

**BIOCHEMICAL STUDIES ON SOME
POLYSACCHARIDES PRODUCED BY
SOME BACTERIAL ISOLATES AS
ANTICANCER AGENTS**

By

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**B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., Egypt, 1999
M.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., Egypt, 2008**

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ABSTRACT

In the course of a screening program for new bioactive polysaccharides, the present study was carried out on 53 isolates of marine bacteria (El-Ein Elsokhna, Sidi Bisher, Marsa-Alam) and 30 isolates of soil samples (El-Kanater, and El-Fayoum). The isolates were obtained using standard serial dilution technique from the original samples. Bacteria were grown in liquid media for three days, the bacterial cells were separated by centrifugation, and proteins were precipitated by trichloroacetic acid (5%). The crude polysaccharides were separated from the supernatant and examined by DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical. The highest antioxidant activities (107%) was recorded for exopolysaccharides from M7 (*Bacillus circulans*) isolate followed by these of M8 (*Bacillus licheniformis*) (105 %), M9 (*Bacillus marinus*) 100.99%, M4 (*Bacillus brevis*) 99.2 %, M3 (*Bacillus insolitus*) 95.11%, M6 (*Bacillus anthracis*) 94.76 %, M2 (*Bacillus alvei*) 92.48 %, M5 (*Staphylococcus saprophyticus*) 85.28 %, S22 (*Bacillus polymyxa*) 84.99 %, K7 (*Bacillus macquariensis*) 74.09 %, E3 (*Bacillus coagulans*) 71.65 %, E8 (*Bacillus firmus*) 71.28 % isolates. As mentioned, the twelve isolates have the higher antioxidant activities and further *in vitro* investigated for their anticancer activities. The most active isolates as anticancer agents is *Bacillus marinus* which selected for further investigation. The crude exopolysaccharides were isolated from *Bacillus marinus* and purified by fractionation with ethanol to three fractions. Determination of the antitumor activities for the three fractions (1, 2, and 3) resulted that fraction (1) has the highest activity against Ehrlich Ascites Carcinoma Cells (EACC) cells (86.38%). Fraction (1) was subjected to further purification studies. The polysaccharides were purified and fractionated by column chromatography on diethylaminoethyl cellulose (DEAE-cellulose). The fractionation processes led to isolation of six sub fractions. Determination of the antitumor activities for six sub fractions (1-6) resulted that sub fraction-6 which gave the highest antitumor activity was subjected to further purification studies.

A structure feature of sub fraction-6 was investigated by a combination of chemical and chromatographic analyses, such as acid hydrolysis, periodate oxidation–Smith degradation, HPLC and IR. The results indicated that sub fraction-6 was composed of glucose: glucouronic acid in a ratio of 3.0:1.0 with molecular weights 500 kDa. Small amount of glycerol and large amount of liberated erythritol partially prove the presence of the β (1→4) linkages between the monosaccharides in the backbone of the polysaccharide. Determination of the biological activities of the purified exopolysaccharide (sub fraction-6) as antimicrobial, antiviral, antioxidant and antitumor activities.

Key words: Exopolysaccharides, isolation, purification, identification, chemical structure antimicrobial, antiviral, antioxidant, antitumor activities.

DEDICATION

I dedicate this work to whom my heart felt thanks; my parents, husband and lovely kids Mustafa and Shahad for their patience and help, as well as to my mother for all the support she lovely offered along the period of my post graduation.

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LIST OF ABBREVIATIONS

A-549	Human alveolar basal epithelial cell line
ALL	Acute lymphoblastic leukaemia
ASFV	African swine fever virus
BRM	Biological response modifier
Caco-2	Cell line is a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells
CAT	Catalase
CP	Cyclophosphamide
CPS	Capsular polysaccharide
DEAE-cellulose	Diethylaminoethyl cellulose
DMEM	Dulbecco's Eagle's medium
DPPH	1,1-diphenyl-2-picrylhydrazyl
EACC	Ehrlich Ascites Carcinoma Cells
EPS	Exopolysaccharide
GPC	Gel permeation chromatography
GPx	Glutathione peroxidase
HAV	Hepatitis A virus
HCT 116	Human colon carcinoma
HSV	Herpes simplex virus
HT 29	Human colorectal adenocarcinoma
IC₅₀	Half maximal inhibitory concentration
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LAB	Lactic acid bacteria
LPO	Lipid peroxide
MCF-7	Human breast adenocarcinoma cell line
MDCK	Madin Darby canine kidney cell line
MDR	Multi drug resistance
MTT	(3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide)
NCI:	National Cancer Institute
NK	Nature killer
NPs	Natural pigments
PBS	Phosphate buffer solution

PSK	Polysaccharide Kureha
PSP	Polysaccharopeptide
ROS	Reactive oxygen species
RPMI 1640	Roswel Park Mark Institute 1640
RSA	The free radical scavenging activity
SEC-LLS	Size exclusion chromatography combined with laser light scattering
SOD	Superoxide dismutase
SPs	Sulfated polysaccharides
TCM	Traditional Chinese medicine
TEAC	Trolox Equivalent Antioxidant Activity
TNF-α	Tumor necrosis factor α
VHSV	Viral hemorrhagic septicemia virus

CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	5
1. Marine floras	5
2. Biological activity of polysaccharides	8
a. Antioxidant activity.....	8
b. Antitumor activity of polysaccharides.....	18
c. Antiviral activity.....	33
d. Antimicrobial activity.....	37
MATERIALS AND METHODS	40
MATERIALS	40
1. Microorganism source.....	40
2. Media.....	40
3. Chemicals.....	42
4. Experimental animals and tumor cells.....	42
5. Solvents.....	42
METHODS	42
1. Isolation of bacteria	42
a. Preparation of samples.....	42
b. Identification of bacteria	43
1. Cell morphology	43
2. Biochemical tests	43
a. Catalase test.....	43
b. Anaerobic growth.....	43
c. Voges-Proskauer (V-P) test	44
d. Acid from carbohydrates	44
e. Utilization of citrate and propionate	45
f. Reduction of nitrate to nitrite	45
g. Production of indole	46
h. Growth in sodium chloride	46
i. Hydrolysis of starch.....	47
2. Production of polysaccharide	47
3. Biological activity	48
a. Antioxidant activity.....	48
b. Assay of the antitumor activity Ehrlich ascites carcinoma cells (EACC)	49
c. Antitumor Activity (using different cell lines)	50
d. Antiviral activity.....	51

e. Antimicrobial activity.....	54
4. Purification of polysaccharides.....	55
5. Chemical analysis.....	57
6. Desulfation of polysaccharides by methanolic hydrogen chloride (MeOH/Cl).....	58
7. Hydrolysis of the exopolysaccharide.....	58
a. Complete acid hydrolysis.....	58
b. Separation and determination of sugars by (HPLC)	59
8. Periodate oxidation of the polysaccharide	59
a. Determination of the reduced periodates.....	59
b. Determination of the released formic acid.....	60
c. Reduction of periodate-oxidized material.....	60
d. Identification of sugar alcohols.....	61
9. Infrared Spectroscopy (I.R).....	61
RESULTS AND DISSCUSSION.....	62
1. Collection and isolation of bacteria	62
2. Biological activity of isolated exopolysaccharides from bacteria.....	63
a. Antioxidant activity.....	63
b. Antitumor activity.....	69
3. Identification of the selected bacteria isolates.....	72
4. Isolation and purification of the exopolysaccharide.....	76
a. Fractionation of crude exopolysaccharides isolated from <i>B. marinus</i> by ethanol.....	76
b. Identification and determination of sulfate.....	77
c. Purification and fractionation of the alcohol precipitated polysaccharides (fraction 1) by DEAE-cellulose column.....	80
5. Molecular weight determination of sub fractions (3-6) by gel chromatography.....	83
6. Infrared (FT-IR) spectra of the sub fractions.....	86
7. Periodate Oxidation and Smith degradation.....	90
8. Biological activities of purified exopolysaccharide.....	93
a. Antimicrobial activities.....	93
b. Antiviral activities.....	103
c. Antioxidant activities.....	109
d. Antitumor activities.....	112
SUMMARY.....	120
REFERENCES.....	125
ARABIC SUMMARY.....

LIST OF TABLES

No	Title	Page
1.	Number of isolated bacteria from different areas in Egypt.....	63
2.	Radical scavenging activity (RSA) of different bacterial exopolysaccharide (40 mg/ml) at different times	66
3.	Radical scavenging activity (RSA) of different bacterial exopolysaccharide (40 mg/ml) at different times	66
4.	Radical scavenging activity (RSA) of different bacterial exopolysaccharide (40 mg/ml) at different times	67
5.	Radical scavenging activity (RSA) of different bacterial exopolysaccharide (40 mg/ml) at different times	68
6.	Radical scavenging activity (RSA) of different bacterial exopolysaccharide (40 mg/ml) at different times	68
7.	The most active isolates as radical scavenging free radical of different bacterial exopolysaccharide (40 mg/ml) at different times	69
8.	Viability of EACC cells for different crude exopolysaccharide (40 mg/ml).....	70
9.	Differential characteristics of the species of the genus	74
10.	The selected bacteria strains as antitumor activity.....	76
11.	Viability of EACC cells for different fraction.....	77
12.	Sulfate percentages in each fraction of exopolysaccharide	79
13.	Chemical components and viability of EACC cells for different sub fractions.....	82
14.	Molecular weights of the sub fractions (3-6).....	84
15.	Periodate oxidation results.....	90
16.	HPLC results of Smith's degradation of sub fraction-6.....	92
17.	Inhibition zones of microbial growth by different concentrations of of the purified exopolysaccharide sub fraction-6	94
18.	The minimum inhibitory concentration (MIC) of sub fraction-6 against test organisms.....	102
19.	Cytotoxicity (%) of different concentrations ($\mu\text{g/ml}$) of the crude exopolysaccharides.....	104
20.	Cytotoxicity (%) of different concentrations ($\mu\text{g/ml}$) of the purified exopolysaccharide (sub fraction-6).....	104

21. Antiviral activity of the crude exopolysaccharides against H1N1.....	107
22. Antiviral activity of the purified exopolysaccharide (sub fraction-6) against H1N1.....	108
23. Radical scavenging activity (RSA) of different concentrations of the purified exopolysaccharides (sub fraction-6).....	111
24. Viability of EACC cells for the purified exopolysaccharide (sub fraction-6).....	113
25. The effect of the purified exopolysaccharide (sub fraction-6) on the proliferation of MCF-7 cells.....	114
26. The effect of the purified exopolysaccharide (sub fraction-6) on the proliferation of MCF-7 cells.....	116

LIST OF FIGURES

NO	Title	Page
1.	Number of isolated bacteria from different areas in Egypt...	63
2.	Viability of EACC cells for different crude polysaccharides.....	70
3.	Viability of EACC cells for different crude exopolysaccharides.....	71
4.	The viability of EACC cells (control).....	71
5.	Microscopic of <i>Bacillus marinus</i> stained with a Gram's stain (10 × 90).....	73
6.	Viability of EACC cells for different fractions	77
7.	IR spectra of fraction (1).....	78
8.	IR spectra of fraction (2).....	78
9.	IR spectra of fraction (3).....	79
10.	Sulfate contents in each fraction of exopolysaccharide.....	80
11.	Elution profile of crude exopolysaccharide on DEAE-cellulose	81
12.	Sulfate contents and viability of EACC cells for different sub fractions	83
13.	Elution profile of sub fraction-3 on Sephadex G-200.....	84
14.	Elution profile of sub fraction-4 on Sephadex G-200	85
15.	Elution profile of sub fraction-5 on Sephadex G-200.....	85
16.	Elution profile of sub fraction-6 on Sephadex G-200.....	86
17.	Infrared spectrum of the sub fraction-3.....	87
18.	Infrared spectrum of the sub fraction-4.....	88
19.	Infrared spectrum of the sub fraction-5.....	88
20.	Infrared spectrum of the original exopolysaccharide sub fraction-6.....	89
21.	Infrared spectrum of the desulfated exopolysaccharide sub fraction-6.....	89
22.	Periodate consumption per anhydrosugar unit and formic acid productions from sub fraction-6	91
23.	Proposal structure of the purified exopolysaccharide isolated from <i>Bacillus marinus</i>).....	93
24.	Antimicrobial activity of sub fraction-6 against <i>B. subtilis</i>	95
25.	Antimicrobial activity of sub fraction-6 against	

<i>B. subtilis</i>).....	95
26. Antimicrobial activity of sub fraction-6 against <i>St. aureus</i>	96
27. Antimicrobial activity of sub fraction-6 against <i>St. aureus</i>	96
28. Antimicrobial activity of sub fraction-6 against <i>P. floureceans</i>).....	97
29. Antimicrobial activity of sub fraction-6 against <i>P. floureceans</i>).....	97
30. Antimicrobial activity of sub fraction-6 against <i>E. coli</i>).....	98
31. Antimicrobial activity of sub fraction-6 against <i>E. coli</i>).....	98
32. Antimicrobial activity of sub fraction-6 against <i>S. cerevisiae</i> .	99
33. Antimicrobial activity of sub fraction-6 against <i>S. cerevisiae</i> .	99
34. Antimicrobial activity of sub fraction-6 against <i>C. albicans</i> .	100
35. Antimicrobial activity of sub fraction-6 against <i>C. albicans</i> .	100
36 The minimum inhibitory concentration (MIC) of sub fraction-6 against test organisms).....	103
37. Cytotoxicity (%) of different concentrations ($\mu\text{g/ml}$) of the crude exopolysaccharides).....	105
38. Cytotoxicity (%) of different concentrations ($\mu\text{g/ml}$) of the purified exopolysaccharides (sub fraction-6)).....	105
39. Antiviral activity of the crude exopolysaccharides against H1N1).....	108
40. Antiviral activity of the purified exopolysaccharide (sub fraction-6) against H1N1).....	109
41. Radical scavenging activity (RSA) of different concentrations of the purified exopolysaccharides (sub fraction-6)).....	112
42. Viability of EACC cells for the purified exopolysaccharide (sub fraction-6).....	113
43. Antitumor activity of the purified exopolysaccharide (sub fraction-6) against MCF-7 cells.....	115
44. Antitumor activity of the purified exopolysaccharide (sub fraction-6) against A-549 cells.....	116

INTRODUCTION

Cancer remains leading cause of death globally. IARC (2010) recently estimated that 7.6 million deaths worldwide. Due to cancer with 12.7 million new cases per year were being reported worldwide. A significant proportion of this burden is borne by developing countries; 63% of cancer deaths are reported to be from developing countries. Cancer is a multigenic and multicellular disease that can arise from all cell types and organs with a multi-factorial etiology (Ferlay *et al.*, 2010 and Jemal *et al.*, 2011).

The clinical treatment methods against cancer include: surgery, which is local excision of tumor; radiotherapy, which eliminate tumor by exposing to radiation; chemotherapy, which relies on drug targeting tumor cells; combined modality therapy, which includes all three former treatments together; and immunotherapy, which evokes an immune response against tumor. Most tumor treatments incur side effects like complications and toxicity, thus patients have to suffer from the pain of treatments. The ultimate reason for the suffering underlies in the similarity between tumor cells and normal cells. It is necessary to find out a treatment that could eliminate malignant cells while protecting normal cells from being killed (Miller *et al.*, 1981). Novel nanomedicine pharmaceutical systems emerged recently to achieve site-specific delivery thereby minimizing non-specific toxicity (Lammers and Smith, 2008).

Chemotherapy is one of the major therapeutic modalities for cancer. Conventional chemotherapeutic agents are highly toxic to normal tissues and are not successful for complete remission of tumors

and to prevent metastasis. During the past three decades, many polysaccharides and polysaccharide protein complexes have been isolated from mushrooms, fungi, yeasts, algae, lichens and plants with medicinal properties. The biological activity of the polysaccharides has attracted more attention recently in the biochemical and medical areas because of their immunomodulatory and antitumor effects. The ability of bioactive polysaccharides and polysaccharide bound proteins to modulate so many important immune functions may be due to the structural diversity and variability of these macromolecules. A successful approach in cancer therapy is to trigger apoptosis but it is often complicated by development of multi drug resistance (MDR) mechanisms (Salgaller and Lodge, 1998). Polysaccharides are able to down regulate *P*-glycoprotein, and reverse MDR (De Vuyst and Degeest, 1999; Ooi and Liu, 2000; Laws *et al.*, 2001; Wei *et al.*, 2008 and Kambourova *et al.*, 2009). Natural products have been the mainstay of cancer chemotherapy for the past 30 years and are likely to provide many of the lead structures, and these will be used as templates for the construction of novel compounds with enhanced biological properties (John, 2002).

An exopolysaccharide (EPS) is a sugar polymer that is produced mainly by bacteria and microalgae, either in a form bound to the cell-wall, a so-called capsular polysaccharide (CPS), or in a free form liberated into the culture medium, known as a slime EPS (Sutherland, 1972). It is believed that the physiological function of EPS is as the first line of biological defense against phagocytosis, phage attack,