Molecular Characterization of the Plasmids in *Vibrio cholerae* strain V58.

Eveline Jutta Bartowsky, B.Sc. (Hons.) (Adelaide)

Department of Microbiology and Immunology

The University of Adelaide

A thesis submitted for the degree of

Doctor of Philosophy

June 1988

To my Mum and Dad,

for their love and

understanding

CONTENTS

ABSTRACT

V58 is one of the prototype strains used in the genetic analysis of *Vibrio cholerae* and has been found to contain three plasmids. The largest of these corresponds to the P sex factor which except for its ability to promote low level transfer of chromosomal markers is cryptic like the other two plasmids.

The aim of this work was to characterize these three plasmids on a molecular and physical basis as well as to investigate their possible functions. The three plasmids have been identified in whole genomic DNA extracts of V58 and sized by agarose gel electrophoresis and electron microscopy. Restriction endonuclease cleavage maps of the three plasmids have been constructed and the sum of the fragment sizes agrees with other measurements: P, 68 kb; large cryptic plasmid (lcp), 34 kb; small cryptic plasmid (scp), 4.7 kb.

Most of the *Eco*RI and *Xba*I fragments of P have been cloned and the proteins encoded within these fragments analysed in both whole cells and minicells. Concerted effort has failed to clone the remaining fragments suggesting they may contain lethal functions in the absence of other regions of P. The number of proteins detected in the subclones does not account for the potential coding capacity suggesting that important regulatory regions/genes have not been cloned on the fragments. Similarly the lcp and scp have been subcloned and the proteins analysed.

Possible properties of P have been examined including resistance to serum and metal ions, but no effect could be seen comparing P^+ and P^- strains. The ability of P to transfer to different hosts and its relationship to the incompatibility type strains has been examined. P is unique in its properties and also could be shown to encode a surface

exclusion system. It has been previously reported that P plays a role in the suppression of virulence of hypertoxinogenic strains. Studies here have demonstrated that the suppression of virulence by P is due to poor colonization of the small intestine. Transposon mutagenesis of P has enabled the regions associated with transfer and surface exclusion to be mapped on the plasmid, however the region(s) responsible for suppression of virulence could not be localized.

The lcp has no phenotype except that it can also be shown to be transferable and is identical to the V plasmid described in non-01 *V. cholerae* and a 31.5 kb (21 MDal) plasmid identified in Classical strains isolated during the sixth pandemic.

A role for the scp has not been identified. It appears to be the same as the 4.5 kb (3 MDal) plasmid detected in Classical strains isolated during the sixth pandemic.

Using subclones of these various plasmids, it has been possible to examine their distribution in other *Vibrio* species by DNA hybridization. None of the plasmids were found in *Vibrio* species other than in *V. cholerae* 01 even though numerous plasmids were detected in non-01 isolates.

STATEMENT

I state that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by any other person, except where due reference is made in the text of the thesis.

Eveline Bartowsky

June 1988

ACKNOWLEDGEMENTS

I would like to thank Dr. Paul Manning for his supervision, guidance and encouragement throughout the period of this study.

My thanks and appreciation also to Dr. Giovanna Morelli and Marion Kamke for their excellent use of the electron microscope and to Drs. Stephen Attridge, Peter Ey and Graham Mayrhofer for their assistance in the immunological work. Helpful discussion with Dr. Jim Hackett was greatly appreciated.

The support of a University of Adelaide Postgraduate Scholarship is gratefully acknowledged.

ABBREVIATIONS

Ap	ampicillin
ATP	adenosine 5'-triphosphate
bp	base or nucleotide pairs
Bla	gene for β-lactamase encoding Ap^R
BSA	bovine serum albumin
cpm	counts per minute
cAMP	cyclic adenosine 3', 5'-monophosphate
CAT	chloramphenicol acetyltransferase
Cm	chloramphenicol
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxynucleoside triphosphate
ds	double stranded
EDTA	ethylenediaminetetraacetic acid
DTT	dithiothreitol
ELISA	enzyme-linked immunosorbent assay
eop	efficiency of plating
EtBr	ethidium bromide
Ig	immunoglobulin
kDal	kilodaltons(s)
kb	kilobase pair(s) or 1000bp
Km	kanamycin
LPS	lipopolysaccharide
MDal	megadaltons
min	minute(s)
MM	minimal medium
NA	Nutrient Agar

NB	Nutrient Broth
pfu	plaque-forming units
PAGE	polyacrylamide-gel electrophoresis
^{R}	resistance
Rif	rifampicin
RNA	ribonucleic acid
rpm	revolutions pre minute
^{S}	(superscript) sensitive
SDS	sodium dodecyl sulphate
sec	second(s)
Sm	streptomycin
Spc	spectinomycin
TB	Tryptone Broth
Tc	tetracycline
Tn	transposon
Tris	Tris (hyroxymethyl)aminomethane
UV	ultraviolet
wt	wild type
[]	designates plasmid-carrier state