IDENTIFICATION OF PATHOGENIC ORGANISMS USING RECOMBINANT DNA METHODS

Thesis

Submitted By

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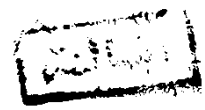

For the Degree of
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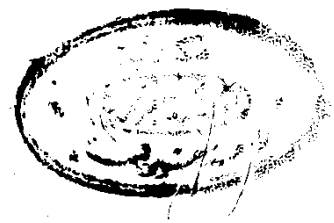

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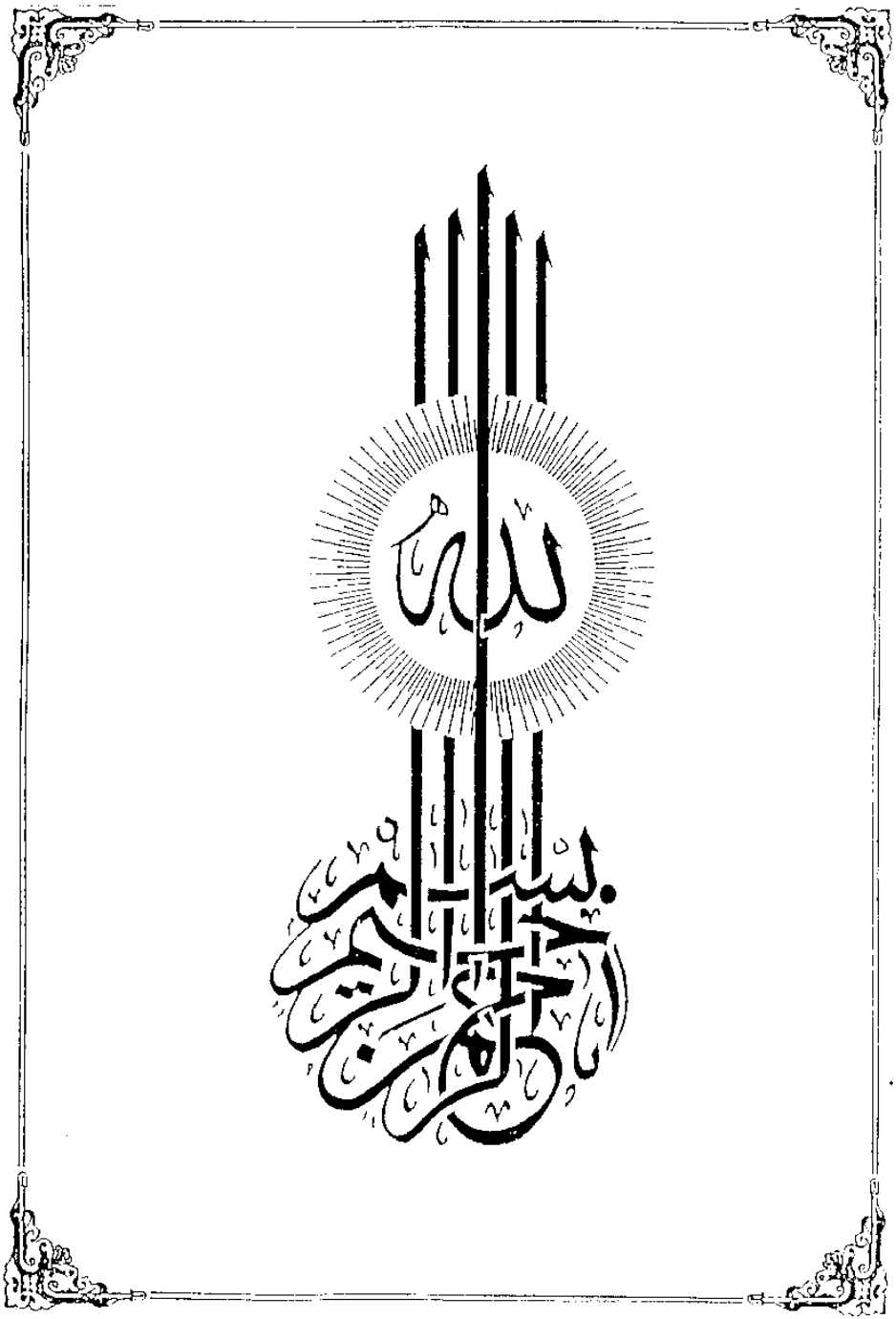

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CONTENTS

ABBREVIATIONS

A	adenine or current ampere
A_{260}	absorbance at 260 nm
AAAF	N-acetoxy-N-2-acetyl-aminofluorene
ABC	avidin DH-biotinylated horseradish peroxidase complex
ABC-AP	avidin DH-biotinylated alkaline phosphatase
ac	acrylamide
ADP	adenosine 5'-diphosphate
AEC	3-amino-9-ethylcarbazole
amm.	ammonium
AMP	adenosine 5'-monophosphate
AP	alkaline phosphatase
Ap	ampicillin
ATP	adenosine 5'-triphosphate
BCIP	5-bromo-4-chloro-3-indolyl phosphate
Bio	biotinylated
Biotin-11-dUTP	8-(2, 4-dinitrophenyl-2, 6- aminohexyl) aminoadenosine-5'-triphosphate or 2'-deoxyuridine-5' triphosphate-5' allylamin biotin
bis, bisacrylamide	N, N'-methylene-bisacrylamide
BM	Boehringer Mannheim
bp	base pairs
Bq	becquerel (1 disintegration/sec)
BRL	Bethesda Research Laboratories
BSA	bovine serum albumin
C	cytosine or carbon
°C	degree centigrade
ccc DNA	covalently closed circular DNA
cDNA	complementary DNA
CHO	Chinese hamster ovary
Ci	Curie= 3.7×10^{10} Bq $=2.2 \times 10^{12}$ disintegrations per minute
Cm	Chloramphenicol
cm	centimetre
Co.	Corporation
Col E1	colhicin
CsCl	cesium chloride
CT	Cholera toxin
DAB	3, 3' diaminobenzidine
dATP	deoxyadenosine triphosphate
DC	direct current
dCTP	deoxycytidine triphosphate

dd	deonized distilled
dGTP	deoxyguanosine triphosphate
dist	distilled
DMF	N,N-dimethylformamide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNP	2, 4 dinitrophenyl
dNTP	deoxynucleoside triphosphate
dpm	disintegrations per minute (60 Bq)
DTT	dithiothreitol
dTTP	deoxythymidine triphosphate
dUTP	deoxyuridine triphosphate
E. coli	*Escherichia coli*
Ed.	editor
EDTA	ethylene diamine tetraacetic acid disodium salt
EHEC	enterohemorrhagic *E. coli*
EIA	enzyme immunoassays
EIEC	enteroinvasive *E. coli*
ELISA	enzyme-linked immunosorbent assay
Ent	enterotoxin plasmids
EPEC	enteropathogenic *E. coli*
ETEC	enterotoxigenic *E. coli*
Eu	Europium
FIG.	figure
G	guanine or gauge
g	gram
GMP	guanosine 5'-monophosphate
^{3}H	tritium
Hly	hemolysin
H_2O	water
H_2O_2	hydrogen peroxide
hr	hour
Hrp	horseradish peroxidase
H_2S	hydrogen sulfide
^{125}I	iodine 125
IgG	immunoglobulin
kb	kilobases (1,000 nucleotide bases)
KCN	potassium cyanide
kg	kilogram
LB	Luria-Bertani medium
Log	logarithm
LT	Heat-labile enterotoxin production
M	molarity
mCi	milliCurie = 3.7×10^{7} Bq $= 2.2 \times 10^{9}$ dpm

Mdal	megadaltons
Mg^{2+}	magnesium
mg	milligram
min	minutes
ml	millilitre
mM	millimolar
mm	millimetre
m mol	millimol
MRHA	mannose-resistant hemagglutination
mRNA	messenger ribonucleic acid
ms	millisecond
M. wt	molecular weight
N	normality or nitrogen
η	density
NAD	nicotinamide adenine dinucleotide
NaCl	sodium chloride
NAMRU-3	Naval Medical Research Unit No. 3
NaOH	sodium hydroxide
NBT	nitro-blue tetrazolium
NC	nitrocellulose
NEN	New England Nuclear
ng	nanogram
no.	number
NTB	nick-translation-buffer
NY	New York
OD	optical density read at a defined wavelength
OH	hydroxyl
ONPG	orthonitrophenyl-β-D-galactopyranoside
p	para or page
^{32}p	phosphorus 32
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
pg	picogram
pI	isoelectric point
pmol	picomol
poly	polymer
ppt	precipitation
prep	preparation
R	deoxyribose 5' triphosphate
RE	restriction endonuclease
RFLPs	restriction-fragment-length-polymorphisms
RL	radiolabeled
RNA	ribonucleic acid
RNase	ribonuclease

rpm	round per minute
rRNA	ribosomal ribonucleic acid
^{35}S	sulphur 35
S & S	Schleicher and Schuell
SBAP	Streptavidin biotinylated alkaline phosphatase
SDS	sodium dodecyl sulfate
sec	second
SNAP^{R}	Synthetic Nucleic Acid Probe
sp.	species
SSB	single-stranded binding protein
SSC	standard saline citrate
ST-H	heat-stable enterotoxin production of human origin
ST-P	heat-stable enterotoxin production of porcine origin
Suppl.	supplement
T	thymine
T_{50}	the actual time required for the successful 50% hybridization of a given probe
Tc	tetracycline
TCA	trichloroacetic acid
TEMED	N, N, N', N' tetramethylethylenediamine
T_i	incubation temperature
TM	transfer membrane
T_m	melting temperature
TR-FIA	time-resolved fluoroimmunoassays
Tris	tris (hydroxymethyl) aminomethane
tRNA	transfer RNA
Tween 20	polyoxyethylene sorbitan monolaurate
U	uridine or unit of enzyme activity
μ	micron
μCi	microCurie = 3.7×10^4 Bq = 2.2×10^6 dpm
μg	microgram
μl	microlitre
μM	micromolar
unpubl.	unpublished
USA	United State of America
UTI	urinary tract infections
UTP	uridine 5'-triphosphate
UV	ultraviolet light
V	volts or volume
vol/vol	volume/volume
w/w	weight/weight
x	times or fold
xg	centrifugal force (x unit gravitational field)

PREFACE

Colony hybridization is an important technique used in recombinant DNA work for clinical and non clinical applications. It is a rapid and simple technique for detecting specific DNA sequences in bacterial colonies with the drawback that adequate sensitivity requires radiolabeling the probe with ^{32}P. An alternative hybridization approach which has gained widespread acceptability over the past few years involves labeling the probe with biotin and enzymatic detection *via* a streptavidin (or avidin) bridge. While such assays are currently available using biotin/avidin systems their limited sensitivity and their high background precludes their current use in colony hybridization assay. We have tried to extend the usefulness of this technique to bacterial colony hybridization. In this respect we have identified the source of high backgrounds in the colony hybridization assay and have worked out a method to eliminate that background.

While the broader scope of the research undertaken in this thesis is to increase the usefulness of the colony hybridization assay for diagnosing infectious diseases. To achieve this goal we are focusing on the detection of enterotoxigenic *E. coli* and the use of a biotin-streptavidin enzymatic detection system.

I. INTRODUCTION