A 98 Megadalton R Factor of Compatibility Group C in a *Vibrio cholerae* El Tor Isolate from Southern U.S.S.R.

By R. W. HEDGES AND A. E. JACOB

Bacteriology Department, Royal Postgraduate Medical School,
London W12 0HS

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INTRODUCTION

The seventh cholera pandemic has been the result of the westward spread of the El Tor biotype of *Vibrio cholerae* from Indonesia reaching the Astrakhan area of the U.S.S.R. about 1970 (Cvjetanovic & Barua, 1972). Initially the bacteria were susceptible to antibiotics, and tetracycline, in particular, was of value in clinical practice (Greenough *et al.* 1964).

Kuwahara *et al.* (1967), Prescott, Datta & Datta (1968) and Rahal, Gerbaud & Chabbert (1973) have observed strains of *V. cholerae* carrying transmissible antibiotic resistance plasmids (R factors). Rahal *et al.* (1973) found that a strain isolated in Algeria carried an R factor (conferring resistance to ampicillin, streptomycin, tetracycline, chloramphenicol, kanamycin and sulphonamides) that was a member of compatibility group 6 which corresponds with the A-C complex in the system of N. Datta and colleagues (see Hedges, 1974).

METHODS

Escherichia coli K12: J53-2 F⁻ *pro met rif^R*; J62 F⁻ *pro his trp lac*; W3110 *thy F⁻*; HfrC Hfr *met* (Bachmann, 1972).

Transfer of R factors and determination of compatibility and fi character were as described by Datta *et al.* (1971), Coetzee, Datta & Hedges (1972) and Dennison (1972).

Radiolabelling and lysis of strains, isolation of R plasmid DNA by caesium chloride-ethidium bromide density gradient centrifugation and neutral sucrose gradient analysis of plasmid DNA were as described by Jacob & Hobbs (1974), modified by Hedges & Jacob (1974).

Calculation of R plasmid molecular weight. The relationship used was of the general form $d_1/d_2 = (M_1/M_2)^n$ (Burgi & Hershey, 1963), where d_1 and d_2 are the distances sedimented from the meniscus by the unknown and reference R plasmid DNA of similar tertiary structure, and M_1 and M_2 are their respective molecular weights. The values of exponent n used were determined empirically by Barth & Grinter (1974) as 0.43 for the covalently closed circular (supercoiled) DNA tertiary form, and 0.36 for the open circular DNA form.

RESULTS

Resistance determinants of V. cholerae P2796

A clinical isolate of *V. cholerae* El Tor, P2796, stably resistant to streptomycin, tetracycline and chloramphenicol, was received from Dr P. I. Anisimov, Director of The Institute 'Microbe', Saratov, U.S.S.R. All three resistances proved to be transferable to *E. coli* K12 as a group, whichever resistance was selected. We concluded that all three were determined by a single plasmid, R994.

Table 1. *Quantitative antibiotic resistance patterns conferred by R994*

	<i>V. cholerae</i> p2796	<i>E. coli</i> J62	<i>E. coli</i> J62 (R994)
Streptomycin	> 100	< 2	10*
Tetracycline	> 100	< 5	> 50, < 100
Chloramphenicol	10	< 2.5	80

Figures refer to minimal inhibitory concentrations in $\mu\text{g/ml}$ in MacConkey agar.

* R994 conferred no increase in resistance to spectinomycin and therefore probably determines production of streptomycin phosphotransferase (Hedges, 1972).

Although all three resistances were transferable from *V. cholerae* the levels of resistance conferred upon the *E. coli* transcient were strikingly different from those of the donor (Table 1). Thus p2796 was fully resistant to 100 μg of streptomycin/ml whereas J62 (R994) was inhibited by concentrations as low as 10 $\mu\text{g/ml}$ (and was only about five times as resistant as the R⁻ strain). This very great difference suggests that p2796 carries chromosomal as well as plasmid-borne streptomycin resistance.

The low level of chloramphenicol resistance of p2796 compared with J62 (R994) suggests that the gene(s) determining this property is expressed relatively inefficiently in the *V. cholerae* host.

Compatibility properties of R994

Hfrc (R994) was visibly lysed by phage MS2 (Davis, Strauss & Sinsheimer, 1961). Therefore, R994 is fi⁻. R994 is transmissible between R⁻ strains of *E. coli* K12 with an efficiency of about 5×10^{-5} /donor/h. The efficiency of transfer into a recipient carrying a plasmid of the A-C complex was less than 1% of this value and the presence of R994 in a recipient culture excluded the transfer of other plasmids of this group.

R994 was incompatible with R40a, an R factor determining resistance to ampicillin, kanamycin and sulphonamides assigned to group C (i.e. *com-6*) (Datta & Hedges, 1972; Chabbert *et al.* 1972). Since there are no common resistance markers on these two plasmids it was possible to show that all the R994 determinants were simultaneously eliminated, confirming the conclusion that they were part of a single plasmid.

Molecular properties of R994

The covalently closed circular (CCC) DNA tertiary form of plasmid R994 was isolated by using dye-buoyant density centrifugation, and its molecular weight was determined by comparing its sedimentation rate through a neutral sucrose gradient with the CCC DNA of R plasmid R1, which has a molecular weight of 60 Mdaltons (Clowes, 1972; P. T. Barth and N. J. Grinter, unpublished), as a reference. The results indicated that R994 is a single plasmid DNA species, with a molecular weight calculated to be 98 Mdaltons.

DISCUSSION

The R factor transferred from an Algerian strain of *V. cholerae* El Tor by Rahal *et al.* (1973) was a member of compatibility group C. The compatibility properties of the R factors transferred from Calcutta *V. cholerae* strains (both El Tor and 'classical' varieties) were not investigated, but all determined resistance to tetracycline and sulphonamides, a pattern frequently observed in plasmids of the A-C complex (Hedges, 1974, and unpublished observations).

Thus, it may be that the *V. cholerae* R factor set is dominated by plasmids of the A-C complex. Yokota *et al.* (1972) showed that a variety of R factors capable of transfer to *V. cholerae* El Tor were unable to maintain themselves stably in growing cultures. However, plasmids of the A-C complex seem to be adapted to existence in a particularly broad range of hosts. A-C complex plasmids have been found in *Pseudomonas aeruginosa* (Chabbert *et al.* 1972; Bryan, Shahrabadi & van den Elzen, 1974), *Aeromonas* spp. (Aoki *et al.* 1971; Hedges & Datta, 1971), *Proteus mirabilis* (Hedges, 1975), *Providencia* (Hedges, 1974) and *Serratia marcescens* (Hedges, Rodriguez-Lemoine & Datta, 1975), as well as in a variety of typical enterobacteria. The very low efficiency of expression of chloramphenicol resistance determinants suggests that even members of this extremely promiscuously distributed group are imperfectly adapted to the *V. cholerae* cell.

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