

**USE OF NOVEL LACTIC ACID BACTERIAL
STRAINS ISOLATED FROM HONEY BEE
WORKERS GUT FOR FERMENTED MILK
PRODUCTION**

By

ASHWAK ABDEL MONEIM HASSAN MOHAMED ALMADAH
B.Sc. Agric. Sci. (Dairy Science), Fac. Agric., Cairo Univ., 2008
M.Sc. Agric. Sci. (Dairy Science), Fac. Agric., Cairo Univ., 2015

THESIS

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APPROVAL SHEET

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Name of Candidate: Ashwak Abdel Moneim Hassan Mohamed El-Madah **Degree:** Ph.D.
Title of Thesis: Use of Novel Lactic Acid Bacterial Strains Isolated from Honey Bee Workers Gut for Fermented Milk Production.

Supervisors: Dr. Hoda Mahmoud Elzeini
Dr. Nasr Fawzy Nasr

Department: Dairy Science

Approval: 16 / 7 / 2019

ABSTRACT

Traditional LAB flora from natural sources is still waits for scientific attention and because of uncontrolled industrialization, the folkloric knowledge in fermented dairy products could be lost in the near future. In this study it was aimed to isolate Lactic acid bacteria from honey bee workers gut as a natural source for getting wild strains. So five isolates were identified and also probiotic properties of isolates were investigated. Two of them are bacilli and the others are cocci. Before investigation the probiotic properties of these isolates, they were identified by Morphological, biochemical characterization techniques and molecular identification by using amplification and 16S rRNA sequencing. Two lactobacilli were identified as *Lactobacillus brevis* HBE2 and *Lactobacillus casei* HBE5, while cocci strains identified as *Enterococcus faecalis* HBE1, HBE3 and HBE4. These isolates showed resistance to stomach pH (pH 2, 5), tolerance against 0, 3 and 0.7% bile concentrations and antimicrobial activity against *Salmonella typhimurium* ATCC14029, *Escherichia coli* O157H:793111 and *Staphylococcus aureus* ATCC 25923. In the light of this study, it is observed that, honey bee workers gut is a source of potential probiotic strains.

Technological properties were investigated with a view towards selection of appropriate starter cultures. Lactobacilli had high rates of acidifying initially (6h), but Enterococci were after (1h) incubation. None of the LAB was found to produce CO₂ from glucose; also the best growth in medium with different NaCl concentrations was maintained by Enterococci strains. All The bacterial isolates gave ropy colonies and were positive for production of exopolysaccharides except *L. casei* HBE5.

LAB strains were selected to prepare the fermented milk by adding single isolated LAB or Mixed starter. Fermented skim milk made with HBE3 exhibited higher WHC, and fermented skim milk made with HBE5 exhibited the lowest. The storage period showed no significant (P>0.05) influence on viable probiotic cells. It was obtained that *L. brevis* HBE2, *En. faecalis* HBE3 and HBE4 were a better choice for probiotic fermented milk products, with better sensory and textural characteristics than the other strains during storage

Key words: Honey bee workers, Intestinal tract, Lactic acid bacteria, Probiotic properties, Fermented milks.

DEDICATION

*I dedicate this work to whom my heart left thanks; to
my mother, my father, my husband and my son Eyad
for their patience and help*

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I wish to express my sincere thanks to Allah who without his aid this work could't be done

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
AOAC	Association of Official Analytical Chemists
ATCC	American type culture collection
Am	Ampicillin
AU	Arbitrary Unit
bp	base pair
buffer AL	lysis buffer
C	Chloramphenicol
°C	Degree Celsius
CFU	Colony-forming unit
Cip	Ciprofloxacin
CLSI	Clinical and laboratory standards institution
Co.Va	Coefficient of Variation
Cp	Centipoises
CTAB	CethylTrimethyl Ammonium Bromide
DF	Degree of freedom
DNA	Deoxyribonucleic Acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
EDTA	Ethylene Diamide Tetra Acetic Acid
EFSA	European Food Safety Authority
En.	Enterococcus
EPS	Exopolysaccharides
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FM	Fermented milk
GIT	Gastrointestinal tract
GRAS	Generally Recognized As Safe
G+	Gram-positive bacteria
HPLC	High pressure liquid chromatography
K	kanamycin
LAB	Lactic Acid Bacteria
L., Lb.	Lactobacillus
LN	Natural logarithm
LSD	Least Significant Differences
ml	Milliliter
µl	Microliter
MRS	De Man, Rogosa and Sharpe

Abbreviation	Meaning
Mpa	Mega Pascal
MS	Mean square
NCBI	National Center for Biotechnology Information
nm	nanometer
OD	Optical Density
OPA	O-phthaldialdehyde
PCR	Polymerase Chain Reaction
pH	Negative decadal logarithm of the [H ⁺] ion concentration
RAPD	Randomly Amplified Polymorphic DNA
rDNA	Ribonucleic Acid
rRNA	Ribosomal RNA
RNA	Ribonucleic Acid
RSM	Reconstituted skim milk
rpm	Round per minute
SDS	Sodium Dodecyl Sulfate
SD	Standard deviation
SEM	Scanning electron microscope
SPIP	Scanning Prop Image Processor (SPIP) program
spp., subsp.	Subspecies
spp	Species
SS	Shear stress
SR	Shear rate
Sku	Surface Kurtosis
Sa	Surface average roughness
TA	Titrateable Acidity
TAC	Total antioxidant capacity
Tc	Tetracycline
ZOI	Zone Of Inhibition
Va	Vancomycin
Vis	Viscosity
v/v	Volume/volume
WHC	Water-holding capacity
WE	whey expelled
WHO	World Health Organization
w/v	weight/volume
WSE	Water-soluble extract