Identification of DNA Homologies Among H Incompatibility Group Plasmids by Restriction Enzyme Digestion and Southern Transfer Hybridization

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Plasmids belonging to the three HI plasmid incompatibility subgroups were characterized by the use of restriction enzymes and Southern transfer hybridization. A diversity of restriction enzyme patterns was noted among the HI subgroups, and a small amount of DNA homology was observed by probing these digests with a nick-translated HI1 plasmid. Within a single subgroup (HI1 and HI2), similar restriction enzyme patterns were noted. Plasmids of all three HI subgroups and the HI group had a guanine plus cytosine content of 49 to 50 mol%. The IncHI1 plasmid pH1508a also showed some homology with the HI1 probe. The DNA homology observed is probably responsible for common phenotypic properties encoded by these plasmids.

Plasmids have been conveniently classified by incompatibility, the inability of related plasmids to stably coexist within the same host cell. In general, plasmids within a given incompatibility group show a high degree of DNA homology. Plasmids belonging to the H incompatibility group, however, do not necessarily demonstrate this property as determined by DNA-DNA hybridization studies with total plasmid DNA (5, 11). Based on these studies, the H plasmid group has been divided into three subgroups designated H1, H2, and H3 such that members within a given subgroup show a high degree of DNA homology with one another, but not with members of the other two subgroups. The H3 subgroup to date comprises only a single member, MIP233 (11).

Despite the lack of homology between members of the H plasmid group, they do share several common features. They are all large (greater than 100 megadaltons [Mdal]), have a thermostable mode of transfer (15), and determine morphologically and serologically related pili (2). These plasmids have played an important role in mediating antibiotic resistance in medically important pathogens (17, 18, 20).

Recently, Bradley et al. (3) have described plasmids that are similar to H plasmids in size, pili, and mediation of resistance to potassium tellurite. Members of this new plasmid group, however, are compatible with reference H plasmids, and their mode of transfer is either thermostable nor repressed like that of H1, H2, and H3 plasmids. These plasmids have been designated IncHII, and their relationship with the H plasmid subgroups now designated HI1, HI2, and HI3 has been likened to that of FI with FII plasmids, which are related by antigenically similar pili.

In the present study we investigated the molecular relatedness of these H plasmid subgroups by the use of restriction enzyme analysis and Southern transfer-hybridization techniques. Representative members of each subgroup were isolated, and their buoyant density and corresponding guanine plus cytosine (G+C) contents were determined. They were then digested with restriction enzymes. Although a diversity of fragment patterns was generated, trends were noted in the frequency of cutting by particular restriction enzymes. Southern transfer hybridization techniques, with a nick-translated HI1 plasmid as a probe, revealed that there is indeed a small, but distinct, amount of homology among members of different H subgroup plasmids. A member of the IncHII plasmid group was included in this study and also revealed some sequence homology with HI1 probe. In addition, five HI1 and five HI2 plasmids were examined; within a subgroup, similar restriction patterns were noted. When the HI1 plasmids were probed with a nick-translated HI1 plasmid, extensive sequence homology was observed.

MATERIALS AND METHODS

Bacterial strains and plasmids. The H plasmids and their relevant properties are listed in Table 1. All of the plasmids were harbored in derivatives of E. coli K-12.

Isolation of plasmid DNA. Plasmid DNA was isolated by the Sarkosyl lysate method. Cultures were grown in enriched minimal medium (8) to an optical density of 0.4 to 0.6 at 600 nm. Lysates were prepared
as described earlier (8) to a final volume of 80 ml. The lysates were then treated with RNase (Boehringer Mannheim Canada Ltd.) at a final concentration of 50 μg/ml for 20 min at 25°C. Lysates were treated with either pronase at 200 μg/ml or with proteinase K at 150 μg/ml for 30 min at 37°C. The proteases were obtained from Boehringer Mannheim. Plasmid DNA was purified in CsCl-ethidium bromide density gradients in a Beckman 75 Ti rotor at 55,000 rpm for 24 h. Plasmid peaks from a first centrifugation were pooled and rebandaged.

Measurement of buoyant density of DNA and determination of corresponding G+C content. Plasmid DNA was isolated as described above and subjected to analytical ultracentrifugation in CsCl with a Beckman model E ultracentrifuge at 44,000 rpm for 24 h at 25°C (16). The relative buoyant densities of the plasmid DNA was determined relative to Micrococcus lysodeicticus chromosomal DNA (buoyant density, 1.731 g/cm³). The base composition (G+C content in moles percent) was then determined graphically from published data (16).

Restriction enzyme digestion. Restriction enzymes were purchased from Boehringer Mannheim and Bethesda Research Laboratories (Rockville, Md.). Samples (0.5 to 1.0 μg) of DNA were digested for 80 min at 37°C under the conditions specified by Davis et al. (4). The reaction was stopped by the addition of EDTA to 12 mM final concentration and heating at 71°C for 10 min.

Agarose gel electrophoresis. Samples were electrophoresed on a horizontal agarose gel apparatus with bromophenol blue as tracking dye at 50 V for 16 to 18 h. The agarose concentration in these gels ranged from 0.5 to 0.8%. The buffer used for electrophoresis was Tris-borate-EDTA (89 mM Tris, 89 mM boric acid, 3.4 mM EDTA, pH 8.3). Gels contained ethidium bromide (Calbiochem, La Jolla, Calif.) at a final concentration of 1 μg/ml. The gels were photographed under UV illumination with a Pentax 35-mm camera and Tri-X film.

Estimation of fragment sizes. Fragment sizes were determined by using the computer program of Schaffer and Sederoff (12), adapted for use on the computer at the University of Alberta.

Southern transfers, nick translation, and hybridization. DNA was transferred to nitrocellulose (BA85; Schleicher & Schuell Co., Keene, N.H.) by the method of Southern (13). A 0.5-μg sample of the HI plasmid pRG1251 was labeled with [α-32P]dCTP by nick translation as described by Rigby et al. (19). Dextran sulfate hybridization was done as described earlier (21). After hybridization, the nitrocellulose membranes were autoradiographed with Kodak XAR film and a Du Pont Lighting-Plus screen for 3 to 18 h at −70°C.

RESULTS

Restriction enzyme digestion of plasmids from the three HI subgroups. A representative member of each of the HI plasmid subgroups, namely pRG1251 (HI1), R478, (HI2), and MIP233 (HI3), was subjected to restriction enzyme digestion. A survey of 12 restriction enzymes (AccI, AvaI, BamHI, BglII, EcoRI, HpaI, HindIII, KpnI, PstI, PvuII, Sau96, and SalI) was undertaken. All three H plasmids behaved similarly with respect to the frequency of cutting with a particular restriction enzyme. In spite of this, no bands common to all three HI subgroups could be identified. AccI, AvaI, AvaII, BamHI, BglII,

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**TABLE 1. H incompatibility group plasmids**

<table>
<thead>
<tr>
<th>Plasmid designation</th>
<th>Incompatibility group</th>
<th>Resistance pattern</th>
<th>Molecular mass (Mdal)</th>
<th>Original host</th>
<th>Place of origin and date</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRG1251</td>
<td>HI1</td>
<td>Ap, Cm, Sm, Su, Tc</td>
<td>120</td>
<td>Salmonella typhi</td>
<td>Thailand, 1972</td>
<td>18</td>
</tr>
<tr>
<td>pRG1271</td>
<td>HI1</td>
<td>Ap, Cm, Sm, Su, Tc</td>
<td>120</td>
<td>Salmonella typhi</td>
<td>Mexico, 1972</td>
<td>18</td>
</tr>
<tr>
<td>pRG1254</td>
<td>HI1</td>
<td>Cm, Sm, Su, Tc</td>
<td>120</td>
<td>Salmonella typhi</td>
<td>Vietnam, 1972</td>
<td>18</td>
</tr>
<tr>
<td>R27</td>
<td>HI1</td>
<td>Tc</td>
<td>120</td>
<td>Salmonella typhimurium</td>
<td>England, 1961</td>
<td>5</td>
</tr>
<tr>
<td>TP123</td>
<td>HI1</td>
<td>Cm, Sm, Su, Tc</td>
<td>123</td>
<td>Salmonella typhimurium</td>
<td>Mexico, 1972</td>
<td>5</td>
</tr>
<tr>
<td>R476b</td>
<td>HI2</td>
<td>Sm, Su, Tc</td>
<td>190</td>
<td>Serratia marcescens</td>
<td>United States, 1969</td>
<td>19</td>
</tr>
<tr>
<td>R826</td>
<td>HI2</td>
<td>Ap, Cm, Gm, Ks, Sm, Tc, Te</td>
<td>150</td>
<td>Serratia marcescens</td>
<td>France, 1974</td>
<td>20</td>
</tr>
<tr>
<td>TP116</td>
<td>HI2</td>
<td>Cm, Sm, Su, Te</td>
<td>144</td>
<td>Salmonella typhimurium</td>
<td>Spain, 1969</td>
<td>5</td>
</tr>
<tr>
<td>MIP235</td>
<td>HI2</td>
<td>Cm, Sm, Su, Tc</td>
<td>190</td>
<td>Salmonella oranienburg</td>
<td>Brazil, 1973</td>
<td>12, 20</td>
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<tr>
<td>R478</td>
<td>HI2</td>
<td>Cm, Km, Tc, Te, Hg</td>
<td>166</td>
<td>Serratia marcescens</td>
<td>United States, 1969</td>
<td>7, 19</td>
</tr>
<tr>
<td>MIP233</td>
<td>HI3</td>
<td>Te, Scr</td>
<td>150</td>
<td>Salmonella ohio</td>
<td>England, 1972</td>
<td>12, 20</td>
</tr>
<tr>
<td>pHII1508a</td>
<td>HII</td>
<td>Sm, Te, Tp</td>
<td>100</td>
<td>Klebsiella pneumoniae</td>
<td>England, 1981</td>
<td>3</td>
</tr>
</tbody>
</table>

*a Resistances are designated as follows: Ap, ampicillin; Cm, chloramphenicol; Gm, gentamicin; Hg, mercuric chloride; Km, kanamycin; Sm, streptomycin; Su, sulfonamide; Tc, tetracycline; Te, tellurium; Tp, trimethoprim; Scr, sucrose fermentation.

*b Determined by electron microscopy for R27, TP123, and TP116 (1, 5) or by agarose gel electrophoresis; for high-molecular-mass plasmids (150 to 190 Mdal) the sizes are approximate (see reference 20).
EcoRI, HindIII, Hpal, PvuII, and Sau96 were frequent cutters, and SalI and KpnI were moderate cutters, whereas PstI was an infrequent cutter. Of the frequent cutters, AvaI, BamHI, HindIII, and Hpal were unsuitable for further analysis because they generated too many fragments. AvaII, PvuII, and Sau96 were also unsuitable because of the large number of small fragments that were unresolvable in an agarose gel system, the largest fragments being in the 4- to 9-kilobase (kb) size range. Of the frequent cutters, EcoRI generated fragments that could be resolved most clearly, giving between 27 and 39 fragments (Fig. 1). SalI (Fig. 2) and KpnI, the moderate cutters, generated between 19 and 29 fragments. PstI gave between 8 and 19 fragments (Fig. 3). The number of fragments obtained after digestion with most restriction enzymes was consistent with the size of the plasmid. The smallest plasmid pRG1251 (183 kb or 120 Mdal) gave the fewest fragments, whereas either R478 (256 kb or 166 Mdal) or MIP233 (230 kb or 150 Mdal) gave the most fragments.

Comparison of plasmids within the same HI subgroups by restriction digestion. Five IncH1 plasmids from different geographic sources (Table 1) were compared by digestion with EcoRI (Fig. 4). All five plasmids had very similar restriction enzyme digestion patterns. EcoRI digestion was also used to compare five IncH2 plasmids (Fig. 5). These plasmids were originally identified in members of different genera, namely Serratia marcescens, Salmonella typhi, and Salmonella oranienburg (Table 1). Although fewer similarities were noted among the restriction digests of the H12 plasmids, common bands were observed, particularly in the lower-molecular-weight range.

Buoyant density and base composition of H group plasmids. The G+C content (corresponding to the buoyant density) of the IncH1 plasmids R27 and pRG1251 was 49 mol%, that of the IncH12 plasmid R478 was 50 mol%, and that of the IncH13 plasmid MIP233 was 48.5 mol%. The H11 plasmid pH1508a had a G+C content of 50 mol%.

DNA homology among H group plasmids determined by hybridization to nick-translated

FIG. 1. EcoRI digests of H plasmids and their corresponding hybridization patterns. Digests were subjected to electrophoresis on a 0.8% agarose gel in Tris-borate-EDTA buffer. The fragments were probed with a nick-translated H11 plasmid, pRG1251. Lanes A through F show ethidium bromide-stained gels: (A) bacteriophage lambda digested with EcoRI, (B) bacteriophage lambda digested with HindIII, (C) pRG1251 (H11), (D) R478 (H12), (E) MIP233 (H13), (F) pH1508a (H11). Lanes G through J show hybridization patterns: (G) pRG1251, (H) R478, (I) MIP233, (J) pH1508a.

FIG. 2. SalI digests of H plasmids and their corresponding hybridization patterns. Digests were electrophoresed on a 0.5% agarose gel in Tris-borate-EDTA buffer. The fragments were probed with a nick-translated H11 plasmid, pRG1251. Lanes A through D show ethidium bromide-stained gels: (A) bacteriophage lambda digested with HindIII, (B) pRG1251 (H11), (C) R478 (H12), (D) MIP233 (H13). Lanes E through G show hybridization patterns: (E) pRG1251, (F) R478, (G) MIP233.
pRG1251 (IncHI1) DNA. Radioactively labeled pRG1251 DNA was used as a probe to hybridize to a series of Southern blots of endonuclease-generated fragments of unlabeled plasmid DNAs. The five HI plasmids, which had very similar EcoRI digestion patterns, yielded EcoRI digestion fragments, the majority of which hybridized to pRG1251, as determined by autoradiography (Fig. 6). These results confirm that pRG1251, pRG1271, and pRG1254 belong to the HI subgroup of H1 plasmids.

EcoRI digests of representative plasmids of the other HI subgroups (R478, MIP233) and the H1I group (pHH1508a) showed hybridization with pRG1251 DNA (Fig. 1). R478 had 10 fragments, ranging in size from 24 to 1.8 kb, to which pRG1251 hybridized, and MIP233 had 7 fragments, varying in size from 23 to 3.7 kb, which hybridized. The pHH1508a plasmid had 5 fragments which hybridized to pRG1251 with sizes from 37 to 3.7 kb.

When SalI digests of R478 and MIP233 were examined for homology with pRG1251 (Fig. 2), three fragments of R478 and four fragments of MIP233 showed hybridization. In contrast, PstI digests of MIP233 showed only three such fragments, 3.1, 3.2, and 2.1 kb. The PstI digest of R478 contained four fragments that showed some homology with pRG1251 (Fig. 3).

DISCUSSION

We have confirmed the observation of Roussel and Chabbert (11) that plasmids of HI incompatibility can be subdivided into three groups on the basis of DNA homology measurements. Our study provides a visual estimate of the molecular relatedness of these plasmids by restriction enzyme fingerprinting and DNA hybridization after Southern transfer. Although a diversity of endonuclease cleavage patterns was noted among H subgroups, similar cleavage patterns were observed within a subgroup. This is particularly true of the HI1 plasmids (Fig. 4), whereas HI2 plasmids gave more diverse patterns (Fig. 5). These results probably reflect the fact that
HI1 plasmids originated in *Salmonella* spp., whereas the HI2 plasmids were obtained from different genera (*Salmonella* spp. and *Serratia* spp.). Restriction enzymes could be divided into three groups based on their ability to fragment the H plasmids. Most enzymes, including *EcoRI*, were frequent cutters, *SalI* and *KpnI* were moderate cutters, and *PstI* was an infrequent cutter.

DNA hybridization of 32P-labeled pRG1251 (IncHII) to restriction digests after Southern blotting demonstrated a lack of DNA homology among plasmids of different H subgroups, but extensive DNA homology within the same subgroup. Specific DNA restriction fragments of the HI2 plasmid R478 and the HI3 plasmid MIP233 showing some DNA homology with pRG1251 were identified.

The distribution and number of fragments showing homology with pRG1251 varied with the enzyme used. The frequent cutter *EcoRI* gave the largest number of fragments that hybridized to the pRG1251 probe, followed by the moderate and infrequent cutters *SalI* and *PstI*. The lack of homology between the plasmids of the HI subgroups was emphasized by the example of MIP233 digested with *PstI*, in which the total molecular weight of the fragments showing homology with pRG1251 was 8.6 kb (4.7% of the total molecular weight of pRG1251). In the case of the other digests of MIP233, this common homology is distributed among more fragments, some of which are large. The hybridization studies presented are unsuitable for quantitative analysis of the percent DNA homology between the HI subgroup plasmids; however, certain qualitative statements can be made. In the case of most digests, some DNA homology was noted in the upper-molecular-weight ranges. Furthermore, in the autoradiograms with the pRG1251 probe the band intensities were much less than those seen with pRG1251 fragments of the same molecular range, indicating that only a small percentage of the fragment showing homology was hybridized with the probe.

All three HI subgroup plasmids are incompatible with each other and encode H pilus production; thus, the genes responsible for these properties may be among the fragments showing homology. Both pRG1251 and R478 encode resistance to tetracycline and chloramphenicol, and thus these determinants may account for some of the observed homology.

Unlike plasmids of the HI incompatibility group, members of the HI group (3) had not been examined for DNA homology with other plasmids. The IncHIII plasmids, such as pHH1508a, do have the tellurium resistance determinant in common with most plasmids of
the H12 and H13 subgroups, although not with H11 plasmids. Our results demonstrate that there is some degree of homology between the H11 plasmid pRG1251 and the H11 plasmid pHH1508a contained within several EcoRI fragments. Although pRG1251 and pHH1508a both encode resistance to streptomycin, in the latter it is probably part of Tn7. It is likely that the homologous DNA fragments contain the genes responsible for pilus production and perhaps other functions related to transfer.

When H plasmids were initially divided into two subgroups, it was observed that H11 plasmids were incompatible with autonomous F factors, whereas H12 plasmids were compatible with F (15). Recent studies have shown that H11 plasmids contain a single EcoRI fragment of approximately 5 Mdal which hybridizes to a probe for the f5 region of the F factor (D. E. Taylor, R. W. Hedges, and P. L. Bergquist, submitted for publication). H1 and H12 plasmids have been further distinguished in that H11 plasmids have been primarily associated with chloramphenicol resistance in Salmonella typhi, whereas H12 plasmids that were originally described in strains of Salmonella spp. have also been found to mediate antibiotic resistance in other enterobacteriaeae including Shigella flexneri (17), Serratia marcescens (18), Citrobacter freundii (20), Enterobacter cloacae (10), and Klebsiella pneumoniae (15). It therefore appears that these plasmid subgroups could have evolved separately in different genera, which would account for the small amount of DNA homology among these groups.

We had hoped that the buoyant density of the plasmids would give some clues as to the bacterial host in which these plasmids had evolved, since DNAs of different bacterial genera often have characteristic G+C values (16). However, we found no measurable difference in the G+C content of plasmids belonging to the three HI subgroups or to the IncHI1 group.

This study represents the first attempt to search for DNA homologies among plasmids of the H incompatibility group by using restriction enzyme analysis and DNA hybridization of nick-translated DNA to Southern transfers of restriction enzyme digests. We have been able to identify some regions of homology by using these techniques. In the case of both IncHI1 and IncHI2 plasmids, very similar restriction enzyme digestion patterns were obtained, and these results could be further extended as a diagnostic tool for the identification of H plasmids belonging to a given subgroup. A considerable amount of work remains to be done to characterize plasmids of this incompatibility group. This includes the preparation of restriction enzyme maps of representative plasmids and unequivocal identification of regions involved in incompatibility, conjugal transfer, and antibiotic resistance.

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**LITERATURE CITED**


