SHORT COMMUNICATIONS

A Variety of Staphylococcal Plasmids Present as Multiple Copies

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(Received 4 June 1973; revised 16 July 1973)

INTRODUCTION

In enteric bacteria estimation of the number of plasmids per chromosome reveals two distinct situations (Clowes, 1972): first, when the number of copies is approximately one; and second, when the number of copies is much greater (generally between 7 and 40). This division may also exist for staphylococcal plasmids (Novick & Bouanchaud, 1971), but to test this possibility more fully we have determined the number of plasmid copies per organism for a range of staphylococcal plasmids. We have found that plasmids detectable as covalently closed circular (CCC) DNA are present in multiple copies, i.e. as at least four copies per organism.

METHODS AND RESULTS

Mitomycin C-induced lysates of clinical strains containing single plasmids were used to prepare the *Staphylococcus aureus* 649 transductants shown in Table 1. The individual plasmids were then isolated as CCC DNA by dye-buoyant density centrifugation (Lacey & Grinsted, 1972). Strain 649N, the recipient in transduction, contains no detectable CCC DNA (Grinsted & Lacey, 1973*a*). From the yield of CCC DNA the number of plasmid copies per organism can be calculated, provided that both plasmid molecular weight and the bacterial DNA content are known. The molecular weights of the individual plasmids were determined from contour-length measurements by electron microscopy (Lang & Mitani, 1970). The cellular DNA content was estimated as follows: bacteria in the stationary phase were washed and resuspended in saline, sonicated for 2 min to disperse clumps, and samples taken for DNA assay (Grinsted & Lacey, 1973*b*) and total bacterial count using a slide counting chamber. Strain 649N contained $(6.6 \pm 0.3) \times 10^{-15}$ g DNA/organism.

All plasmids analysed existed as at least four copies per organism (Table 1), which is probably a minimum estimate since a proportion of plasmid DNA may not be in the CCC form and therefore not detectable by this assay procedure. The majority of plasmids (with molecular weights between six and 35 megadaltons) were present as about six to ten copies per organism, but the 'tetracycline plasmid' (mol. wt 2.9 megadaltons) was present as about 40 copies per organism. However, the ratio of plasmid to total DNA was relatively constant for all strains (Table 1), which may indicate control of the total quantity of plasmid DNA per organism.

DISCUSSION

Our data are not in complete agreement with those of Novick & Bouanchaud (1971), who found that a penicillinase plasmid of molecular weight about 20 megadaltons exists only in two to three copies per organism, although a small plasmid (specifying tetracycline

Table 1. *The number of copies per organism and transduction frequencies of a variety of staphylococcal plasmids*

Cultures of *Staphylococcus aureus* 649 containing the plasmids shown below were incubated with (Me-³H)-thymidine (10 µCi/ml, 5 Ci/mmol) and analysed for CCC DNA by ethidium bromide/CsCl-gradient centrifugation (Lacey & Grinsted, 1972). Contour-length measurements by electron microscopy were made on plasmid fractions cleared of ethidium by extraction with *n*-octanol followed by dialysis against TES buffer (0.05 M-tris, 0.05 M-NaCl, 0.005 M-EDTA, pH 8.0). Transduction and transfer in mixed culture were performed as previously described (Grinsted & Lacey, 1973a; Lacey, 1971).

Plasmid marker(s)*	Molecular weight (megadaltons)†	Percentage of total DNA‡	Number of estimations	Copy no.§	Transduction frequency	Transfer frequency in mixed culture¶
Cd, Hg, As	34.9±0.9	5.0±1.4	10	4-7	2.4×10 ⁻¹⁰	<10 ⁻¹⁰
Cd, Hg, As, P	20.0±0.5	5.2±2.2	4	6-15	1.5×10 ⁻⁷	3.3×10 ⁻⁷
Cd, P, F	14.6±0.2	4.5±0.5	3	11-14	2.0×10 ⁻⁷	4.0×10 ⁻⁷
Cd, P, F	13.5±0.2	3.3±1.3	3	6-13	8.5×10 ⁻⁶	4.2×10 ⁻⁷
N	5.9±0.2	1.9±0.5	5	9-16	6.0×10 ⁻⁶	5.3×10 ⁻⁵
T	2.9±0.1	3.1±0.8	7	31-47	2.5×10 ⁻⁵	4.5×10 ⁻⁴

* Nomenclature: Cd, Hg and As are markers for resistance to cadmium, mercuric and arsenate ions respectively; F, N and T resistance to fusidic acid, neomycin and tetracycline respectively; P = penicillinase production.

† Calculated from contour lengths using a mass-to-length ratio for double-stranded DNA of 2.07 megadaltons/µm (Lang, 1970).

‡ % of radioactivity in satellite peak to chromosomal peak. Quench correction was performed by the Channels Ratio method.

§ Calculated from the quoted molecular weights and the yields of plasmid DNA using the value 6.6×10⁻¹⁵ g DNA/649N organism.

|| Transduction frequency/plaque-forming unit. Performed by propagation of phage 53 on the 649 strains carrying individual plasmids and using *Staphylococcus aureus* 649N as recipient.

¶ Number of resistant recipients/total recipients. Transfer in nutrient broth between *Staphylococcus aureus* 609 carrying the individual plasmids and *Staphylococcus aureus* 6936.

resistance) is present as multiple copies. Although the discrepancy may result from differences in the staphylococcal strains used, our results are comparable with those obtained for *Escherichia coli*, in which plasmids of molecular weight of about 30 megadaltons or less are present in multiple copies (Clowes, 1972).

The existence of staphylococcal plasmids in multiple copies per organism could account for their high transduction frequencies both *in vitro* and *in vivo*. But if the number of plasmid copies per organism was the major factor involved, then the frequency of transduction of plasmids with similar numbers of copies per organism should be equal. However, neither transduction frequency nor frequency of transfer by transduction in mixed cultures was constant for those plasmids present as about ten copies per organism, but rather the frequencies of transduction increased with plasmids of decreasing size (Table 1). Thus plasmid size is probably the important factor in transduction. Analysis of mutants with increased numbers of plasmid copies per organism could establish this point. Attempts to obtain such mutants have so far been unsuccessful.

This work was supported by grants from the Medical Research Council to Professor M. H. Richmond.

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