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Characterization of *Lactobacillus* Species Recovered from Raw Dromedary Milk in Relation to its Antimicrobial Activity

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ABSTRACT

Dromedary milk (camel's milk) is high in nutritious than cow milk due to its high content of antibacterial agents and vitamin C. The current research goal is to characterize and estimate the antimicrobial activity of Lactobacillus species (spp.) recovered from Dromedary milk samples. Fifty raw dromedary milk samples were obtained from camels raised in five Egyptian Governorates. The isolates were further identified using morphological, biochemical, and PCR. Twenty-two isolates (44%) were identified as *Lactobacillus* spp., *Lactobacillus plantarum* (18%), *L. acidophilus* (14%), *L. fermentum* (4%), *L. casei* sub spp. *Pseudoplantarum* (4%), *L. paracasei* (2%) and *L. brevis* (2%). The antibacterial properties of Lactobacillus spp, were conducted against several foodborne pathogens such as *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus*, and *listeria monocytogenes*, as well as *Aspergillus flavus*. *L. plantarum* isolates were found to possesses the highest inhibitory activity versus *S. aureus* and *Salmonella* Typhimurium. High antibacterial activity was observed by *L. acidophilus* on *S. aureus* and *S.* Typhimurium although, *Lactobacillus plantarum* showed the highest inhibition of fungal growth followed by *L. acidophilus*, while *L. fermentum* and *L. casei* sub spp.. *Lactobacillus plantarum* showed antibacterial and antifungal activities, and further investigations are needed to be used as a potential probiotic-like organism.

Key words: Dromedary milk, *Lactobacillus* spp., *Lactobacillus plantarum*, Foodborne pathogen, Probiotics.

INTRODUCTION

Camelus dromedary (Humped camel) is one of several animal species in the desiccated regions of Africa, especially in East African countries (Sudan, Somalia, Ethiopia, Djibouti, and Kenya) and Asia. Camel is unique adapted livestock species to the arid and hot environment than other domestic animals (Dioli 2020). Africa has 32 million of the 35 million world's camels (FAO 2019).

Dromedary milk is nutritional food for people living in deserts and dry areas of Africa and central Asia (Sani et al. 2019). Dromedary milk microflora possesses a main therapeutic effect (enhancing the digestion properties and antimicrobial character) by its fermentation character (Akhmetsadykova et al. 2015). Lactic acid bacteria (LAB) form the base of probiotics, and lactobacilli are the principal group (Rivera-Espinoza and Gallardo-Navarro 2010). LAB are non-spore-forming Gram-positive bacteria that lactic acid is the prime fermentation output of carb wherefore they are used as a starter culture (Singh and Sharma, 2009). LAB are food-grade bacteria harmless and beneficial to take in that can suppress pathogenic bacteria through contesting for binding sites and nutrients. (Saputri et al. 2018). Recently, there were many works on lactobacilli as probiotics recovered from dromedary milk (Monteagudo-Mera et al. 2012; Sharma et al. 2021). LAB act as probiotic microorganisms by secreting compounds that enhance the immune system, have anti-mutagenic effects and increase the activity and delivery of enzymes (Zommiti et al. 2020).

The current study aimed to isolation and characterization of *Lactobacillus* spp. from Dromedary milk as an essential probiotic bacterium in addition to evaluating its antimicrobial activity of again several foodborne microorganisms; *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus* and *listeria monocytogenes* and *Aspergillus flavus*.

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MATERIALS AND METHODS

Sampling

Fifty raw milk specimens were collected from camels raised in the desert (Mars Matrouh, Aswan, and Sinia) and from local farms in some governorates of Egypt (Cairo, Giza, and Alexandria). Collected samples were kept in sterile bottles, stored in a cooler, and transferred back as soon as possible to the lab for isolation and identification of *Lactobacilli*.

Recovering and Characterization of *Lactobacilli* from Dromedary's Milk

Recovering was carried out according to Ashmaig et al. (2009) using de Man Rogosa and Sharpe broth (MRS broth, Oxoid) for enrichment at 37°C for 48h then a loopful from enrichment broth was streaked onto de Man Rogosa and Sharpe agar (MRS agar, Oxoid). Plates were anaerobically incubated using anaerogen bags (AnaeroGen, Oxoid) at 37°C for 48h. Suspected colonies were being typed according to Gram's stain uptake, ability to form spore, oxidase, and catalase activities. All Gram-positive rods and catalase-negative colonies suspected to be *Lactobacillus* spp. and further examined by Vitek 2 compact system method according to the manufacture's instruction (Pincus 2006).

Molecular Identification of Recovered *Lactobacillus plantarum* Isolates

DNA extraction of suspected *Lactobacillus plantarum* isolates was done using a QIAamp DNA mini kit (Catalogue # 51304) according to its instructions. The used Oligonucleotide primers in PCR were get from Metabion (Germany) (Table 1), Agarose gel electrophoreses was done according to Green and Sambrook (2012).

Antimicrobial Inhibition Potency

Antibacterial activity of *Lactobacillus* spp. was assessed against four pathogenic bacterial strains; *Escherichia coli* (ATCC 25922), *Salmonella* Typhimurium (ATCC 14028), *Staphylococcus aureus* (ATCC 25923), and *listeria monocytogenes* (ATCC7644) by agar well diffusion method (Abbas and Mahasneh 2014).

One fungal strain *Aspergillus flavus* generously - provided from the Microbiology Department, Faculty of Veterinary Medicine, Cairo University-was used to detect the antifungal activity of *Lactobacillus* spp. using the agar overlay method (Magnusson and Schnu¨rer 2001).

Statistical Analysis

Data were analysed using Statistical excel (Microsoft 2013). The results were shown as the mean±SD.

RESULTS

Lactobacilli Recovery from Dromedary's Milk

Out of 50 milk samples, 22 isolates (44%) were recovered and classified as Gram-positive rods, non-spore formers, catalase and oxidase negative and tentatively suspected to be *Lactobacillus* species confirming the selectivity of MRS agar.

These isolates were identified by Vitek 2 compact system method as nine isolates of *Lactobacillus plantarum*, seven *L. acidophilus* isolates, two isolates of *L. fermentum*, two *L. casei* subspp. *Pseudoplantarum* isolates each, one isolate of *L. paracasei* and one isolate of *L. brevis.* These isolates were identified by Vitek 2 compact system method as nine isolates of *Lactobacillus plantarum*, seven *L. acidophilus* isolates, two isolates of *L. fermentum*, two *L. casei* subspp. *Pseudoplantarum* isolates each, one isolate of *L. paracasei*, and one isolate of *L. brevis* with a percentage of 18, 14, 4, 4, 2 and 2%, respectively.

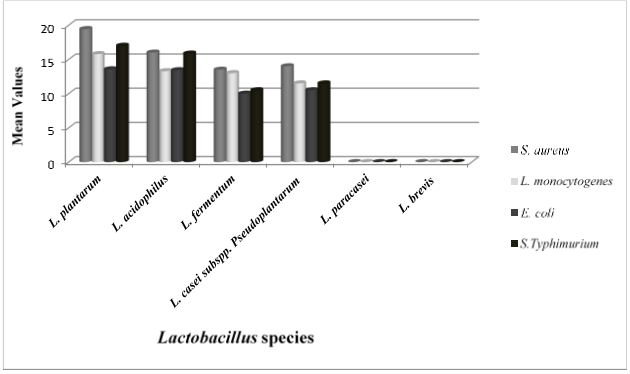

Fig. 1: Antibacterial activity of *lactobacillus* spp. isolated from raw camel milk against pathogenic strains using agar well diffusion method expressed as Mean Values of ZDI.

Antimicrobial Inhibition Potency of *Lactobacillus* spp. Isolates against Pathogenic Microorganisms

Results illustrated in Table 2 and Fig. 1 showed that *L. plantarum* possesses the highest inhibitory activity on *S. aureus* and *S.* Typhimurium growth while having moderate to low effect on *L. monocytogenes* and *E. coli.* Strong antibacterial activity was observed by *L. acidophilus* on *S. aureus* and *S.* Typhimurium but moderate antibacterial activity was detected on *L. monocytogenes* and *E. coli*. Both *L. fermentum* and *L. casei* subspp. *Pseudoplantarum* have moderate to low antibacterial potency on the four tested foodborne bacteria. However, *L. paracasei* and *L. brevis* didn't show antimicrobial potency against all of the examined bacteria. *Lactobacillus* spp. were potent to decrease the growth of *Aspergillus flavus* in vitro. Compared to control group, the highest inhibition of fungal growth belonged to *Lactobacillus plantarum* isolates followed by *L. acidophilus*, while *L. fermentum* and *L. casei* sub spp. *Pseudoplantarum* showed minimal inhibition of fungal growth. Although, both *L. paracasei* and *L. brevis* strains didn't show inhibitory potency on *A. flavus.*

DISCUSSION

Probiotic are essential bacteria improve health profit of their host and are generally vital for human health and nutrition. The most widespread probiotics type is *Lactobacillus* spp. Ayivi et al. (2020). After examination of different samples of dromedary milk found that 22 isolates (44%) were *Lactobacillus* spp. which were *L. plantarum, L. divergens, L. brevis, L. fermentum, L. animalis, L. rhamnosus, L. gasseri, L. paracasei, L. alimentarium* and untyped *Lactobacillus* spp., this result was conducted to Khedid et al. (2009) who found that *Lactobacillus* spp. Which isolated from Dromedary milk account were 37.5%. Another study on Dromedary milk referred to the existence of some LAB such as *L. pentosus, L. lactis* and *L. plantarum*

Table 1: Oligonucleotide primers sequences source (Wang et al. 2016)

Target Organisms	Targeted gene	Primer sequence (5'-3')	Cycling
Lactobacillus plantarum	*rec*A gene	F:CAGAATTGAGCTGGTGGTGG R:TGTTACTTTCGCAACCAGAT	1. Denaturation at 94°C for 5min and 35 cycles of 94°C for 30s 2. Annealing 55°C for 30s 3. Extension 72°C for 30s 4. Final extension at 72°C for 7min

Length of amplified product= 210bp

Table 2: Antibacterial activity of *Lactobacillus* spp. isolated from raw camel milk

Lactobacillus isolates	Tested bacterial strains (ZDI±SD)			
	Gram positive bacteria		Gram negative bacteria	
	Staphylococcus aureus	*Listeria monocytogenes*	*E. coli*	*Salmonella* Typhimurium
Lactobacillus plantarum	19.44±2.01	15.78±2.49	13.56±1.58	17±2.5
L. acidophilus	16±2.31	13.28±2.43	13.43±2.07	15.86±2.41
L. fermentum	13.5±2.12	13±1.41	10±1.41	10.5±0.707
L. casei subspp. Pseudoplantarum	14±1.41	11.5±2.12	10.5±0.70	11.5±2.12
L. paracasei	0±0	0±0	0±0	0±0
L. brevis	0±0	0±0	0±0	0±0

ZDI: Zone diameter of inhibition (mm); SD: Standard deviation.

Table 3: Growth inhibition by *Lactobacillus* spp. on *Aspergillus flavus*

Lactobacillus spp.	*Aspergillus flavus*
Lactobacillus plantarum	+++
L. acidophilus	++
L. fermentum	+
L. casei subspp. *Pseudoplantarum*	+
L. paracasei	-
L. brevis	-

(++):no fungal growth on >8% of plate area per bacterial streak: (++): no fungal growth on 3 to 8% of plate area per bacterial streak: (+): no fungal growth on 0.1 to 3% of the plate area per bacterial streak; (-): no suppression.

in raw dromedary milk (Yateem et al. 2008). Furthermore Sharma et al. (2021) could isolate *Lactococcus lactis*, and *Lactobacillus plantarum* from Dromedary milk.

In the current research, the most predominant species in this group is *Lactobacillus plantarum*, which agreed with Bettache et al. (2012) who reported that *Lactobacillus plantarum* is the predominance species of the genus Lactobacilli in almost all examined samples. Ruiz et al. (2009) reported that *Lactobacilli* showed a broad antimicrobial potency on some human and animal pathogens. In this research, the Lactobacilli isolates showed inhibition activity against the growth of *S. aureus*, *E. coli*, *S.* Typhimurium, and *L. monocytogenes*. The highest inhibitory effect was reported on *S. aureus* followed by *S.* Typhimurium then, *L. monocytogenes*, and the lowest inhibitory effect on *E. coli* as shown in Table 2 and Fig. 1. The result obtained is covenant with Boris et al. (2001) and Karami et al. (2017) that found that *Lactobacillus* strains obtained from milk products inhibit *Pseudomonas aeruginosa*, *S. aureus*, *E. coli*, *S.* Typhimurium, and *Bacillus subtilis* and the best suppression effect was on *S. aureus.* Also, Prabhurajeshwar and Chandrakant (2019) reported that *Lactobacillus* strains had an antagonistic effect on some bacteria, such as *S. aureus, E. coli, E. faecalis, S. Typhi* and *Shigella* spp.. In addition, Shehata et al. (2020) observed the antimicrobial activity of *Lactobacillus* and *Bifidobacteria* isolates on *E. coli, E. coli* MC1400, *S. aureus, P. aeruginosa, L. ivanovii,* and *Candida Albicans.*

In the current study, *L. plantarum* possesses the highest inhibitory activity on *S. aureus* and *S.* Typhimurium this result matches Soleimani et al. (2010) that mentioned that the *L. plantarum* ATCC 8014 had great activity against *S. aureus* isolated from bovine mastitis and *S. aureus* ATCC 25923 and Coeuret et al. (2004) who found that *L. plantarum* was highly active against Salmonella spp. The same finding of a considerable effect of *L. plantarum* isolates against Gram-negative pathogen was reported by Yateem et al. (2008) and Sankar et al. (2012). Davati et al. (2015) recorded that *L. casei* could inhibit the growth of *S. aureus* subsp. *aureus* ATCC 25923 and *B. cereus* ATCC 10876.

This study showed the potential antifungal effect of *L. acidophilus, L. plantarum, L. casei* subspp. *Pseudoplantarum,* and *L. fermentum* in the control group of *Aspergillus flavus* growth in vitro. That, the most species had an antifungal effect against *Aspergillus flavus* were L. *plantarum* strains. Also, *L. acidophilus* strains had moderate antifungal activity on Aspergillus *flavus.* A minimal activity of *L. fermentum* and *L. casei* sub spp. *Pseudoplantarum* isolates on inhibition of *Aspergillus favus* growth. These findings harmonize with those observed by Shehata et al. (2020), which mentioned that the isolated eight probiotic strains (*Lactobacillus plantarum, L. rhamnosus, L. acidophilus, L salivarius,* and *L. paracasei, Bifidobacterium longum, B. adolescentis,* and *B. breve*) could inhibit the pathogenic *Aspergillus* species (*A. niger, A. flavus* and *A. fumigatus*) growth. Also, Aryantha and Lunggani (2007) detected that *L. plantarum, L. fermentum,* and *L. delbrueckii* strains considerably decrease the *A. flavus* growth and production of AFB1 and also agreed with (Gerbaldo et al. 2012), which noticed antifungal effectiveness of *L. fermentum* L23and *L. rhamnosus* L60 on aflatoxigenic fungal isolates.

Eddine et al. (2018) mentioned that LAB *L. plantrarum, L. paracasei subsp. paracasei,* and *L. brevis,* have shown an antifungal activity on growth and spore germination of *Penicillium* sp. and *Aspergillus* ssp. The antifungal activity of Lactobacillus strains may be due to the production of secondary metabolites. *Lactobacilli* are producers of bacteriocins, H2O2, and organic acids (Ruiz et al. 2009). Also, Acetic and lactic acids (the main end-products of LAB fermentation of carb) make its antimicrobial action by penetrating the target organisms' membrane and lowering the pH of the cytoplasm, which

leads to cell destruction (Dalie´ et al. 2010). At the same time, the potent antifungal activity may be due to challenges in-between LAB and *A. flavus* species in batch conditions as LAB are simpler organisms with a faster metabolism. Therefore, bacteria can utilize the original substrate early to produce more cell biomass.

Conclusion

The study was carried out to recover of *Lactobacillus* spp. from dromedary's milk and its classification on the species level as an essential probiotic bacterium as well as, evaluation its antimicrobial activity of against several foodborne microorganisms; *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *listeria monocytogenes* and *Aspergillus flavus.* In the current work, the obtained *Lactobacillus* spp. from dromedary's milk had shown a wide range of antimicrobial properties against foodborne pathogen (bacterial and fungal) and can be used as bio-preservatives in food production. The most effective inhibitory organism was *Lactobacillus plantarum*, which can be used as a probiotic with antibacterial and antifungal activities.

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Authors contribution

Hemat Khyralla: Methodology and Writing, Heidy Abo- El yazeed; Supervision and Data curation, Sherif Marouf: Editing and Data curation, Mai H. Hanafy, and Atef Hussein; Editing and Data curation, Aalaa Saad: Writing and follow up.

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