		3.13	Soft rot disease in cabbage, potato, onion and other crops	(Lee *et al.*, 2013)
Pseudomonas aeruginosa		9.38	Bacteremia nosocomial pneumonia	(Jeong *et al.*, 2014; Micek *et al.*, 2015)
Serratia marcescens	9.68	6.25	Opportunistic nosocomial pathogen and spread in hospitalized patients	(Khanna *et al.*, 2013)
Staphylococcus lugdunensis	3.23		A culprit in skin and soft tissue infections and cause urinary tract infection	(Lourter-Hascoet *et al.*, 2016; Manica and Cohen, 2017; Chiu *et al.*, 2020
Staphylococcus pseudintermedius	3.23		A major bacterial pathogen causing canine skin and ear infections	(Bannoehr and Guardabassi, 2012)
Staphylococcus saprophyticus	3.23		Leading cause of cystitis in young women and an agent in urinary tract infection	(Svanborg, 1998; Raz *et al.*, 2005)
Staphylococcus epidermidis	3.23	3.13	Surgical wound infections and bacteremia in immunocompromised patients.	(Blum and Rodvold, 1987)
Stenotrophomonas maltophilia	6.45		Multidrug-resistant global opportunistic pathogen. Nosocomial infection	(Brooke, 2012)

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DISCUSSION

This study was designed to determine the culturable bacterial communities associated with *P. americana* and *Pheidole rugaticeps* scavenging around human dwellings through culture-dependent techniques. Both American cockroach and *Pheidole* ants are omnivorous insects (Yun *et al.*, 2014) that scavenge around homes. The current analysis of the 16S rRNA gene sequences revealed 64 bacterial sequences from *P. rugaticeps* (31) and *P. americana* (33) and this number is incomparable to those obtained from high throughput 16S rRNA gene sequencing such as those of Tinker and Ottesen (2016), Ashigar and Ab Majid (2020b), and Martins and Moreau (2020). This may be due to low percentage recovery (1 – 10%) of the culture-dependent techniques of bacteria studies (Pace, 1997; Hugenholtz *et al.*, 1998). However, from the present study, some bacterial taxonomic groups were common to both *P. americana* and *Pheidole rugaticeps*. Similarly, previous studies involving ants revealed similarities of the bacterial communities associated with ant species of the same group (Anderson *et al.*, 2012).

From the analyses of the sequences results obtained from the two insect species, Firmicutes and Proteobacteria were the most dominant phyla isolated and this finding is constant with previous culture-independent studies of ants (Martis and Moreau, 2020) and cockroaches (Tinker and Ottesen, 2016; Guzman and Vilcinskas, 2020). In particular, Firmicutes was the most predominant phyla in both *P. rugaticeps* (46.88%) and *P. americana* (68.75%). As described by Guzman and Vilcinskas (2020) and Tinker and Ottesen (2020), Firmicutes is the second most predominant bacterial taxa cultivated from cockroaches but the most abundant phylum in culture-independent studies (Dietrich *et al.*, 2014). This bacterial phylum is highly abundant in the midgut than the foregut and hindgut of the cockroaches particularly due to its alkaline nature (Vinokurov *et al.*, 2007), several alkaliphilic, aerobic bacterial genera such as *Bacillus* flourish in there (Yumoto *et al.*, 2011). Similarly, in ant groups such as *Camponotus*, *Oecophylla*, and *Pheidole*, Firmicutes has also shown to be one of the dominant bacterial taxa (Hosmath and Timmappa, 2019; Martins and Moreau, 2020).

Bacillus was the predominant bacterial genus from both *P americana* (59.49%) and *Pheidole* ants (22.59%) samples. According to several earlier studies, alkaliphilic *Bacillus* flourish in cockroach midgut especially due its alkaline nature (pH 6.1—8.9) (Vinokurov *et al.*, 2007; Yumoto *et al.*, 2011). *Bacillus cereus*, *B. subtilis* and related strains can readily be cultured from cockroaches (Guzman and Vilcinskas, 2020). In this study, *B. subtilis* was the predominant strain isolated from the cockroach samples and the result corroborate with Guzman and Vilcinskas (2020). *Bacillus cereus* isolate PaDASH2 was also isolated from cockroach collected in hospital environment. Notably, *Bacillus* isolated from cockroaches demonstrates biotechnological potentials due to their ability to produce bioactive metabolites (Um *et al.*, 2013) and industrially useful enzymes. They can also be a platform for producing recombinant proteins (van Dijl and Hecker, 2013). *Bacillus* strain (isolate 29K) cultured from *P. americana* demonstrated strong keratinolytic and proteolytic activities (Sharma *et al.*, 2019). Nevertheless, *Bacillus* strains such as *B. cereus* are associated with food poisoning (Granum and Lund, 1997) and several earlier studies have isolated *B. cereus* from cockroaches (Rahmaet-Alla and Rowley, 1990; Pai *et al.*, 2004; Solomon *et al.*, 2018) and ants (Beatson, 1972; da Costa *et al.*, 2006; Lima *et al.*, 2013). *Bacillus cereus* and others like *B. anthracis*, *B. megaterium* and *B. pumilus* were also linked with various disease in human and other animals including insects due to their ability to secrete lytic enzymes and toxins (Ehling-Schulz *et al.*, 2019).

Other members of the Firmicutes such as *Lysinibacillus* and *Staphylococcus* are prevalent in cockroaches according to molecular studies (Schauer *et al.*, 2014; Mikaelyan *et al.*, 2015; Lampert *et al.*, 2019). In the present study, both *Lysinibacillus* and *Staphylococcus* have been isolated from both *P. americana* and *Pheidole rugaticeps*. Prior studies have described antibiotic-resistant strains of *Staphylococcus* from some cockroaches (Menasria *et al.*, 2014; Islam *et al.*, 2016; Abdolmaleki *et al.*, 2019) and ants (Oliveira *et al.*, 2014) and ants collected from hospital settings (Lise *et al.*, 2006). *Staphylococcus epidermidis*, a bacterial pathogen associated with surgical wound infections and bacteremia in immunocompromised patients (Blum and Rodvold, 1987) was isolated from both *Pheidole rugaticeps* (3.23%) and *P. americana* (3.13%) samples collected in this study. Other bacterial pathogen like *Staphylococcus pseudintermedius* that is associated with skin and ear infections in canine (Bannoehr and Guardabassi, 2012) were isolated from the *Pheidole rugaticeps*.

Likewise, other genera such as *Acinetobacter* (3.28%), *Burkholderia* (9.84%) and *Serratia* (8.20%) belonging to the phyla Proteobacteria have also been isolated from both *P. rugaticeps* and *P. americana.* The genus, *Acinetobacter* have frequently been cultured from both cockroaches (Guzman and Vilcinskas, 2020) and ants (Fowler *et al.*, 1993; Lise *et al.*, 2006). From this study *A. baumannii*, a member of the genus *Acinetobacter* causing bacteremia and nosocomial infections (Khanna *et al.*, 2013) has been isolated from the *P. americana* samples. Although no strain of this bacteria was isolated from the *Pheidole rugaticeps* samples, but other studies have previously cultivated *A. baumannii* from ants (Wong *et al.*, 2017; Alharbi *et al.*, 2019). Similarly, *Serratia marcescens* strain belonging to the genus *Serratia* has been isolated from both ants (PrPHC, PrGRA1 and PrAKS6 isolates) and cockroach samples (PaPHC2 and PaLHE1 isolates). *Serratia marcescens* have frequently been isolated from insects collected from hospital and houses (Pai *et al.*, 2004; da Costa *et al.*, 2006; Lima *et al.*, 2013; Solomon *et al.*, 2018; Alharbi *et al.*, 2019).

Moreover, *P. aeruginosa* strains (isolates PaLHE3, PaPHC3 and PaKRK2), another member of the phyla Proteobacteria that have frequently been cultivated from

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cockroaches in the genus *Pseudomonas* (Lampert *et al.*, 2019; Guzman and Vilcinskas, 2020; Zhang *et al.*, 2020) was also isolated from the cockroaches examined in this study. Although, *P. aeruginosa* have not been isolated from the *Pheidole* ant samples but several studies have cultured it from ants collected from houses and hospital environment (Wong *et al.*, 2017; Alharbi *et al.*, 2019).*Pseudomonas aeruginosa* are clinically important group of bacteria that cause human infections that are difficult to treat due to antibiotic resistance (Alanis, 2005; Demain, 2009; Nikaido, 2009; WHO, 2014). However, drug resistance has not been found in *P. aeruginosa* strains cultured from cockroaches (Zarei *et al.*, 2018). Some species of *Pseudomonas* are vital in biotechnology due to production of bioactive metabolites (Gross and Loper, 2009), their usage in bioremediation (Wasi *et al.*, 2013) and as source of lytic enzymes like proteases and lipases for industrial activities. For example, the strain *P. aeruginosa* BGf-2 (Zhang *et al.*, 2020), isolated from *Blattella germanica* have shown antifungal activity against *Beauveria bassiana*, an entomopathogenic fungus.

Stenotrophomonas maltophilia is a bacterial strain have previously been cultured from several cockroach species (Le Guyader *et al.*, 1989; Elgderi *et al.*, 2006; Mpuchane *et al.*, 2006; Ozdal *et al.*, 2016). In this study, *S. maltophilia* was isolated from the *Pheidole rugaticeps* as shown in Table 2. This bacterium is a highly versatile and widely distributed in a wide range of habitats and the bacterium has useful biocontrol and bioremediation properties (Anzai *et al.*, 2000) and protease production (Wang *et al.*, 2016). A strain isolated from Oriental cockroach was revealed to degrade endosulfan (an organochlorinated pesticide) and uses its sulfur source, and then convert it to lesser toxic metabolites (Ozdal *et al.*, 2017). Other studies also cultured *S. maltophilia* strain from foodstuff (Geng *et al.*, 2010) and the bacterium is linked with increasing food spoilage (Prieto *et al.*, 2007; Ercolini *et al.*, 2009; Silvetti *et al.*, 2010; Böhme *et al.*, 2011). Opportunistic pathogenicity and nosocomial infection and multidrug-resistant were also reported from *S. maltophilia* (Brooke, 2012).

Other important bacterial strains isolated from the present study were *D. solani* and *P. carotovorum* with former bacterial strains causing potato yield loss (Toth *et al.*, 2011; Kutsuna *et al.*, 2018; Rossmann *et al.*, 2018) and has been cultured from *Pheidole* in this study. Similarly, *P. carotovorum* also causes soft rot disease in cabbage, potato, onion and other crops (Lee *et al.*, 2013) and was isolated from *P. americana* in the present study. Both *Pheidole* ants and *P. americana* are omnivorous insects scavenging in kitchens and related food storage areas and may perhaps be a source of stored food contamination (Toth *et al.*, 2011; Kutsuna *et al.*, 2018; Rossmann *et al.*, 2018). The bacterial pathogens may adhere to the insect body surfaces such as legs and mandibles (Hughes *et al.*, 1989; De Zarzuela *et al.*, 2005; Zurek and Gorham, 2008) while they move around filthy environments like pit latrines (Zurek and Gorham, 2008). They might then be deposited on dishes and other food contact surfaces and eventually mixed up with poorly stored foods stuff or ready-to-eat food (Simothy *et al.*, 2018).

CONCLUSION

This study cultured a plethora of importance bacterial groups like *Bacillus* and *Pseudomonas* that have been suggested to have biotechnological importance because of their ability to produce bioactive metabolites, their usage in bioremediation, as well as a source of lytic enzymes like proteases and lipases for engineering and industrial usage. However, other bacterial strains like *B. cereus*, *B. subtilis*, *S. epidermidis*, *S. pseudintermedius*, *A. baumannii*, *B. cepacia*, *P. aeruginosa* with the history of human infections were isolated from some of the insects' specimens. Therefore, the presence of these insect species around household can be a source of serious concern as they are potentials source of transmitting diseases not only to human diseases but also plants and other animals. This study also suggests that cockroaches and ants scavenging especially around kitchens, food stores, toilets or hospital environments should effectively be kept at bay to avert diseases related to bacteria species they are capable of transmitting.

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CONFLICT OF INTEREST

We author(s) declare no conflict of interest.

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SUPPLEMENTARY INFORMATION

Table S1: Bacterial isolates from *P. rugaticeps* and *P. americana* based on 16S rRNA gene sequences using identification threshold of similarity greater than 99%.

Phylogenetic group	Bacterial isolate	Bacterial species (Top match)	Accession number	Nucleotide identity (%)
Proteobacteria	PrPHC1	*Stenotrophomonas maltophilia*	MW267005	100
Proteobacteria	PrPHC2	*Sphingomonas* sp.	MW267006	100
Proteobacteria	PrPHC3	*Burkholderia cenocepacia*	MW267007	99.40
Firmicutes	PrPHC4	*Serratia marcescens*	MW267008	100
Firmicutes	PrLHE2	*Staphylococcus pseudintermedius*	MW267011	99.70
Firmicutes	PrLHE3	*Lysinibacillus* sp.	MW267012	99.69
Proteobacteria	PrGRA1	*Serratia marcescens*	MW267014	100
Firmicutes	PrGRA2	*Bacillus licheniformis*	MW267015	100
Proteobacteria	PrGRA4	*Dickeya solani*	MW267017	100
Firmicutes	PrKRK2	*Staphylococcus saprophyticus*	MW267019	99.09
Actinobacteria	PrKRK3	*Microbacterium laevaniformans*	MW267020	99.10
Proteobacteria	PrKRK4	*Burkholderia cepacia*	MW267021	99.38
Firmicutes	PrKRK5	*Bacillus cereus*	MW267022	100
Actinobacteria	PrKRK6	*Brevibacterium linens*	MW267023	99.06
Firmicutes	PrKDR1	*Staphylococcus lugdunensis*	MW267024	99.69
Firmicutes	PrKDR2	*Bacillus* sp.	MW267025	99.69
Firmicutes	PrKDR3	*Bacillus megaterium*	MW267026	99.38
Proteobacteria	PrKDR4	*Brevundimonas* sp.	MW267027	100
Proteobacteria	PrKDR5	*Stenotrophomonas maltophilia*	MW267028	99.69
Firmicutes	PrAKS1	*Bacillus pumilus*	MW267030	100
Firmicutes	PrAKS3	*Lysinibacillus pakistanensis*	MW267032	100
Proteobacteria	PrAKS4	*Burkholderia cepacia*	MW267033	99.41
Firmicutes	PrAKS5	*Staphylococcus epidermidis*	MW267034	100
Proteobacteria	PrAKS6	*Serratia marcescens*	MW267035	100
Firmicutes	PrAKZ1	*Bacillus* sp.	MW267036	99.40
Proteobacteria	PrAKZ3	*Acinetobacter* sp.	MW267038	100
Proteobacteria	PrAKZ4	*Burkholderia cepacia*	MW267039	99.67
Firmicutes	PrAKZ5	*Staphylococcus nepalensis*	MW267040	100
Actinobacteria	PrAKZ6	*Brachybacterium saurashtrense*	MW267041	99.68
Firmicutes	PrAKZ7	*Bacillus pumilus*	MW267042	100
Proteobacteria	PaPHC2	*Serratia marcescens*	MW267754	100
Proteobacteria	PaPHC3	*Pseudomonas aeruginosa*	MW267755	99.36
Firmicutes	PaPHC4	*Bacillus anthracis*	MW267756	99.04
Proteobacteria	PaLHE1	*Serratia marcescens*	MW267757	100
Firmicutes	PaLHE2	*Bacillus* sp.	MW267758	99.69
Proteobacteria	PaLHE3	*Pseudomonas aeruginosa*	MW267759	99.38
Proteobacteria	PaDASH1	*Burkholderia cepacia*	MW267761	99.67
Firmicutes	PaDASH2	*Bacillus cereus*	MW267762	99.37
Firmicutes	PaDASH4	*Bacillus subtilis*	MW267764	100
Firmicutes	PaDASH5	*Bacillus pumilus*	MW267765	99.70
Firmicutes	PaDASH6	*Bacillus amyloliquefaciens*	MW267766	99.07
Proteobacteria	PaGRA2	*Burkholderia cepacia*	MW267768	100

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Firmicutes	PaKRK1	*Bacillus subtilis*	MW267769	99.70
Proteobacteria	PaKRK2	*Acinetobacter baumannii*	MW267770	99.06
Proteobacteria	PaKRK3	*Pectobacterium carotovorum*	MW267771	100
Proteobacteria	PaKRK5	*Pseudomonas aeruginosa*	MW267773	100
Firmicutes	PaKDR1	*Lysinibacillus* sp.	MW267774	99.10
Firmicutes	PaKDR3	*Bacillus toyonensis*	MW267776	99.06
Phylogenetic group	Bacterial isolate	Bacterial species (Top match)	Accession number	Nucleotide identity (%)
Firmicutes	PaKDR4	*Bacillus anthracis*	MW267777	99.69
Firmicutes	PaKDR5	*Bacillus subtilis*	MW267778	100
Proteobacteria	PaKDR6	*Bacillus megaterium*	MW267779	99.40
Proteobacteria	PaKDR7	*Burkholderia cepacia*	MW267780	99.04
Firmicutes	PaKDR8	*Staphylococcus epidermidis*	MW267781	100
Firmicutes	PaCAKS1	*Bacillus subtilis*	MW267782	100
Firmicutes	PaCAKS2	*Bacillus* sp.	MW267783	100
Firmicutes	PaCAKS3	*Bacillus amyloliquefaciens*	MW267784	99.68
Firmicutes	PaCAKS4	*Bacillus pumilus*	MW267785	100
Firmicutes	PaCAKS5	*Bacillus mycoides*	MW267786	100
Firmicutes	PaCAKS7	*Bacillus subtilis*	MW267788	100
Firmicutes	PaAKZ2	*Bacillus* sp.	MW267790	99.38
Firmicutes	PaAKZ3	*Bacillus anthracis*	MW267791	99.68
Firmicutes	PaAKZ4	*Paraclostridium bifermentans*	MW267792	99.69
Firmicutes	PaAKZ5	*Lysinibacillus* sp.	MW267793	99.69

*Isolates that starts with Pr are from *P. rugaticeps* while those beginning with Pa are from *P. americana*. The table includes the top (closest) match using the NCBI databases.

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