



CHARACTERIZATION OF BIFIDOBACTERIA IN THE INDIGENOUS HONEYBEE OF SAUDI ARABIA

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ABSTRACT

Bifidobacteria were isolated from the intestinal tract of the indigenous honeybee of Saudi Arabia, *Apis mellifera jemenitica*, and investigated for potential application as a probiotic agent against some drug multi-resistant human pathogen, based on the findings of *in vitro* inhibition assays. A total of 05 bifidobacteria strains (designated as KsuBF01-KsuBF05) were isolated using a culture-dependent method and their 16S rRNA gene sequences were analysed. The KsuBF isolates belonged to three distinct bifidobacterial phylotypes that were similar to those found in the Japanese honeybee, *Apis cerana japonica*. Although the Saudi Arabian and Japanese honeybees are distinct species with different traits and habits, the observation that they share highly similar bifidobacterial phylotypes suggests that bifidobacteria are conserved among honeybee species. Despite having extremely high 16S rRNA gene sequence similarities, the KsuBF isolates had markedly different carbohydrate fermentation profiles. In addition, *in vitro* growth inhibition assays revealed that the cell-free supernatants of all KsuBF isolates exhibited antagonistic effects on Drug multi-resistant *E. coli*, *P. aureginosa*, *B. subtilis* and *S. aureus* growth. These results indicate that the bifidobacteria isolated from the gut of indigenous Saudi Arabian honeybee could potentially be employed to produce some probiotics against some human pathogens.

METHODOLOGY

In March 2013, 10 adult workers of *A. m. jemenitica* were collected at the entrance of a hive in the Al-Baha area of Saudi Arabia and their digestive tracts were dissected aseptically. Wilkins-Chalgren medium (Oxoid, UK) was used as selective medium for isolating bifidobacteria (Ferraris et al., 2010). Colonies grown on the plates were selected according to small size and white color. A total of nine bifidobacterial colonies were obtained and repeatedly grown on Wilkins-Chalgren agar plates.

The pure isolated colonies were used as templates for polymerase chain reaction (PCR) amplification of 16S rRNA genes. The 16S rRNA genes of bifidobacteria were amplified using primers Im26 (50-GAT TCT GGC TCA GGA TGA ACG-30) and Im3 (50-CGG GTG CTI CCC ACT TTC ATG-30) (Kaufmann et al., 1997). Biochemical typing of each of the nine bifidobacterial colonies was performed using an API 50CH biochemical kit with the supplied medium (BioMerieux).

In vitro growth inhibition assay was performed and nine bifidobacteria strains were cultured anaerobically on Wilkins-Chalgren liquid medium at 35 C for 48 h. The cell-free supernatant (CFS) was recovered by centrifugation at 4000g for 4 min at 10 C and filtered (pore size: 0.22 µm). Drug multi-resistant human pathogens were cultured on nutrient agar plates at 35 C. Antagonistic activity was performed using cell diffusion assay.

Scanning electron microscopy (SEM) (Leo 435, Cambridge, USA) was performed to examine the morphological characteristics of the isolated bifidobacteria.

Bifidobacterium isolates/Human Isolates	Mean (±SD) diameter of inhibition zone against different Human Isolates (in mm)		
	<i>E. coli</i>	<i>P. aureginosa</i>	<i>S. aureus</i>
ksuBF-1	20.25 ± 1.2	21.50 ± 1.4	21.25 ± 1.1
ksuBF-2	12.25 ± 0.7	19.25 ± 1.0	12.00 ± 0.6
ksuBF-3	11.75 ± 0.5	12.50 ± 0.4	13.25 ± 0.4
ksuBF-4	10.75 ± 0.6	12.25 ± 0.4	14.50 ± 0.5
ksuBF-5	10.75 ± 0.7	10.75 ± 0.3	04.50 ± 0.4

Table: *In vitro* antagonistic effect of gut Bifidobacterial isolates against different human drug multi-resistant isolates

INTRODUCTION

The honeybee (*Apis mellifera*) is a highly valued resource worldwide and is of great relevance for humans and the entire ecosystem (vanEngelsdorp & Meixner; 2010). The total annual global economic worth of pollination amounts 202 billion USD, representing 10% of the value of the global agricultural production. However, very unfortunately, this honeybee is facing enormous threat worldwide including Saudi Arabia from a wide range of infectious diseases, including American foulbrood (AFB), European foulbrood (EFB), Chalkbrood disease, Nosema disease, and a variety of viral pathogens (Al-Ghamdi, 1990). Hence, the declines of both managed and wild pollinators are of increasing concern (vanEngelsdorp & Meixner; 2010) because of food security issues.

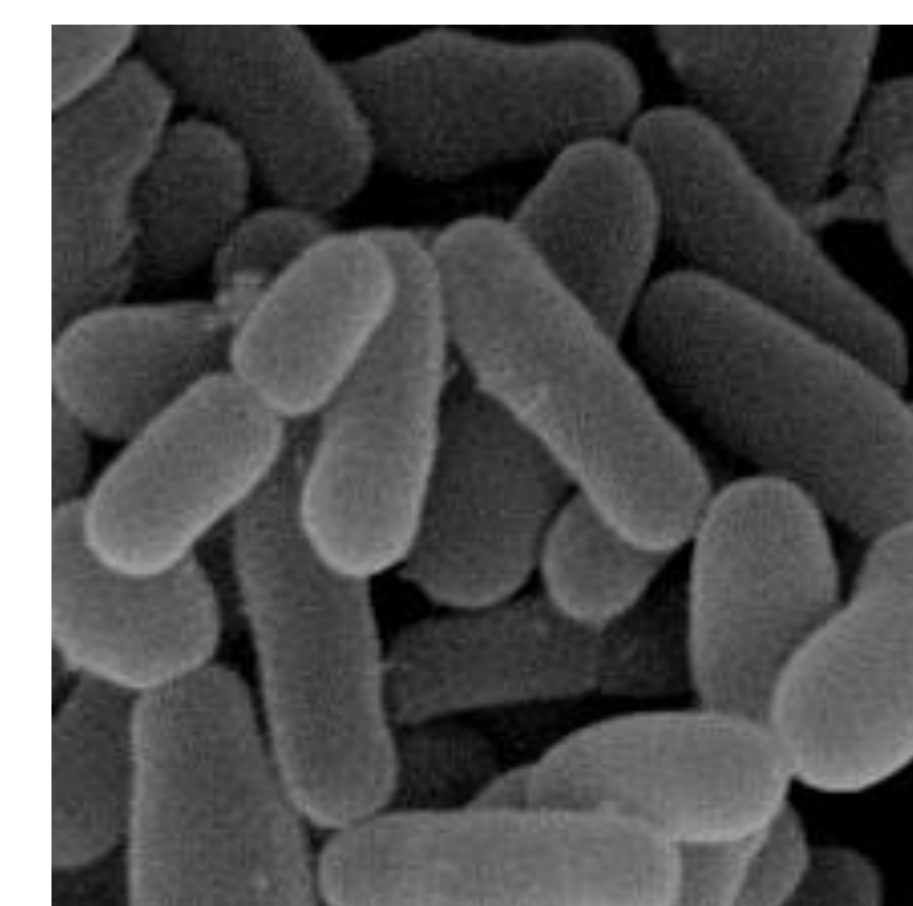
Bifidobacteria are the principal commensal microflora of the human and animal gastrointestinal tracts. They are widely used as human and animal probiotics because of their potential benefits to the host. Three *Bifidobacterium* species have been so far isolated from the insect digestive tract: *Bifidobacterium asteroides* and *Bifidobacterium coryneforme* are the two species found in the *Apis mellifera* intestine, and *Bifidobacterium indicum* in the intestine of *Apis cerana* and *Apis dorsata* from the Philippines and Malaysia (Scardovi & Trovatelli; 1969). Three distinct bifidobacterial phylotypes were isolated from the digestive tract of the Japanese honeybee, *Apis cerana japonica* (Wu et al. 2013).

The indigenous bee race of Saudi Arabia, *A. m. jemenitica* Ruttner has been used in apiculture throughout the Arabian Peninsula since at least 2000 BC. It is the smallest race of *A. mellifera*, and well established in local harsh environmental conditions, including high temperatures and low rainfall and tolerates hunger for long periods of time (Alqarni et al. 2011). Given the lack of information on the incidence of bifidobacteria in this honeybee species, we characterized bifidobacteria isolated from the intestinal tract of the Saudi Arabian honeybee and investigated the potential antagonistic effect of these bacteria on some Drug Multi-Resistant human pathogens.

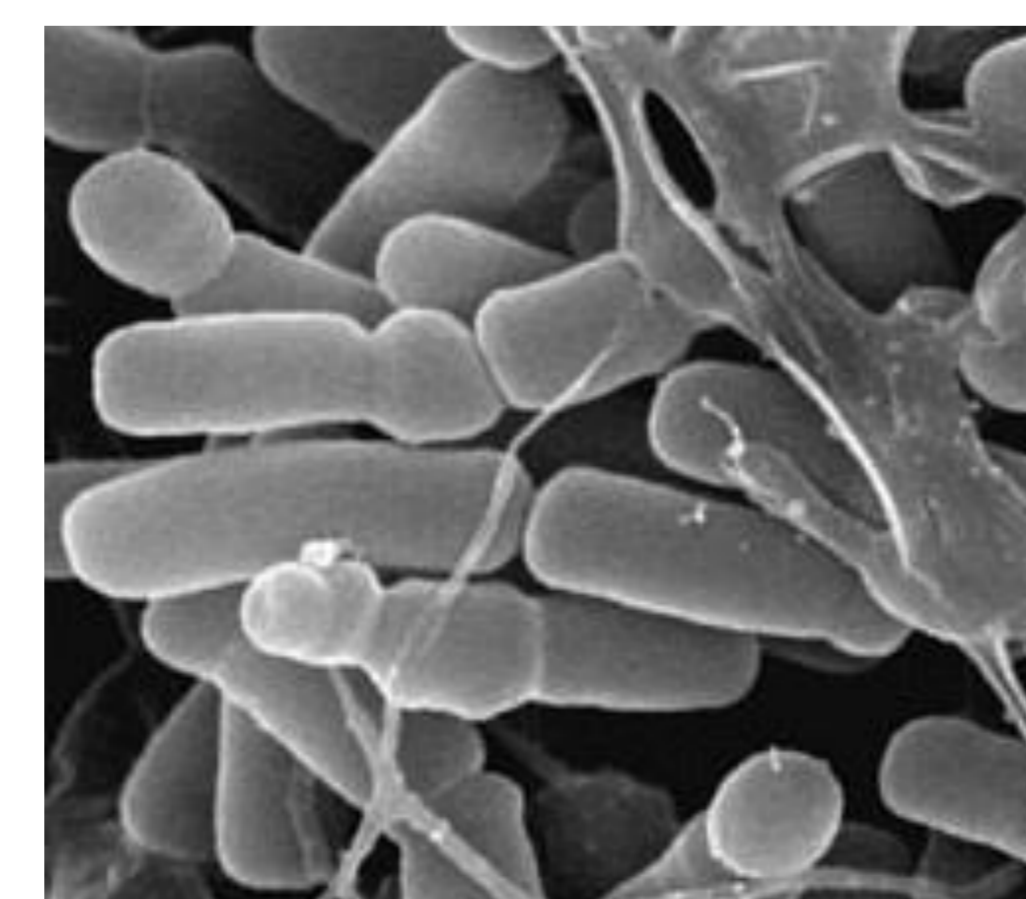
RESULTS



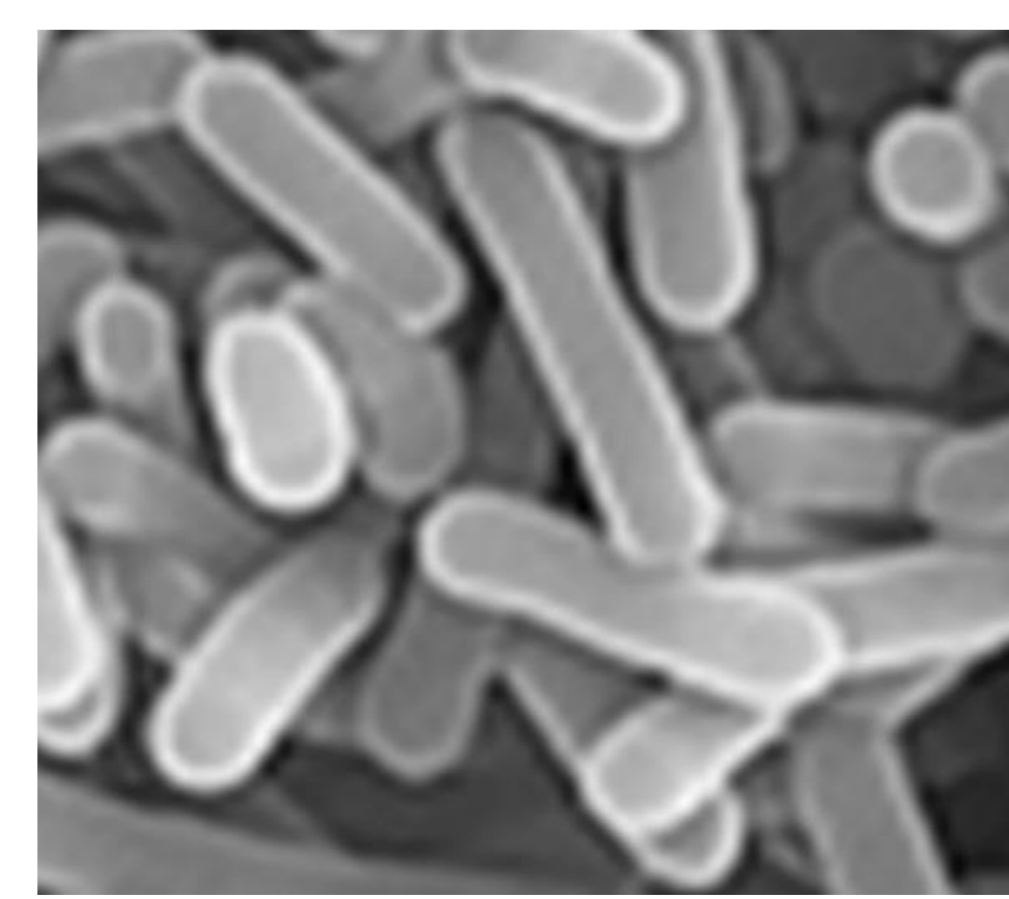
ksuBF-1



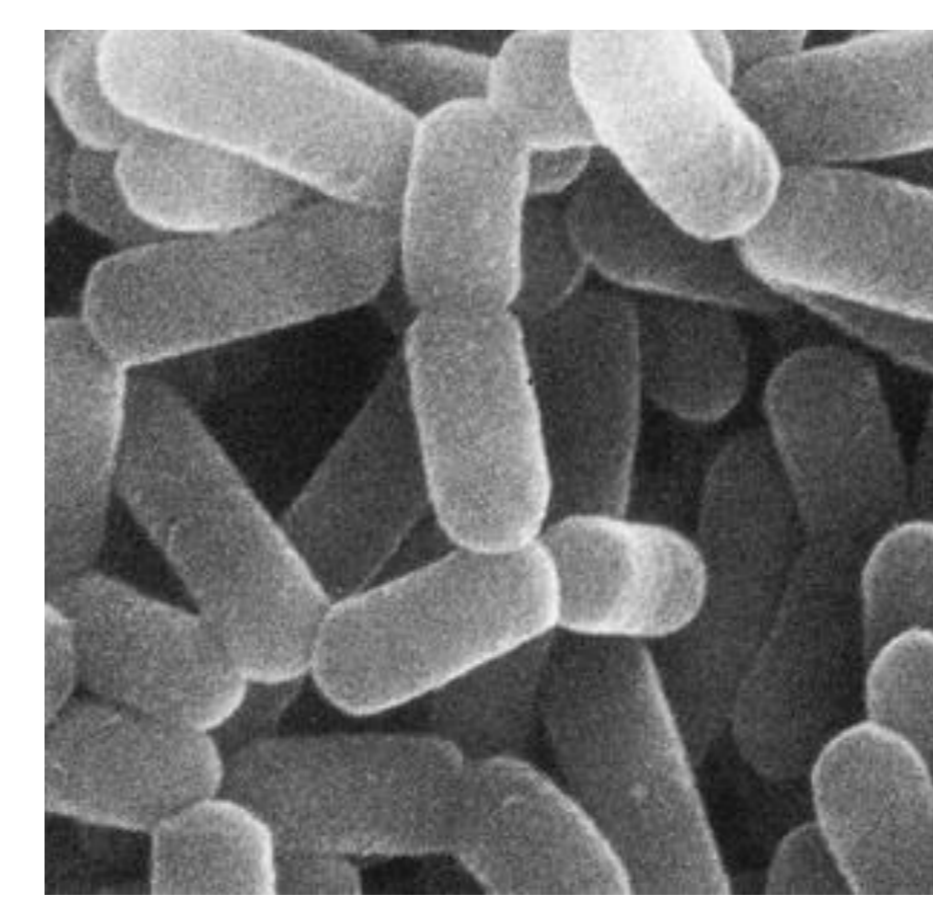
ksuBF-2



ksuBF-3



ksuBF-4



ksuBF-5

FIG. 1: Scanning electron micrographs of ksuBF isolates (ksuBF-1 - ksuBF-5)

CONCLUSIONS

The results presented in this paper demonstrate that most of the honeybee gut Bifidobacteria have potential antibacterial activities against different drug multi-resistant human pathogens. Bacteria associated with the native Saudi Arabian honeybee, *A. m. jemenitica* could be used to develop various pathogen management strategies. The use of symbiotic gut bacteria could represent a natural alternative to the use of synthetic antibiotics in the control of various humandisease caused by pathogenic bacteria, which should therefore reduce antibiotic resistance and the levels of antibiotic residues. Further research must be conducted on these beneficial bacteria to isolate the active antagonistic compound that kill these human pathogens.

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