

CONTENTS

	<u>Page</u>
INTRODUCTION	1-4
REVIEW OF LITERATURE	5-21
CHAPTER 1	22-48
<b>MATERIALS AND METHODS</b>	
Bacterial strains	24
Isolation, identification and characterization of <u>V.cholerae</u> strains	24
Description of the strains of the different species of <u>Vibrio</u> , under study	26
Maintenance of strains	27
Cell-growth measurement	27
Bacterial culture conditions for L-asparaginase production	27
Intracellular and extracellular L-asparaginase preparation	28
Preparation of media	28
Study on the enzyme biosynthesis of the <u>V.cholerae</u> strains	29
Re-induction and starvation of bacterial cells	30
L-Asparaginase assay	30
L-Glutaminase assay	31
Protein estimation	31
Determination of toxigenicity of <u>V.cholerae</u> strains	32
Serial passage in RIL model	32
Determination of the enzyme stability	32
Enzyme inhibition study	33
Thermal-inactivation of enzyme	33

	<u>Page</u>
Statistical analysis	33
Spheroplast formation	33
Cell-free supernatant and whole-cell enzyme preparation	35
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitation	35
MnCl <sub>2</sub> precipitation	36
Acetone precipitation	36
pH and temperature profiles of the enzyme of the CFSP, PP and CP	37
Thermal-inactivation of the enzyme of the CFSP, PP and CP	37
Substrate catalytic efficacy of the CFSP, PP and CP	37
<b>RESULTS</b>	
Characterization of <u>V.cholerae</u> strains	38
Optimization of culture conditions	38
L-Asparaginase production of the strains	38
Constitution of the TAYG medium	39
Effect of growth culture pH	39
Enzyme yield for successive generation of culture	39
L-Asparaginase activity and toxigenicity of <u>V.cholerae</u> strains	40
L-Asparaginase activity after serial animal passage of the selected 3 <u>V.cholerae</u> strains	40
Effect of exogenous cAMP and glucose on inducible L-asparaginase activity of <u>V.cholerae</u> strains	41
Stability of the enzyme of <u>V.cholerae</u> strains	41
Influence of the constituents of medium and pH of growth culture upon biosynthesis of L-asparaginase of two different toxigenic <u>V.cholerae</u> strains	42

	<u>Page</u>
Effect of chloride and phosphate salts of Na <sup>+</sup> and K <sup>+</sup> on L-asparaginase biosynthesis by the <u>V.cholerae</u> strains	43
Effect of molarity of the buffers on L-asparaginase production of the strains	43
Effect of the substrate stimulation, re-induction and starvation in the bacterial cells, upon biosynthesis of L-asparaginase of the two <u>V.cholerae</u> strains	44
Effects of the sulfhydryl reagents, amino acids, and carbohydrates addition in medium upon biosynthesis of L-asparaginase in the two <u>V.cholerae</u> strains	44
Changes of the effect of the reagents upon activity of L-asparaginase produced by the <u>V.cholerae</u> strains grown in two separate medium	45
Changes in stability and thermal-inactivation of the enzyme of the two <u>V.cholerae</u> strains when grown separately in the two media	45
Location of L-asparaginase activity	46
Precipitations of L-asparaginase of CFS, periplasm and cytoplasm lyzates of two <u>V.cholerae</u> strains	47
pH and temperature profiles of L-asparaginase of CFSP, PP and CP of the two <u>V.cholerae</u> strains	47
Thermo-inactivation and L-asparagine catalytic efficacy of L-asparaginase of CFSP, PP and CP from the two <u>V.cholerae</u> strains	47
Inhibition of L-asparaginase activity of CFSP, PP and CP of each strain	48
CHAPTER 2	49-79
<b>MATERIALS AND METHODS</b>	
Purification of L-asparaginase from <u>V.cholerae</u> strain 569B	
Harvest of cells	50
Cell-disruption	50

	<u>Page</u>
Ammonium sulphate fractionation	50
DEAE-cellulose column chromatography	50
Sephadex G-100 gel filtration	51
Sephadex G-200 gel filtration	51
DEAE-Sephadex column chromatography	52
Polyacrylamide gel electrophoresis	52
Gel-filtration for MW determination of the L-asparaginase	53
Sodium dodecyl sulphate (SDS) - polyacrylamide gel electrophoresis	54
Characterization of the purified L-asparaginase	
Influence of temperature, pH and strength of buffer on enzyme velocity	55
Incubation period	55
Variation of enzyme and substrate concentration	55
Variation of incubation temperature, pH and strength of buffer (in hydroxylaminolysis method)	56
Correlation of hydroxylaminolysis and hydrolysis reactions	56
Determination of substrate specificity	56
Determination of toxigenicity in enzyme preparation	
Suckling (or infant) mouse model	57
Rabbit skin vascular permeability test	57
Animals (care and diet)	57
Tumor-cells and maintenance	58
Tumor-cells count	58
The enzymes used	59
Tumor-inhibition assay ( <u>in vitro</u> study)	59

	<u>Page</u>
Tumor-inhibition study ( <u>in vivo</u> experiments) ....	59
Plasma-clearance test ....	60
Lymphocytes (T-cells) isolation from mice spleen ....	60
E-Rosette formation and count ....	61
Preparation for scanning electron microscopy ....	62
Antiserum preparation ....	62
Serological cross-reaction study ....	63
 <b>RESULTS</b>	
Purification of L-asparaginase from <u>V.cholerae</u> strain 569B	
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> fractionation ....	64
DEAE-cellulose column chromatography ....	64
Sephadex G-100 gel filtration ....	64
Sephadex G-200 gel filtration ....	65
DEAE-Sephadex column chromatography ....	65
Estimation of protein ....	65
Polyacrylamide gel electrophoresis ....	65
Molecular weight (MW) of the L-asparaginase ....	66
Sub-unit MW of the L-asparaginase ....	66
Homogeneity of the purified L-asparaginase ....	66
Characteristics and properties of the purified L-asparaginase	
Effect of temperature, pH and strength of buffer upon the reaction velocity (by nesslerization procedure) ....	67
Effect of incubation period on the enzymatic reaction velocity ....	67
Effect of enzyme concentration on reaction velocity (co-relation curve) ....	68
V <sub>max</sub> and K <sub>m</sub> value determination by nessleri- zation method ....	68

	<u>Page</u>
Heat-stability and storage-stability of the L-asparaginase ....	69
Effect of temperature, pH, and strength of the buffers upon reaction velocity (by hydroxylaminolysis method) ....	69
$K_m$ and $V_{max}$ determination-by hydroxylaminolysis of L-asparagine in relation to deamidation of L-asparagine ....	70
Substrate specificity of the purified L-asparaginase ....	71
Toxicogenicity check in the enzyme preparations ....	71
Effect of the purified <u>V.cholerae</u> -L-asparaginase and the <u>E.coli</u> -L-asparaginase upon tumor-cells ( <u>in vitro</u> study)	
Effect on Dalton's lymphoma-tumor cells ....	71
Effect on Ascites fibrosarcoma-tumor cells ....	72
Effects of the purified <u>V.cholerae</u> -L-asparaginase and the <u>E.coli</u> -L-asparaginase upon tumor ( <u>in vivo</u> study)	
Effects on Dalton's lymphoma ....	72
Effects on Ascites fibrosarcoma ....	73
Effects of the enzymes upon grown-tumor in mice ....	74
Plasma-clearance property of L-asparaginase ....	75
Immunological aspects of L-asparaginase treatment	
The immunocytoadherence study (E-Rosette formation) ....	76
Changes of spleen-size ....	76
Ultrastructural characters of splenic T-lymphocytes ....	77
Immunological inhibition of the enzyme activity ....	78
Serological cross-reaction study	
The immunoprecipitation reaction of the L-asparaginases ....	78

		<u>Page</u>
DISCUSSION	....	80-109
SUMMARY	....	110-114
REFERENCES	....	115-128