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Characterization of Concatemeric Plasmids of *Neisseria gonorrhoeae*

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Three strains of *Neisseria gonorrhoeae* carried novel plasmids of 7.8 megadaltons (mdal) molecular mass in addition to plasmids previously observed in this organism. The presence of the 7.8-mdal plasmids was not accompanied by any distinguishable phenotype in the strain possessing them. Analysis of plasmid DNA with restriction endonucleases showed that these plasmids were composed of three directly repeated copies of a 2.6-mdal cryptic plasmid frequently found in *N. gonorrhoeae*. In addition, the 7.8-mdal plasmids exhibited characteristics common to the 2.6-mdal plasmid, structural lability and sites resistant to cleavage with *Hpa*II. The concatemeric forms of the cryptic plasmid appear to be stable in these strains and do not undergo internal recombination to produce the 2.6-mdal monomer, nor were higher concatemers detected.

Since the emergence of penicillinase-producing *Neisseria gonorrhoeae* (PPNG), the range of plasmids found in these strains has remained relatively constant. The majority of strains possess a 2.6 × 10^6-megadalton (Mdal) cryptic plasmid, and, in rare instances, they may also possess a 24.5-Mdal plasmid (3, 6, 11). If the strains are PPNG, they will also possess either the 4.4-Mdal R plasmid characteristic of Asian PPNG or the 3.2-Mdal R plasmid commonly found in West African PPNG (10, 14, 16). Those strains that harbor the 4.4-Mdal R factor also frequently contain the 24.5-Mdal plasmid, which has been shown to conjugally mobilize the R plasmid (14). However, PPNG strains that possess both the conjugative plasmid and the 3.2-Mdal R factor have been found, although less frequently (4).

While screening both PPNG and non-PPNG strains, we encountered strains of both types which contained novel plasmids of 7.8 × 10^6 Mdal molecular mass, as determined by agarose gel electrophoresis and electron microscopy of purified plasmid DNA. These plasmids were found singly and in combination with other plasmids previously observed in gonococci, among them the 4.4-Mdal plasmid, which encodes beta-lactamase production (Table 1 and Figure 1) (9, 13, 14, 16). As expected, both PPNG strains exhibited resistance to only penicillin and ampicillin as expected, but strain 79C′, which was a non-PPNG isolated from a patient with pelvic inflammatory disease, did not show resistance to chloramphenicol, tetracycline, spectinomycin, kanamycin, or erythromycin. In addition, no relationship was observed between auxotype and the presence of a 7.8-Mdal plasmid, nor did all the strains fall into the same serogroup as determined by the W antigen coagglutination typing scheme described by Sandstrom et al. (12, 15).

The 7.8-Mdal plasmids and the 2.6-Mdal cryptic plasmid of strain 746 showed nearly identical restriction profiles when purified plasmid DNA was digested with *Eco*RI, *Ava*II, *Hin*fI, and *Ava*II followed by *Hin*fI (Fig. 2A). These results suggested that the 7.8-Mdal plasmids are identical and that they are concatemers composed of three directly repeated copies of the 2.6-Mdal plasmid. Only the larger *Ava*II/*Hin*fI fragment of the plasmid from strain 416 differed slightly in size from the corresponding fragment of the plasmids of the other strains. Similar results were obtained for the 2.6-Mdal cryptic plasmid isolated from strain 416 (Fig. 2B). The increased size of this fragment from both plasmids suggested that the two plasmids might be related.

Both the 2.6-Mdal cryptic plasmid of strain 746 and the 7.8-Mdal plasmid of strain 79C′ were extremely resistant to digestion with *Hpa*II, as reported by other investigators for the 2.6-Mdal plasmid (2, 5). Only two sites on the 2.6-Mdal plasmid appeared readily susceptible, whereas *Hpa*II rapidly generated four fragments from the larger plasmid (Fig. 3). However, prolonged digestion with 7 U of *Hpa*II was necessary to completely digest the *Hpa*II fragment III of the concatamer, which indicated the presence of *Hpa*II-resistant sites on this fragment. Although plasmid DNA isolated from strain 79C′ exhibit-

TABLE 1. Properties of *N. gonorrhoeae* strains

Strain	Plasmids (mol wt × 10^6)	β-Lactamase[a]	Auxotype[b]	Serogroup
79C′	7.8	−	Arg^-	W I
416	2.6, 4.4, 7.8, 24.5	+	Pro^-	W II
486	7.8, 4.4, 24.5	+	Wild type	W II
746	2.6, 24.5	−	Wild type	ND[c]
CDC9	2.6	−	Pro^-	ND

[a] β-Lactamase was detected with both the microiodometric assay (8) and chromogenic cephalosporin (9).
[b] Auxotyping was performed on gonococcal genetic medium as described previously (13).
[c] ND, Not done.

ed resistance to *Hpa*II and the partial digestion products from all plasmids were similar, it was not as resistant as the monomeric 2.6-Mdal plasmid from strain 746. It is not clear whether this distinction was related to the modification properties of the host strain or whether the size of the concatemer resulted in greater exposure of *Hpa*II cleavage sites in the concatemer.

Since the 7.8-Mdal plasmid is apparently tandem, direct repeats of the 2.6-Mdal plasmid, it was possible that recombination could take place within the concatemer to produce the monomeric 2.6 Mdal-plasmid with subsequent disappearance of the trimeric plasmids. However, neither strain 79C′ nor strain 486 contained detectable monomer-sized plasmids; thus such events should be easily observed.

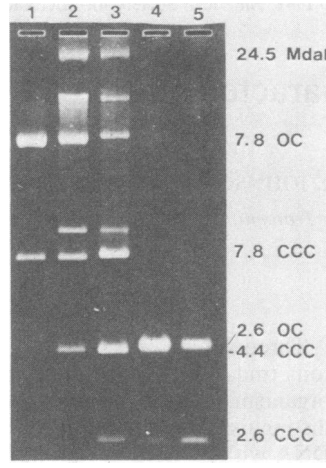

FIG. 1. Agarose gel electrophoresis of purified plasmid DNA. Lane 1, strain 79C′; lane 2, 486; lane 3, 416; lane 4, 746; and lane 5, CDC9. Plasmid purification and agarose gel electrophoresis were performed as described previously (1, 7).

Cultures of either T_2 or T_3 colony type were obtained from both strains. These were serially passaged six times on Gonococcal Base agar, and crude plasmid DNA was extracted from 100 mg (wet weight) of cells and examined by electrophoresis for plasmid content. The 2.6-Mdal plasmid was not present in the unselected, unpassaged controls or in the passaged type T_2 or T_3 cultures (Fig. 4).

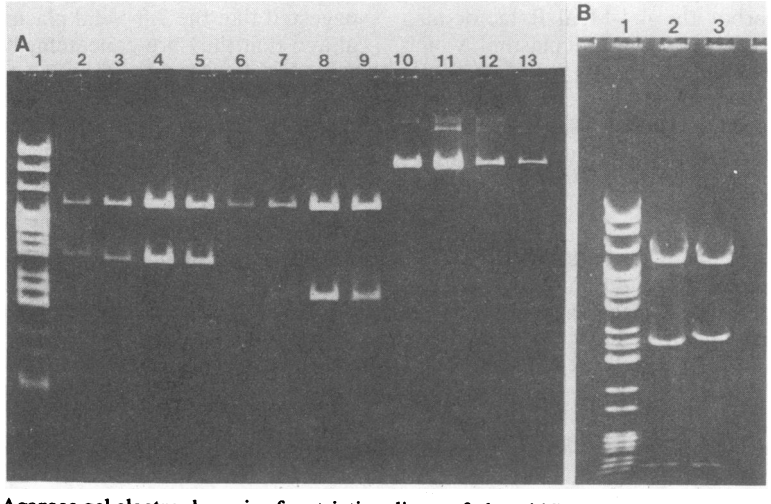

FIG. 2. (A) Agarose gel electrophoresis of restriction digest of plasmid DNA. The order is as follows: lanes 2, 6, and 13, plasmid DNA from strain 416; lanes 3, 7, and 12, plasmid DNA from strain 486; lanes 4, 8, and 11, plasmid DNA from strain 79C′; and lanes 5, 9, and 10, DNA from strain 746. Lanes 2 through 5 were digested with *Ava*II, lanes 6 through 9 with *Ava*II and *Hin*fI, and 10 through 13 with *Hin*fI only. Lane 1 contains Lambda phage DNA digested with *Hinc*II. (B) Comparison of *Hin*fI/*Ava*II digests of 2.6-Mdal plasmid DNA isolated from strain 746 (lane 2) and 2.6-Mdal plasmid DNA from strain 416 (lane 3).

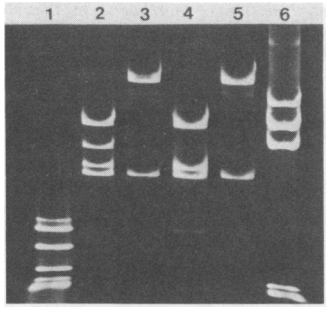

FIG. 3. *Hpa*II digests of plasmid DNA isolated from strains 746 and 79C'. Lane 1, pBR322 digested with *Hpa*II; lanes 2 and 4, 7.8-Mdal plasmid DNA (79C') digested with 2 U of *Hpa*II for 25' and 7 U of *Hpa*II for 120'; lanes 3 and 5, 2.6-Mdal plasmid DNA digested with 2 U of *Hpa*II for 25' and 7 U of *Hpa*II for 120'; lane 6, pBR322 digested with *Ava*II.

The origin of the 7.8-Mdal concatemers is not known, and it is not clear whether their occurrence is a property of the strains or of the particular plasmids which they harbor. The strain exhibited no obvious phenotypic properties to suggest that they possessed a common background that would denote or account for the presence of a 7.8-Mdal plasmid rather than the more common 2.6-Mdal plasmid. This phenomenon is probably not generally related to plasmid replication and maintenance for two reasons. First, the 4.4-Mdal R factor was not altered in size, and second, there were no concatemers of a distribution of sizes, as might be expected if they resulted from plasmid replication. However, results obtained from *Hin*fI/*Ava*II double digests of the 7.8- and 2.6-Mdal plasmids of strain 416 clearly suggested that the two plasmids are related and that one probably arose from the other.

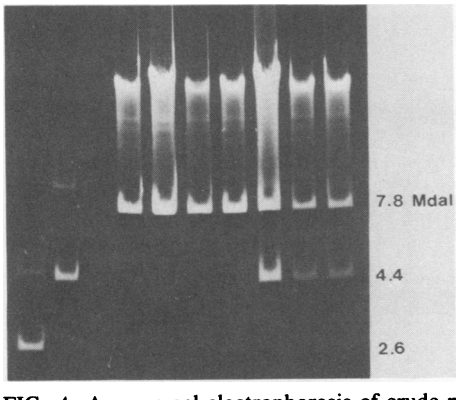

FIG. 4. Agarose gel electrophoresis of crude plasmid DNA from cleared lysates (2) isolated from independently passed T_2 and T_3 colony type cultures of strains 79C' and 486. Lanes 1 and 2, 2.6- and 4.4-Mdal markers, respectively; lane 3, unpassaged 79C' control; lane 4, 79C' T_3 colony type; lanes 5 and 6, 79C' T_2 colony types; lane 7, 486 T_3 colony type; lane 8, 486 T_2 colony type; lane 9, 486 unpassaged control.

Interestingly, there did not appear to be any evidence for intramolecular recombination, which would have generated plasmids of 5.2 and 2.6 Mdal from the 7.8-Mdal plasmids. This observation was of special interest since it might indicate a basic defect in host-mediated recombination in these strains. Although this would not explain the formation of the 7.8-Mdal trimer, it offers an explanation for the persistence of the concatemer. In addition, the apparent stability of the 7.8-Mdal plasmid indicated that this plasmid may be suitable as an epidemiological marker.

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