

**ISOLATION, IDENTIFICATION AND
CHARACTERIZATION OF PROPHYLACTIC AND
THERAPEUTIC PROBIOTICS**

BY

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B.Sc. Agric. Sci., (Biotechnology), Fac. Agric., Cairo Univ., Egypt, 2008

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SUPERVISION SHEET

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DEDICATION

I dedicate this work to whom my heart felt thanks; to my husband for all the support. Also I dedicate this work to my lovely family which support me along the period of my under and post graduation.

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ABSTRACT

The concept of management of beneficial bacteria with a view to replace harmful microbes with useful ones has been invigorated by the probiotic concept. Probiotic approach has shown promising results in prophylaxis and biotherapy. This study aims to isolate probiotics from feces of healthy fed-breast babies; characterize the isolates phenotypically based on morphology physiology, examine the isolates for the presence of probiotic properties such as resistance to acid, bile and pancreatic fluid, adhesive properties, antibacterial activity, and the safety respecting to antibiotic susceptibility and hemolytic activity; search for the additional capability between selected isolates by screening them for the presence of two valuable antitumor genes, the L-asparaginase encoding gene *ansA* and arginine deiminase-encoding gene *arcA* and determine the enzymes activity to averment the expression of genes; finally molecular identification of the isolates that have antitumor genes. Out of the 60 obtained isolates, 42 were preliminary selected as putative *Lactobacillus* genus. These isolates were able to: grow at 15 and 45 °C, tolerate NaCl at concentrations over the range 2 - 8 %; produce CO₂ from glucose except 4 isolates. Most of the tested isolates were able to survive for 3 and 6 h exposure: at pHs 1.5, 3 and 5, the higher the pH value, the higher the percentage of viability; at 0.3 and 0.7 % bile salts with variable survival rates being higher at 0.3%; in pancreatic juice with variable rates depending on the strain and exposure time, where 17 strains inhibited completely after 6h. A great heterogeneity among test strains in their hydrophobicity ranged between superior to low hydrophobic, however only one strain was unable to adhere. All the tested *Lactobacillus* strains have antimicrobial potency; 38 strains showed wide spectrum activity against all tested Gram positive and negative bacteria. Among the tested strains, 7, 14, 20, 30, 32 and 42 were resistant to the tested antibiotics ampicillin, cefotaxime, vancomycin, chloramphenicol, tetracycline and kanamycin. in the respective order. There was a relatively high frequency of multidrug resistance among the isolates, where 7 strains showed resistance to all tested drugs, 3 resistant to 5 drugs, 3 to 4 drugs, 20 to 3 antibiotics, 7 to 2 antibiotics and two strains exhibited resistance for only one drug. All the tested strains showed no hemolytic activity. PCR screening isolates for the *ansA* gene and *arcA* gene revealed that only 2 strains (nos. 5 and 9) gave amplicons of the expected sizes. The preliminary bioinformatics analysis showed strains nos. 5 and 9 are found to be close to that from *Lactobacillus gasseri* and *Lactobacillus fermentum*, respectively. They were deposited in the GenBank under the accession numbers JQ653281 and JQ867180, respectively. Enzyme activity in both cell-free supernatants and cell-free extracts of the identified strains *L. gasseri* and *L. fermentum* were 5.78, 3.56 nmol ammonia/min/mg protein; 3.89 and 4.01 nmol citrulline/min/mg protein for L-asparagenase (EC 3.5.1.1) and arginine deiminase (EC 3.5.3.6), respectively.

Keywords: Probiotics, *Lactobacillus*, PCR, 16S rRNA sequencing, antitumor genes.

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