بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

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Faculty of Science

Microbiology Dept.

Biochemical Studies on Microbial L-Glutaminase And Its Applications

ATHESIS

Submitted for the Master degree of Science (MSc) in Microbiology

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وَقُل رَّبِّ زِدْنِي عِلْمًا
جلالی

Dedication

I dedicate my humble efforts to the memory of ***My father****, who always believed in my ability to be successful in the academic arena. He was gone but his belief in me made the journey possible.*

I also dedicate ***My mother****, whose affection, love, encouragement and prays of day and night make me able to get such success and honor.*

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Declaration

This thesis has not previously submitted for any other universities

Sara Mohammed Abdel-Haleem El-Sousy

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ABSTRACT

L-glutaminase has utmost practical importance in many fields such as medicine, pharmaceutical and some industries as an effective antioxidant, anticancer, flavor enhancer and used as an analytical reagent in the determination of glutamate and glutamine. The objective of the present article was to formulate the production medium and to pinpoint the proper growth conditions for the most potent microorganism producing highly active glutaminase enzyme. The general properties of the crude enzyme and the partially purified enzyme preparation were determined to detect the proper conditions for enzyme activity. Under the specified conditions, the capability of the two enzyme forms for antimicrobial and antioxidant activities were investigated and anticancer activity of the partially purified L-glutaminase was determined. Twelve recommended microbial strains were screened for highly active L-glutaminase enzyme production, Factors influencing the production of L-glutaminase enzyme were optimized and the important properties of the crude enzyme were pinpointed. Finally, biological activities of the crude enzyme were investigated as a preliminary index for the validity of the partially purified L-glutaminase form for medical applications. Among all tested microorganisms, *Bacillus subtilis* NRRL 1315 was the most potent producer for L-glutaminase enzyme. The maximum glutaminase production was obtained after

48 h of incubation on a rotatory shaker (150 rpm) from medium contained (g/L) 5 glucose, 0.1 sodium nitrate and 10 L-glutamine at 37^{o}C and pH 7.5. The important properties of L-glutaminase enzyme were duly pinpointed as follows: optimum enzyme protein concentration and substrate concentration were investigated for the crude and the partially purified enzyme as 2 mg/mL and 40 mM, respectively, and optimum reaction pH and temperature for both enzyme forms were 7.5 and 37^{o}C, respectively. The partially purified enzyme form was highly stable at pH 7 even after 120 min. of incubation and the enzyme retained more than 81% of the original activity also the enzyme was stable at 37^{o}C and after 120 min of incubation it retained more than 84% of the original activity. The partially purified L-glutaminase Michaelis constant (K_m) and maximum velocity constant (V_{max}) were 2.6 mM and 37.14 U/reaction, respectively, applying the Woolf plot. Each of Mg^{+2} and Mn^{+2} activated the partially purified enzyme. On the other hand, EDTA and Hg^{+2} at 100 mM inhibited the enzyme activity. Under the specified conditions the crude enzyme and the partially purified enzyme preparations exhibited considerable DPPH radical scavenging activity. The partially purified enzyme form had cytotoxic activity against the three human tumor cell lines examined namely Hep-G2 (Human Hepatocellular Carcinoma Cell Line), MCF -7 (Breast Cancer Cell Line) and HCT 116 (Colon Cell Line).

List of Abbreviations

C	Carbon
CF	Culture filtrate
^{o}C	Degree centigrade
DMSO	(Dimethyl sulfoxide)
F	Fraction
Fig.	Figure
gm	Gram
gls A	glutaminase A
gls B	glutaminase B
h	Hour
IC_{50}	Half Inhibitor Concentration
L	Liter
mg	Milligram
mg/culture	Milligram/ 50 milliliter culture
µg	Microgram
min.	Minute
mL	Milliliter
mM	millimolar
m	Mole
(MSG)	Mineral Salts Glutamine medium
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)
N	Nitrogen
rpm	Rotation per minute
Na, K Tartarate	Sodium potassium tartarate
U	Unit

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