



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

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To my Mum and Dad,
for their love and
understanding

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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 *EcoRI* and *XbaI* 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.

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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 (hydroxymethyl)aminomethane |
| UV | ultraviolet |
| wt | wild type |
| [] | designates plasmid-carrier state |