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Original article

Investigation of gut microbial communities associated with indigenous honey bee (*Apis mellifera jemenitica*) from two different eco-regions of Saudi Arabia

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ABSTRACT

The microbial communities associated with the alimentary tract of honey bees are very important as they help with food digestion, provide essential nutrients, protect the host from pathogens, detoxify harmful molecules, and increase host immunity. In this study, the structural diversity of the gut microbial communities of native honey bees, *Apis mellifera jemenitica* from two different geographical regions (Riyadh and Al-Baha) of Saudi Arabia was analyzed by culture-dependent methods and 16S ribosomal RNA (rRNA) gene sequencing. In this study, 100 bacterial isolates were cultivated and phylogenetic analyses grouped them into three phyla: Proteobacteria, Firmicutes, and Actinobacteria. Bacteria in the phylum Proteobacteria were the most dominant (17 species), followed by Firmicutes (13 species) and Actinobacteria (4 species). Some of the identified bacteria (*Citrobacter* sp., *Providencia vermicola*, *Exiguobacterium acetylicum*, and *Planomicrobium okeanokoites*) were reported for the first time in the genus *Apis*, while others identified bacteria belonged to the genera *Proteus*, *Enterobacter*, *Bacillus*, *Morganella*, *Lactobacillus,* and *Fructobacillus*. To the best of our knowledge, this is the first study on the gut microbiota of the local honey bees in Saudi Arabia.

1. Introduction

The honeybee is a highly valued insect throughout the world, not only for honey production but also for its great importance to humans and ecosystems as pollinator of many economically important crops and wild flora (vanEngelsdorp and Meixner, 2010). The apiculture industry in Saudi Arabia and throughout the world is experiencing massive economic losses. The main drivers of these losses are environmental stresses, high summer temperatures and low winter temperatures (Naug, 2009), pollution, exposure to chemical compounds (Anderson et al., 2011), infectious diseases caused by different pathogens, and infectious diseases, parasites, including the Varroa mite (Hamdi et al., 2011).

One approach to address these challenges is to increase bee health by studying the interactions between gut bacteria and the host (Moran et al., 2012). Gut bacteria play significant roles in health and vitality (Dillon and Dillon, 2004), contribute enormously to host immunity (Mazmanian et al., 2005), ameliorate nutrient-deficient diets, degrade recalcitrant food ingredients, and protect host from parasites, and pathogens (Engel and Moran, 2013a). The indigenous gut microbial community also has the ability to restrain the growth of exogenous microbes through a process called colonization resistance (Dillon and Charnley, 2002).

Most of the bacteria in the gut microbial community of the adult honey bee (*A. mellifera*) belong to phyla Firmicutes, Actinobacteria, and Proteobacteria (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria) which comprise more than 95% of the detected 16S rRNA sequences and are considered the core gut bacteria (Martinson et al., 2011).

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Culture-independent studies based on 16S rRNA sequences and metagenomic surveys of adult honey bees (*A. mellifera*) demonstrated the existence of distinct gut microbial communities comprising eight core bacterial phylotypes belonging to the Acetobacteraceae, Betaproteobacteria, Gammaproteobacteria, and Firmicutes (Cox-Foster et al., 2007; Kwong and Moran, 2016; Moran et al., 2012; Martinson et al., 2011). In addition to the core bacteria, highly diverse, transient bacteria are also found in honey bees and within the hive environment, which may be transmitted by environmental sources (McFrederick et al., 2013). Gut bacteria play significant roles in host survival and fitness and can be transmitted either vertically or horizontally (Engel et al., 2012).

Beekeeping is an enduring practice in the rural communities of the Kingdom of Saudi Arabia, and it contributes significantly to the incomes of the rural residents. Two honey bee races, *A. m. jemenitica* (native honey bees) and *A. m. carnica* (exotic hybrid honey bees), are typically reared in Saudi Arabia. Native bees are preferred by beekeepers, since they are better adapted to the local arid climate than imported bees (Al-Ghamdi et al., 2012). It is hypothesized, that the distinct gut bacteria of native honey bees may be the reason for their adaptation to the harsh environment of Saudi Arabia. Although, the literature on the gut bacteria of honey bees is continuously increasing, there is no detailed information about the bacterial communities associated with the alimentary tract of the native honey bees in Saudi Arabia. Therefore, the aim of this study was to explore and characterize the bacteria colonizing the alimentary tract of the native honey bees, using culture-dependent methods, and compare the results to the gut microbiota from other honey bees.

2. Materials and methods

2.1. Sample collection

Incoming workers of *A. m. jemenitica* were collected with sterile forceps from two different geographic regions (Riyadh and Al-Baha) in March–April 2015–2016. Five apiaries each from Al-Baha, Al-Makhwa, and Biljurashi (in Al-Baha) and five from the Riyadh region were selected (Fig. 1). Three bee colonies from each apiary were chosen randomly, and 20 worker bees from each colony were collected in separate sterile 50 mL centrifuge tubes (Corning®) each containing 35 mL of sterile physiological saline (0.9% [wt/vol] NaCl, 0.1% [wt/vol] Tween 80, and 0.1% [wt/vol] Peptone) (Olofsson and Vásquez, 2008).

2.2. Isolation of gut microbiota

Prior to gut dissection, bees were disinfected to remove external microbes with a 1% aqueous solution of sodium hypochlorite. Each honey bee was immersed for 2 min and then rinsed three times in sterile purified water (Engel et al., 2013). The whole gut, from the ventriculus to the rectum, was aseptically dissected with sterile forceps under a laminar flow hood. Isolated guts (n = 10) from each colony were homogenized with a pestle in 10 mL of phosphate-buffered saline (PBS). Sterilized wooden cotton applicators (Shanghai Channelmed Co., Ltd. China) were used to spread the homogenate on five agar plates of each of the following selective media, Brain Heart Infusion (BHI) agar (Oxoid Ltd., Basinstock, Hampshire, England), Tryptic Soy Agar Blood Base (Hardy Diagnostics), Lactobacilli MRS agar (Neogen Corporation, Lansing, MI, USA), and BSM agar (Sigma–Aldrich, St. Louis, MO, USA), and incubated aerobically at 36 °C and 80% relative humidity for 2 days. Bacterial colonies grown on agar plates were selected based on size, color, and morphology. Selected colonies were repeatedly streaked individually on fresh agar plates to obtain pure bacterial culture.

2.3. DNA extraction

DNA was extracted from pure bacterial colonies with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

2.4. PCR and DNA sequencing

The primers used in the PCR to amplify the 16S rRNA gene, were 28F 5′-GAGTTTGATCNTGGCTCAG-3′ (Mattila et al., 2012) and 1392R 5′-ACGGGCGGTGTGTRC-3′ (Moran et al., 2012). The PCR conditions consisted of an initial denaturation step at 95 °C for 2 min, followed by 25 cycles of denaturation at 95 °C for 20 s, annealing at 53 °C for 40 s, and elongation at 70 °C for 2 min, followed by a final 10-min elongation step at 72 °C. PCR products from different bacterial isolates were sequenced by BGI Tech Solutions Co., Ltd. (Hong Kong). Sequences obtained were deposited to National Center for Biotechnology Information –NCBI (accession numbers KY027100-KY027195, KT901802-KT901804, KX268228).

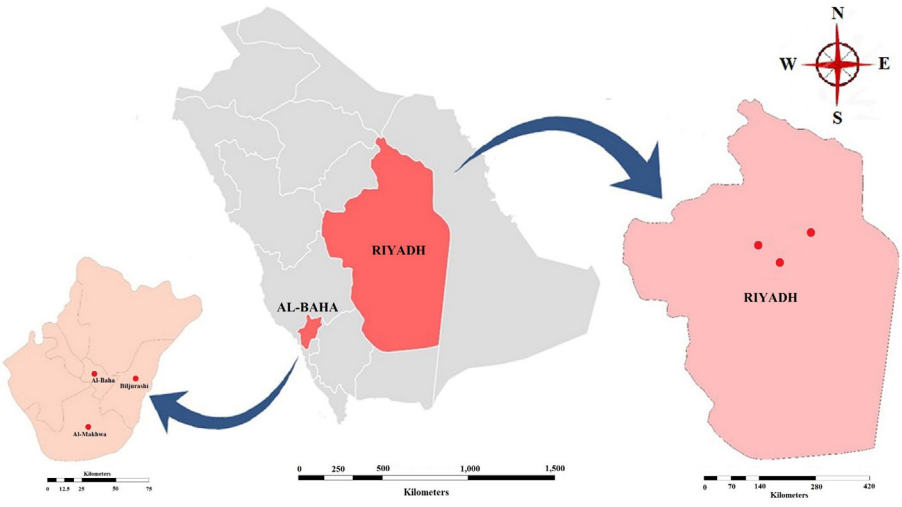

Fig. 1. Map of Saudi Arabia (center) showing the collection sites in the Al-Baha (left) and Riyadh (right) regions. Areas marked by circles are the sites for collection of *Apis mellifera jemenitica* workers.

2.5. Phylogenetic analysis

To study the diversity and evolutionary context of the gut microbiota, a phylogenetic analysis was performed. Closely related partial 16S rRNA sequences were retrieved from the National Center for Biotechnology Information (NCBI) database by BLAST-N (https://blast.ncbi.nlm.nih.gov). Multiple sequence alignments were generated and edited manually in Bio Edit (Hall, 1999). Phylogenetic and molecular evolutionary analyses of the sequences obtained from Riyadh and Al-Baha and sequences retrieved from GenBank were conducted using MEGA version 6 (Tamura et al., 2013) by the neighbor joining method with 1000 bootstrap replicates. All gaps and missing data were removed from the dataset by the complete deletion option.

2.6. Comparing the gut bacteria from Riyadh and Al-Baha

Gut bacterial isolates from the Riyadh and Al-Baha regions were compared based on the percentages of unique and shared species. All the bacterial species from these regions were inserted into an interactive online tool for comparing lists with Venn diagrams, Venny 2.1 (Oliveros, 2015).

3. Results

3.1. Isolation and characterization of gut bacteria

Two hundred forty-two bacterial colonies were isolated from the alimentary tract of *A. m. jemenitica* foragers. Based on colony color, size, and morphological differences, 100 colonies (36 from Riyadh and 64 from Al-Baha) were selected for molecular characterization. Characterization of the gut microbes from the Riyadh region of Saudi Arabia led to the identification of three phyla,12 genera, and 18 species.

Bacterial species isolated from the gut of honey bees in Riyadh, and their abundances are shown in Fig. 2. Of the identified species, eight species (44.45%) from five genera were in the phylum Proteobacteria, nine species (50.00%) from six genera were in the phylum Firmicutes, and one species was in the phylum Actinobacteria (5.55%; Fig. 2). Species within the respective phyla and the relative percentages of the bacterial genera or species in each phylum are shown. Similarly, the culture-dependent characterization of gut microbes from honey bees in the Al-Baha region led to the identification of 21 species from 15 genera in three phyla. There were 11 bacterial species (52.38%) from nine genera in the phylum Proteobacteria, eight species (38.01%) from five genera in the phylum Firmicutes, and two species (9.51%) from one genus in the phylum Actinobacteria (Fig. 3).

3.2. Phylogenetic analysis

Separate phylogenetic trees were constructed for bees from the Riyadh and Al-Baha regions. Thirty-six partial 16S rRNA gene sequences obtained from the Riyadh region and 49 sequences from honey bee gut bacteria identified in previous studies conducted in different countries obtained from NCBI were used to construct the phylogenetic trees (Fig. 4). In the neighbor-joining tree, three main phyla were observed. In the main group of Firmicutes, bacterial isolates Amj-2, AMJ107, AMJ110, AMJ121, and AMJ125 were clustered with *Bacillus* strains, that were previously isolated from honey bees in the USA and Japan. AMJ108 and AMJ122 were clustered with *Staphylococcus* and were more closely related to isolates from the USA than to those from Japan. Amj-3, AMJ102, and AMJ112 were also grouped with *Bacillus* and were related to the isolates from the USA. AMJ112 was clustered with *Exiguobacterium acetylicum* from China. AMJ106, AMJ116, AMJ124, and AMJ127 formed a separate group in the *Fructobacillus.* One bacterial isolate, AMJ114 was grouped in a cluster with *Lactobacillus* and was more closely related to isolates from the USA than to those from Sweden. In the second main group in the phylum Proteobacteria, four bacterial isolates from Riyadh were clustered with a *Proteus mirabilis* strain that was previously isolated from the gut of American honey bees.

Isolate AMJ130 was clustered with *Pantoea agglomerans*, which was isolated from honey bees in Norway and 15 isolates, AMJ101, AMJ129, AMJ115, AMJ105, AMJ103, AMJ111, AMJ132, AMJ128, AMJ113, AMJ126, AMJ120, AMJ117, AMJ104, AMJ118, and AMJ109 were clustered with *Enterobacter* and were closely related to previously described isolates from the USA and Japan. Isolate Amj-6 belonged to the phylum Actinobacteria and was distantly

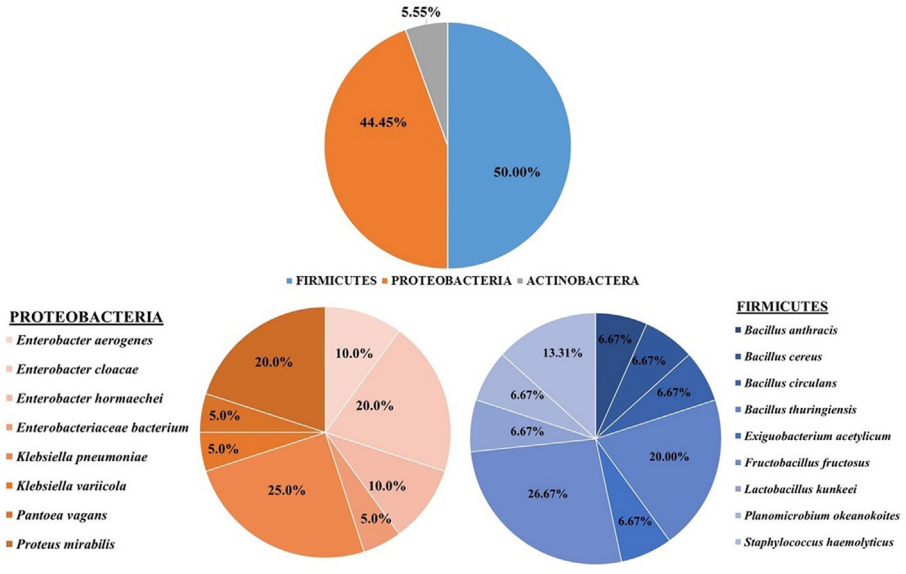

Fig. 2. Taxonomic allocation of the 16S rRNA gene sequences of the cultured gut bacteria from local honey bees (*Apis mellifera jemenitica*) in the Riyadh region of Saudi Arabia. The percentages of bacterial species within their respective phyla and the relative percentages of the genera or species in each phylum are shown.

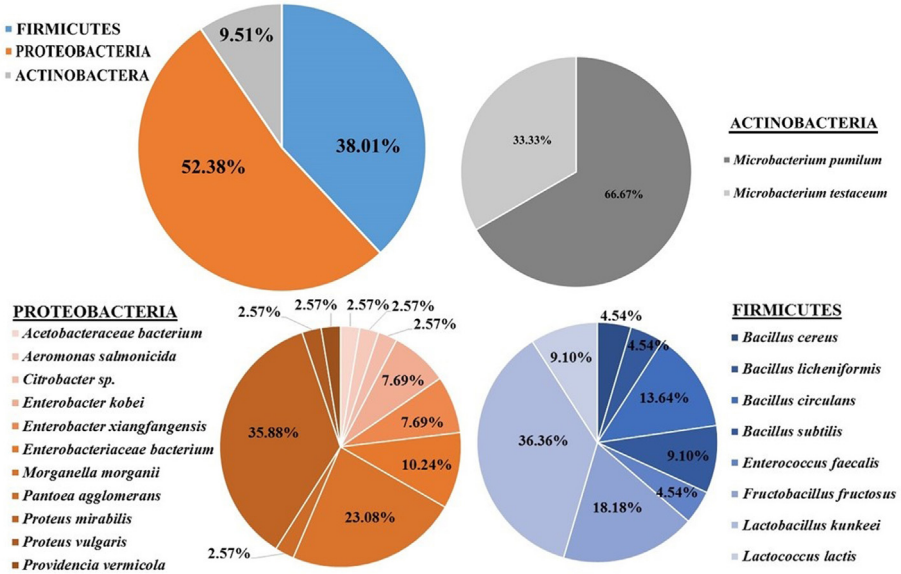

Fig. 3. Taxonomic allocation of the 16S rRNA gene sequences of the cultured gut bacteria from local honey bees (*Apis mellifera jemenitica*) in the Al-Baha region of Saudi Arabia. The percentages of bacterial species within their respective phyla and the relative percentages of the genera or species in each phylum are shown.

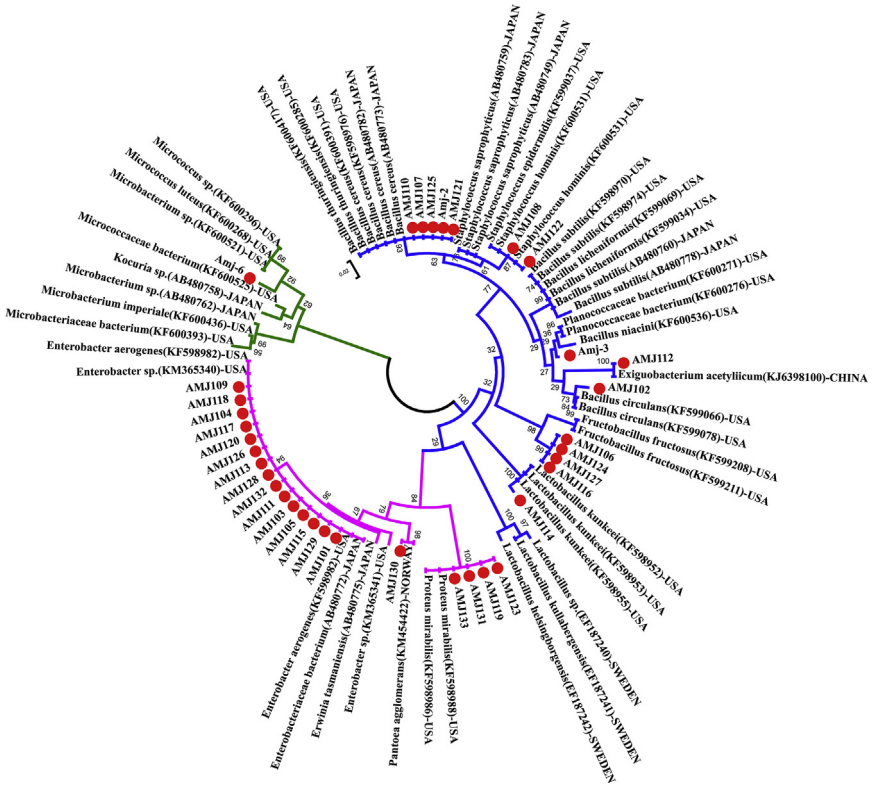

Fig. 4. Phylogenetic tree based on the 16S rRNA gene sequences of gut bacteria isolated from the local honey bees in Riyadh, Saudi Arabia and closely related sequences retrieved from NCBI databases. The tree was constructed by neighbor-joining method using MEGA (V 6.0.6). Bootstrap values, expressed as percentages of 1000 replicates, are shown at each branch. GenBank accession numbers are shown in parentheses.

grouped with a *Micrococuus* strain from the USA. Similarly, a phylogenetic tree was constructed for 64 partial 16S rRNA gene sequences from the gut bacteria from the honey bees in the Al-Baha region and 53 selected related sequences of honey bee gut bacteria isolated in other countries that were obtained from NCBI (Fig. 5). Three phyla Firmicutes, Proteobacteria, and Actinobacteria (with sub clusters), were observed in the tree. In the main phylum, Proteobacteria, 15 bacterial isolates (AMJ205, AMJ249, AMJ233, AMJ230, AMJ217, AMJ211, AMJ207, AMJ209, AMJ213, AMJ222, AMJ227, AMJ239, AMJ256, AMJ255, and AMJ259) were clustered with strains of *Proteus* previously isolated in the USA. AMJ240 was clustered with a *Providencia alcalifaciens* strain isolated from Japanese honey bees, and nine isolates (AMJ201, AMJ214, AMJ229, AMJ238, AMJ248, AMJ254, AMJ258, AMJ261, and AMJ263) were closely related to previously described isolates of *Morganella* from the USA.

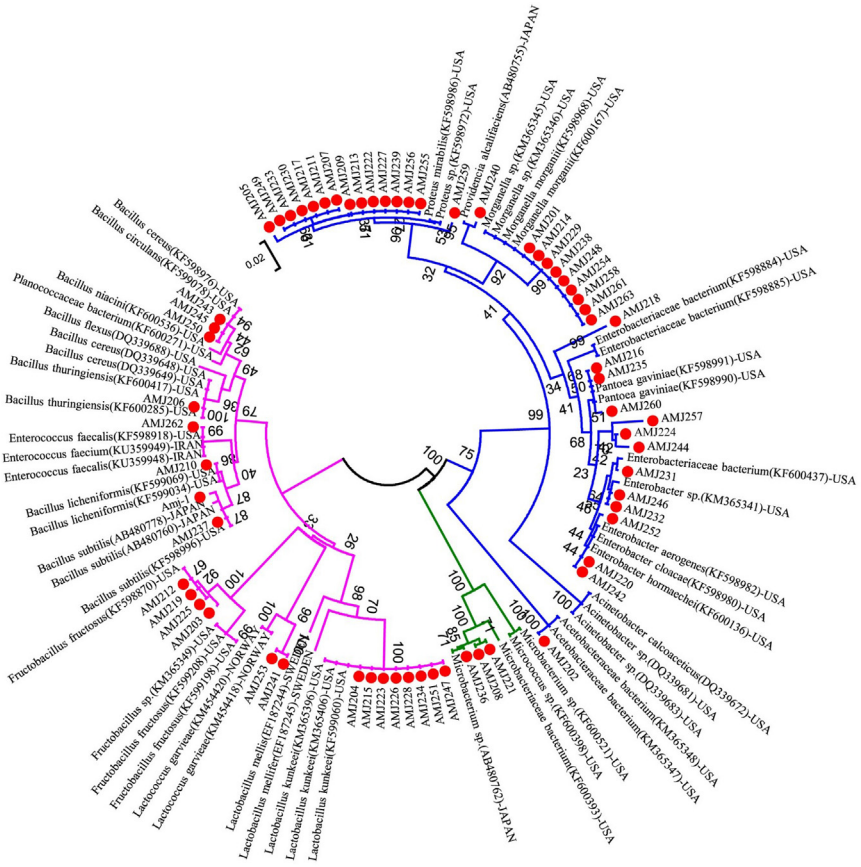

Fig. 5. Phylogenetic tree based on the 16S rRNA gene sequences of gut bacteria isolated from the local honey bees in Al-Baha, Saudi Arabia and closely related sequences retrieved from NCBI databases. The tree was constructed by the neighbor-joining method using MEGA (V 6.0.6). Bootstrap values, expressed as percentages of 1000 replicates, are shown at each branch. GenBank accession numbers are shown in parentheses.

Species in the genera *Enterobacter*, *Enterobacteriaceae*, and *Pantoea* were in the third phylum, Proteobacteria. Three bacterial isolates (AMJ216, AMJ235, and AMJ260) were related *Pantoea* isolates from the USA. AMJ257, AMJ224, and AMJ244 were distantly related to the *Pantoea*, *Enterobacteriaceae*, and *Enterobacter* and formed a separate group. AMJ231 was clustered with *Enterobacteriaceae* from the USA, and five other isolates (AMJ246, AMJ232, AMJ252, AMJ220, and AMJ242) were grouped with *Enterobacter* from the USA. AMJ202 was clustered with *Acetobacteraceae* from the USA. In phylum Actinobacteria, the three isolates (AMJ208, AMJ221, and AMJ236) were more closely related to gut bacteria from the genus *Microbacterium*, that were previously isolated from Japanese honey bees than those isolated in the USA.

In the Firmicutes, eight bacterial isolates (AMJ204, AMJ215, AMJ223, AMJ226, AMJ228, AMJ234, AMJ247, and AMJ251) were clustered with *Lactobacillus*. These were more closely related to gut bacteria isolated from honey bees in the USA and distantly related to isolates from Sweden. AMJ241 and AMJ253 belonged to the *Lactococcus* and were related to previous isolates from honey bees in Norway. Four bacterial isolates (AMJ203, AMJ225, AMJ219, and AMJ212) formed a distant group within the cluster containing *Fructobacillus* isolates from US honey bees. In the third phylum, Firmicutes, there were sub-clusters in the genera *Bacillus* and *Enterococcus*. AMJ243, AMJ245, and AMJ250 were grouped with *B. circulans* and *B. cereus* isolates from honey bees in the USA. AMJ206 was grouped with *B. thuringiensis* and *B. cereus* isolates from honey bees in the USA. AMJ262 was grouped with *Enterococcus* isolates from honey bees in the USA and Iran. AMJ210 was grouped with *B. licheniformis* isolates from honey bees in the USA. Two bacterial isolates (AMJ237 and Amj-1) were grouped with *B. subtilis* isolates from honey bees in the USA and Japan.

3.3. Comparison of honey bee gut microbiomes between Riyadh and Al-Baha

The relative abundance of the bacterial isolates in native honey bees was high in Al-Baha, with 15 bacterial species (45.5%) that were unique to this region, whereas 12 species (36.4%) were unique to Riyadh. Seven bacterial species (18.2%) were common in honey bees from Riyadh and Al-Baha (Fig. 6).

4. Discussion

This study was focused on the isolation and characterization of bacteria from the alimentary tracts of worker honey bees (*A. m. jemenitica*) in Saudi Arabia. Significant diversity was observed in the gut bacteria. The results of the analysis revealed that *A. m. jemenitica* foragers from Riyadh harbored gut bacteria belonging to the taxonomic groups of Actinobacteria, Firmicutes, and Gammaproteobacteria, whereas foragers from the Al-Baha region had one additional bacterial class, Alphaproteobacteria. However, Betaproteobacteria and Bacteroidetes were absent, and these classes were also missing from the culture-dependent studies of Mohr and Tebbe (2007). Some of the gut bacteria detected in the study (*Citrobacter* sp., *Providencia vermicola*, *Exiguobacterium acetylicum*, and *Planomicrobium okeanokoites*) have not been previously reported in the genus *Apis*. In this study, we used 100 gut bacterial

Fig. 6. Venn diagram showing the percentage of unique and shared gut bacterial species. The diagram compares the bacterial species isolated from the gut of local honey bees (*Apis mellifera jemenitica*) in the Riyadh and Al-Baha regions of Saudi Arabia.

isolates, and most of their 16S rRNA gene sequences were identical or highly similar to sequences that were previously found in culture-dependent studies of honey bees (Anderson et al., 2013; Corby-Harris et al., 2014b; Evans and Armstrong, 2006; Ludvigsen et al., 2015; Olofsson and Vásquez, 2008; Tajabadi et al., 2011b; Yoshiyama and Kimura, 2009). However, this study did not find 16S rRNA gene sequences that were similar to some core gut bacteria species that were previously detected in culture-independent studies of honey bees. Similarly, some researcher from Japan, Yoshiyama and Kimura (2009) and Wu et al. (2014) could not isolated some core gut bacteria from *A. cerana japonica* with culture-dependent methods. In addition, Tajabadi et al. (2011b), were not able to isolate core gut bacteria from *A. dorsata* in Malaysia by culture-dependent methods. Similarly, Evans and Armstrong (2006) were not able to isolate core-gut bacteria from *A. mellifera* larvae by culture-dependent methods. In this study, Proteobacteria and Firmicutes composed 90.39% and 94.45% of the isolates in bees from the Al-Baha and Riyadh regions, respectively. This was in accordance with the results of the study by Ahn et al. (2012), who reported the dominance of Proteobacteria and Firmicutes (91.7% and 100% respectively) in the honey bee gut.

Honey bees in Riyadh and Al-Baha shared 18.2% gut bacterial isolates in common. The overall complexity of the microbial communities in honey bees in the Riyadh region was lower (36.4%) than that of Al-Baha (45.5%). This difference in the gut microbial communities might be due to difference in the gut physiological conditions, such as pH, etc. (Yoshiyama and Kimura, 2009), the presence of environmental bacteria from nectar and pollen sources (Anderson et al., 2013), the age of the honey bees, and the season or prevailing characteristics of the environments that affect the geographical location (Gilliam, 1997).

The gut microbial diversity of *A. m. jemenitica* in this study was higher than that in previous culture-dependent studies of *A. mellifera* (Evans and Armstrong, 2006; Gilliam, 1997; Piccini et al., 2004), but was comparable to that of other studies (Wu et al., 2014; Yoshiyama and Kimura, 2009) in which the microbial diversity of *A. c. japonica* was enumerated by using culture-dependent methods. However, the microbial diversity of bees from both regions was lower than that of previous studies in which culture-independent methods were used for microbial isolation (Ahn et al., 2012; Anderson et al., 2013; Babendreier et al., 2007; Corby-Harris et al., 2014a; Cox-Foster et al., 2007; Engel and Moran, 2013b; Engel et al., 2012; Horton et al., 2015; Martinson et al., 2011; Mohr and Tebbe, 2006; Moran et al., 2012).

Bacterial species of the genus *Bifidobacterium* were not detected in the alimentary tract of *A. m. jemenitica* in Riyadh and Al-Baha, in agreement with the results of Babendreier et al. (2007), Disayathanoowat et al. (2012), Evans and Armstrong (2006), Mohr and Tebbe (2006), Saraithong et al. (2015), Tajabadi et al. (2011a), Wu et al. (2014), Yoshiyama and Kimura (2009). However, the non-detection of *Bifidobacterium* in this study contradicts the results of Ahn et al. (2012), Anderson et al. (2016, 2013), Corby-Harris et al. (2014a), Cox-Foster et al. (2007), Engel and Moran (2013b), Engel et al. (2012), Gilliam (1997), Horton et al. (2015), Jeyaprakash et al. (2003), Ludvigsen et al. (2015), Martinson et al. (2011), Moran et al. (2012), Olofsson and Vásquez (2008), Sabree et al. (2012). The possible reason for non-detection of *Bifidobacterium* may be its leower abundance (Horton et al., 2015; Kwong and Moran, 2016), as it comprised only 1.6–3.9% of the bacteria in adult *A. mellifera* and 0.1–0.4% in *A. cerana* (Ahn et al., 2012).

Lactobacillus Firm-4 and Firm-5 are considered to be core gut bacteria of *A. mellifera*, and they have been consistently detected irrespective of geography, environment, and subspecies (Martinson et al., 2011). Evans and Armstrong (2006) did not detect these bacteria in *A. mellifera* larvae by culture-dependent methods. *Lactobacillus* were also not detected in the gut of *A. c. japonica* (Wu et al., 2014; Yoshiyama and Kimura, 2009). In the present study, *Lactobacillus* Firm-4 and Firm-5 were also detected however, *L. kunkeei* was detected in bees from Riyadh and Al-Baha. These detected strains were phylogenetically close to the 16S sequences detected in previous studies (Anderson et al., 2013; Olofsson and Vásquez, 2008; Vásquez et al., 2012). Olofsson and Vásquez (2008) and Vásquez et al. (2012) detected a *L. kunkeei* strain Fhon2, which was consistently detected in honeybees and fresh honey samples. Vojvodic et al. (2013) isolated *L. kunkeei* from the gut of Africanized and European honey bee larvae, even first instar larvae, and observed a greater abundance in managed honey bees compared to the abundance in bees that

had access to a pollination environment. Saraithong et al. (2015) observed abundant *L. kunkeei* in the gut of *A. florea* larvae. Anderson et al. (2013) found that *L. kunkeei* was the most frequent bacterium in the gut of honey bees, and was also present in pure honey, floral nectar, and beebread. However, they suggested that the abundance of *L. kunkeei* was due to culturing bias, as these bacteria were absent or rare in other culture-independent studies (Corby-Harris et al., 2014a; Engel and Moran, 2013b; Engel et al., 2012; Horton et al., 2015; Martinson et al., 2011; Moran et al., 2012; Sabree et al., 2012). The existence of *L. kunkeei* in the gut of *A. m. jemenitica* (this study), and larval guts and flower nectar (Vojvodic et al., 2013), honey bees and fresh honey (Olofsson and Vásquez, 2008), and the gut of *A. florea* larvae (Saraithong et al., 2015) demands further study to clarify the position of *L. kunkeei* as a core or non-core gut bacterium.

Most of the Gammaproteobacteria from the Riyadh and Al-Baha regions were similar to those that were detected in previous culture-dependent (Anderson et al., 2013; Gilliam, 1997; Ludvigsen et al., 2015; Mohr and Tebbe, 2007; Vojvodic et al., 2013; Yoshiyama and Kimura, 2009) and culture-independent (Ahn et al., 2012; Corby-Harris et al., 2014a; Cox-Foster et al., 2007; Disayathanoowat et al., 2012; Horton et al., 2015; Martinson et al., 2011; Sabree et al., 2012) studies. This variance in Proteobacteria diversity and numbers among the two regions may be associated with the effects of season and/or the pollination landscape. Ludvigsen et al. (2015) observed that the numbers of Gammaproteobacteria are influenced by season.

In previous studies, culture-dependent methods used to isolate gut bacteria identified many bacterial phylotypes belonging to the genus *Bacillus* (Evans and Armstrong, 2006; Gilliam, 1997; Mohr and Tebbe, 2007; Wu et al., 2014; Yoshiyama and Kimura, 2009). *Bacillus* bacteria grow well at pH 6 or higher, whereas the intestines of adult bees have typically less than pH 5.0 (Mohr and Tebbe, 2006). This difference in pH should discourage the growth of *Bacillus* species in the gut. hence, these bacteria were either absent or found in low numbers in culture-independent studies. In the Riyadh and Al-Baha honey bee samples, strains of the genus *Bacillus* were detected, and our results verified those of previous culture-dependent methods. The possible reason for their detection may the presence of *Bacillus* spores within the gut and subsequent spore germination on growth medium (Mohr and Tebbe, 2006).

F. fructosus strains, which prefer fructose over glucose, were present in almost equal numbers in the two regions. *F. fructosus* was also isolated by Vojvodic et al. (2013) from the gut of Africanized and European honey bee larvae and by Endo and Salminen (2013) from *A. mellifera*. Anderson et al. (2013) detected these bacteria and many other Firmicutes, but not in honey bee gut. They isolated these bacteria from the pollination environment, in flower nectar and honey bee sources. *L. kunkeei* and *F. fructosus* are undetectable in honey bee crop and food stores, and their detection seems to be associated with flower type or season (Martinson et al., 2011).

5. Conclusions

Adult honey bees (*A. m. jemenitica*) from Riyadh harbored gut bacteria belonging to the Actinobacteria, Firmicutes, and Gammaproteobacteria, whereas bees from the Al-Baha region harbored one additional bacterial class, the Alphaproteobacteria. Gut bacteria belonging to the genera *Citrobacter*, *Providencia*, *Exiguobacterium*, and *Planomicrobium* were detected for the first time in the genus *Apis*. Bees in Riyadh and Al-Baha shared 18.2% of their gut bacterial isolates. The complexity of the gut microbiomes in bees from the Riyadh region was lower (36.4%) than that in bees from Al-Baha (45.5%). This difference in gut microbial communities might be due to differences in gut physiological conditions, the presence of transient bacteria in the pollination environment, the age of the honey bees, and/or the season or prevailing characteristics of the environments that are affected by these geographical locations.

Authors contribution

Khalid Ali Khan and Mohammad Javed Ansari contributed equally to this work.

Declaration of interest

The authors confirm that there is no conflict of interests and are also liable for the content and writing of this article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sjbs.2017.01.055.

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