بسم الله الرحمن الرحيم

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PRODUCTION OF BIOACTIVE COMPOUNDS AS ANTICANCER FROM PROBIOTICS

BY

MAI NOSSAIR HASSAN AHMED AMER

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2008
M.Sc. of Agri. Sci. (Microbiology), Fac. Agric., Cairo Univ., 2014

THESIS

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APPROVAL SHEET

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Title of Thesis: Production of Bioactive Compounds as Anticancer From Probiotics.
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ABSTRACT

Probiotics have shown promising results in prophylaxis and safe biotherapy. The search for a biologically active strain able to produce bioactive compounds of applied importance to meet the growing needs was the main target. To attain this target, buffalo colostrum samples was used as an isolation source of LAB. Out of the 53 LAB isolates, only 16 produced exopolysaccharides; of which, one isolate attained the highest productivity (3.8 g l^{-1}) and was also able to produce L-asparaginase (ASNase). Such isolate was molecularly identified as *Weissella paramesenteroides* MN2C2 and deposited in the GenBank under number MK530206. The optimum conditions for maximal productivity of bioactive compounds were 24 h incubation at 35 °C and pH 6.5 for EPSs; 48 h at 37 °C, pH 7 and 10% inoculum concentration for ASNase. EPSs molecules were partially purified (PP) and physicochemical characterized; UV, FTIR spectra showed absorption bands related to many functional groups. ^{1}HNMR polysaccharide spectrum consists mainly of three regions: ring proton of several sugar residues of the polysaccharides, anomeric proton region and the alkyl proton region. HPLC elucidated EPSs structure as consisting of 80% fructose, 9.3% glucose and 5.6% sucrose. SEM showed a three-dimensional structure of irregular highly compacted lumps with different sizes and a smooth surface. Energy dispersive X-Ray and mapping analyses revealed that PP-EPSs composed of carbon, nitrogen, oxygen, phosphorous and sulfur in high ratios of its weight reached 42.31, 10.11, 42.68, 4.13 and 0.78 %, respectively. As indicated by TEM and DLS analyses, the nanoparticle (NP) sizes of EPSs prepared from crude or PP using ultrasonication ranged from 37.3 to 105 nm for the crude CEPSs-NPs, and from 45.7 to 204 nm for the PPEPSs-NPs, PP-ASNase has a specialized activity of 72 units mg^{-1} protein and a molecular weight of ≈ 36 kDa using SDS-PAGE technique. PP-EPSs proved its superiority as anticancer with highly selectivity indices against liver HepG-2, colon (Caco-2) and breast MCF-7 cancer cells. The CEPSs-NPs had significant activity against breast cancer MCF-7; and the toxicity was increased in the nanoparticles prepared from partially purified polysaccharides. Both CEPS-NPs and PPEPSs-NPs also showed stronger DPPH antioxidant activity than CEPSs, PP-EPSs and PP-ASNase, respectively. Both PP-EPSs and CEPSs reduced Coxsackievirus (CVB3) yield by > 99%. The probiotic *W. paramesenteroides MN2C2* were characterized by its survivability for 3 and 6 hours at pH ranging from 1.5 to 9 and 0.3% of bile salts; in the presence of pancreatic juice, under simulated stomach and intestinal conditions; besides its ability to adhere to intestinal walls. It also showed antimicrobial activity against *B. subtilis* and *P. aeruginosa*, with inhibition zones of 25 and 19 mm, respectively. The strain is considered as safe because of the absence of analytical activity in human blood and the high sensitivity to antibiotics tetracycline, fusidic acid, ampicillin and resistance to vancomycin and kanamycin. Also, *W. paramesenteroides* MN2C2 was able to ferment milk when mixed with probiotic commercial starter strains to produce a promising milk product that is suitable, practical, effective, preventative and with safe therapeutic properties.

Keywords: Probiotics, EPSs, EPSs-NPs, ASNase, Anticancer, Antioxidant, Antiviral.

DEDICATION

I dedicate this work to whom my heartfelt thanks; to my husband and my children 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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*In the name of **ALLAH**, most gracious and most merciful. All praises be to **ALLAH**, the cherisher and sustainer of the world ….*

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List of Abbreviations

Abbreviation	Interpretation
µl	Micro liter
A549	Lung cancer cell line
ABT	Acidophilus bifidus thermophilus
*ans*A	L- asparaginase gene
ASNase	L- asparaginase
ATCC	American type culture collection
BLAST	Basic local alignment search tool
Caco-2	Colorectal adenocarcinoma cell line
CEPSs	Crude EPSs
CEPSs-NPs	Crude EPSs nanoparticles
CFSs	Cell free supernatants
CFU	Colony forming unite
cm	Centimeter
CVB3	Coxsackievirus type B3
DLS	Dynamic light scattering
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDX	Energy dispersive x-ray
EPSs	Exopolysaccharides
EPSs-NPs	EPSs nanoparticles
FBS	Fetal bovine serum
Fig.	Figure
FT-IR	Fourier transform - infrared
gm	Gram
h	Hour
HepG-2	Hepatocellular carcinoma cell line
HPLC	High performance liquid chromatography
IC_{50}	50% inhibition concentration
IZ	Inhibition zone
K Da	Kilo dalton
LAB	Lactic acid bacteria
MCF-7	Breast cancer cell Line
MEM	Minimum essential medium
mg	Mill gram