Diversity of *Pseudomonas* plasmids: To what extent?

Alexander M. Boronin

*Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia*

Received 11 June 1992
Accepted 27 June 1992

Key words: *Pseudomonas*; Plasmid; Incompatibility group

1. SUMMARY

Results obtained in studies of the biology of *Pseudomonas* plasmids are presented here as a mini-review. These data indicate that plasmids are ubiquitous in *Pseudomonas*, but the frequency of their occurrence varies greatly in particular species, or groups of species and in different microbial habitats. Some species of *Pseudomonas*, for instance *P. aeruginosa*, possess great diversity of plasmids both from the viewpoint of their incompatibility properties and their ability to endow bacteria with additional features such as resistance to antibiotics or heavy metals, degradation of xenobiotics or inhibition of phage development.

2. INTRODUCTION

Plasmids control various properties of bacterial cells, including such practically important features as antibiotic resistance and ability to degrade toxic pollutants. Plasmids promote the horizontal transfer of genetic information between bacteria belonging to different taxonomic groups and play an important role in the evolution of bacteria [1].

Owing to the rapid progress of plasmid research, a specialized field of research concerned with the the biology of plasmids has evolved. This field covers the occurrence of plasmids in various bacteria and their contribution to the phenotype of the bacterial hosts, classification, natural history and possible uses. There is even a tendency to consider plasmids as, in a sense, organisms [2].

The genus *Pseudomonas* comprises a vast and rather diverse group of bacteria [3]. The fluorescent pseudomonads which include, among others, the species *Pseudomonas aeruginosa*, *P. fluorescens*, *P. putida* and *P. syringae* can be found in a variety of natural environments.

It is hoped that research into plasmids of the fluorescent pseudomonads, which occupy a diversity of ecological niches, will contribute significantly to the development of the biology of plasmids as a whole.

The following relevant points can be considered: (1) the extent of the occurrence of plasmids in fluorescent pseudomonads inhabiting specific
natural environments; (2) the phenotypic features determined by these plasmids; (3) the diversity of the plasmids determining similar and different features of bacteria; and (4) the diversity of the plasmids that occur in the bacterial populations of particular species.

3. R PLASMIDS OF P. AERUGINOSA

Two decades of research into P. aeruginosa R plasmids have illustrated the wide occurrence of antibiotic resistance plasmids in bacteria obtained from surgical patients and those with urinary tract infections and severe burns.

The R plasmids of P. aeruginosa have been classified into 13 incompatibility groups [4]. Most of the 260 P. aeruginosa R plasmids that we have investigated (Table 1) could be classified into one of these 13 incompatibility groups. About one-third of the plasmids belong to the IncP2 group [5], which is characterized by a large DNA size. We made an attempt to detect new R plasmids with small-size DNA with the aim of using them subsequently as cloning vectors. However, all the small plasmids that we isolated belonged to the IncP4 group [6], although some plasmids differed from RSF1010, which can be considered a prototype of plasmids in this incompatibility group.

In a search for new pseudomonad R plasmids, we investigated Pseudomonas strains isolated from the waste waters of antibiotic-manufacturing plants. Of 132 Pseudomonas strains, 114 were P. aeruginosa isolates which carried 19 plasmids belonging to incompatibility groups P1, P2, P3, P4, and P5. Two plasmids, pBS221 (Tc) (IncP1) [7] and pBS227 (Cb Km Tc Te) (IncP2) [8] were found in strains of P. denitrificans and P. desmoliticum respectively.

Several plasmids did not belong to any of the known incompatibility groups. The broad-host-range plasmid pBS222 was assigned to a new incompatibility group, P14. Another broad-host-range plasmid pBS52 differs from all known plasmids in its incompatibility properties. However, detailed studies have shown it to be the replicon of plasmid RSF1010 fused with part of the replicon of another plasmid carrying tra genes [9].

Thus, most R plasmids of P. aeruginosa belong mainly to the incompatibility groups P1 through P5. Some R plasmids of P. aeruginosa could possibly be the products of replicon interactions of these and other plasmids carrying various combinations of the same rep (inc) genes.

4. DEGRADATIVE PLASMIDS

Many degradative (D) plasmids are known to control degradation of various organic compounds including xenobiotics [10,11]. A number of D plasmids that control the degradation of particular compounds, such as e-caprolactam [12,13], or naphthalene and salicylate [14–17] have

<table>
<thead>
<tr>
<th>Inc group</th>
<th>Plasmid</th>
<th>Properties</th>
<th>Size (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 c</td>
<td>pBS223</td>
<td>Tc Tra +</td>
<td>61</td>
</tr>
<tr>
<td>P2</td>
<td>pBS12</td>
<td>Sm Cm Hg Mer Te Uv Tra + ~ 400</td>
<td></td>
</tr>
<tr>
<td>P3 c</td>
<td>pBS73</td>
<td>Sm Cm Tc Km Hg Su Tra + 88</td>
<td></td>
</tr>
<tr>
<td>P4 c</td>
<td>pBS95</td>
<td>Sm Su Ap Tra + 13</td>
<td></td>
</tr>
<tr>
<td>P5</td>
<td>pBSII</td>
<td>Sm Su Hg Pmr Cr Tra + 200</td>
<td></td>
</tr>
<tr>
<td>P6 c</td>
<td>Rms149</td>
<td>Sm Gm Cb Su Tra + 49</td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>pBS14</td>
<td>Cm Tra + 140</td>
<td></td>
</tr>
<tr>
<td>P9</td>
<td>R2</td>
<td>Sm Su CB Uv Tra + 68</td>
<td></td>
</tr>
<tr>
<td>P10</td>
<td>pBSR1</td>
<td>Km Gm Su Tp Hg Pmr Tra + 65</td>
<td></td>
</tr>
<tr>
<td>P11</td>
<td>R151</td>
<td>Km Gm Sm Sp Su Cb Tp Tra + 81</td>
<td></td>
</tr>
<tr>
<td>P12</td>
<td>R716</td>
<td>Sm Hg Tra + nd</td>
<td></td>
</tr>
<tr>
<td>P13</td>
<td>pM625</td>
<td>Sm Km Gm Su Cb Tp Bor Tra + nd</td>
<td></td>
</tr>
<tr>
<td>P4/P5 c</td>
<td>pBS52</td>
<td>Sm Su Cb Tra + 38</td>
<td></td>
</tr>
<tr>
<td>P14 c</td>
<td>pBS222</td>
<td>Tc Tra + 17.2</td>
<td></td>
</tr>
</tbody>
</table>

a The prefix BS indicates the plasmids and their derivatives isolated at the Laboratory of Biology of Plasmids, Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pBS plasmids are according to refs. 5,6; other plasmids are as given in ref. 4.

b Abbreviations: Ap, ampicillin; Bor, borate; Cb, carbenicillin; Cm, chloramphenicol; Cr, potassium bichromate; Gm, gentamicin; Hg, mercuric chloride; Km, kanamycin; Pmr, phenylmercuric acetate; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline; Te, potassium tellurite; Tp, trimethoprim; Uv, UV irradiation. Tra +, transfer deficient.

c Inc group harboring broad-host-range plasmids.

nd = not determined.
been isolated. Specifically, of 190 ε-caprolactam-degrading strains isolated from various sources, 70 strains belonged to the genus *Pseudomonas*. About 90% of them bear CAP plasmids which determine degradation of ε-caprolactam [12,18]. In 16 out of 30 strains of *Pseudomonas* spp. growing on naphthalene, we found conjugative plasmids (NAH) controlling the oxidation of this compound [16,17]. A more detailed investigation of CAP and NAH plasmids revealed great diversity in molecular size, conjugal transfer, resistance to heavy metal ions and assignment to particular incompatibility groups (Tables 2–4). However, all of these plasmids belong to a limited number of incompatibility groups (P2, P9 and P7); the NAH plasmids belong to incompatibility groups P7 and P9 (Table 4).

In addition to being incompatible with plasmids of the IncP7 group, some NAH plasmids exhibit partial incompatibility with the IncP2 plasmids [17]. A similar phenomenon is observed with some IncP9 CAP plasmids which are incompatible with IncP7 plasmids [21]. The NAH plasmids are transferred to *P. aeruginosa* PAO (usually used in the incompatibility studies of R plasmids) at a very low frequency, and thereafter many of them become non-conjugative. To classify D plasmids by their incompatibility properties, we used *P. putida* BSA [22]. Studies of *P. aeruginosa* R plasmid incompatibility properties in this system yield results identical to those obtained for the *P. aeruginosa* PAO system [22].

The naphthalene catabolic systems are thermosensitive and do not function in *P. aeruginosa* PAO at temperatures above 37-38°C. At 40–41°C, we observed a sharp increase in the instability of NAH plasmids, as well as formation of abnormal elongated partially lysed cells of *P. aeruginosa* PAO [29].

The data presented above suggest that most degradative plasmids belong to incompatibility groups P9 and P7, because the plasmids of these incompatibility groups have evolved and naturally occur in the fluorescent pseudomonads of the species *P. putida*, *P. fluorescens* and the like. This would explain the incapability of the genetic systems of the plasmids, controlling naphthalene degradation, to functionally express and stably maintain the Nah phenotype at temperatures not characteristic of the mesophilic *P. putida* and *P. fluorescens*.

### Table 2

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Molecular size (MDa)</th>
<th>Incompatibility group</th>
<th>Resistance to heavy metals</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBS262</td>
<td>~ 300</td>
<td>P9</td>
<td>Hg</td>
</tr>
<tr>
<td>pBS266</td>
<td>~ 300</td>
<td>P2</td>
<td>Te, Sn</td>
</tr>
<tr>
<td>pBS271</td>
<td>~ 300</td>
<td>P2</td>
<td>Te, Hg, Cr</td>
</tr>
<tr>
<td>pBS265</td>
<td>100</td>
<td>P9</td>
<td>Hg</td>
</tr>
<tr>
<td>pBS267</td>
<td>100</td>
<td>P9</td>
<td>Hg, Sn</td>
</tr>
<tr>
<td>pBS268</td>
<td>70</td>
<td>P9</td>
<td>Hg, Sn</td>
</tr>
<tr>
<td>pBS270</td>
<td>70</td>
<td>P7, P9</td>
<td>Hg, Sn</td>
</tr>
<tr>
<td>pBS276</td>
<td>50</td>
<td>P7, P9</td>
<td>—</td>
</tr>
</tbody>
</table>

*a* Hg, mercuric chloride; Te, potassium tellurite; Sn, organotin compounds; Cr, potassium bichromate.

### 5. MEGA-SIZE PLASMIDS

Of special interest are the large plasmids, belonging to the IncP2 group (Table 5). Some of them carry genes for antibiotic resistance; others, degradative genes. As a rule, they also bear genes for heavy metal resistance. A characteristic feature of these plasmids is that most of them code for resistance to tellurite. Several plasmids were found to carry the genes controlling resistance to organotin compounds [30].

### Table 3

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Transfer frequency</th>
<th>Molecular size (MDa)</th>
<th>Incompatibility group</th>
<th>Pathway of catechol oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBS3</td>
<td>10⁻⁴</td>
<td>110</td>
<td>P7</td>
<td>meta</td>
</tr>
<tr>
<td>pBS219</td>
<td>10⁻⁴</td>
<td>130</td>
<td>P7 (P2)</td>
<td>meta</td>
</tr>
<tr>
<td>pBS244</td>
<td>10⁻⁴</td>
<td>100</td>
<td>P9</td>
<td>meta</td>
</tr>
<tr>
<td>pBS2</td>
<td>10⁻⁴</td>
<td>100</td>
<td>P7</td>
<td>meta</td>
</tr>
<tr>
<td>pBS216</td>
<td>10⁻³</td>
<td>60</td>
<td>P9</td>
<td>meta</td>
</tr>
<tr>
<td>NPL-1</td>
<td>10⁻³</td>
<td>58</td>
<td>P9</td>
<td>absent</td>
</tr>
<tr>
<td>pBS</td>
<td>115</td>
<td>115</td>
<td>P7</td>
<td>Naphthalene oxidation via gentisic acid</td>
</tr>
</tbody>
</table>

*a* Silent genes of the *meta* pathway.
Table 4
Incompatibility groups of degradative plasmids

<table>
<thead>
<tr>
<th>Incompatibility group</th>
<th>Plasmid</th>
<th>Substrate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2</td>
<td>OCT</td>
<td>Octane</td>
<td>[20]</td>
</tr>
<tr>
<td></td>
<td>pBS263, pBS264</td>
<td>e-Caprolactam,</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>pBS266, pBS271</td>
<td>e-Aminocapronic acid</td>
<td>[21]</td>
</tr>
<tr>
<td>P7</td>
<td>pBS2, pBS3, pBS211, pBS213, pBS214, pBS217, pBS243</td>
<td>Naphthalene</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>pBS4</td>
<td>Naphthalene</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>TOL</td>
<td>Xylene, toluene</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>SAL</td>
<td>Salicylate</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>pND50</td>
<td>p-Cresol</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>NAH</td>
<td>Naphthalene</td>
<td>[23]</td>
</tr>
<tr>
<td>P9</td>
<td>pND140, pND160</td>
<td>Naphthalene</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>pWW60</td>
<td>Naphthalene</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>NPL-41</td>
<td>Naphthalene</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>pBS212, pBS216</td>
<td>Naphthalene</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>pBS240, pBS244, pBS248</td>
<td>Naphthalene</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>pBS262, pBS265, pBS267, pBS268, pBS269</td>
<td>e-Caprolactam, e-Aminocapronic acid</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>pBS1004</td>
<td>p-Toluensulfonic acid</td>
<td>[28]</td>
</tr>
<tr>
<td>P7 (P2)</td>
<td>pBS218, pBS219</td>
<td>Naphthalene</td>
<td>[17]</td>
</tr>
<tr>
<td>P9 (P2)</td>
<td>pBS270, pBS276</td>
<td>e-Caprolactam, e-Aminocapronic acid</td>
<td>[21]</td>
</tr>
</tbody>
</table>

Plasmids of various incompatibility groups inhibit the growth of *P. aeruginosa* and *P. putida* bacteriophage. Phage whose growth was inhibited on strains carrying plasmids of the P5, P6, P9 and P10 incompatibility groups were found. However, most often, growth of the phage was shown to be inhibited on plasmids of the P2 group.

Table 5
Pseudomonas plasmids of P2 incompatibility group

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Plasmid</th>
<th>Incompatibility group</th>
<th>Properties a</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>pBS79</td>
<td>P2</td>
<td>Sm Km Tc Gm Hg Te Tra+</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>pBS10</td>
<td>P2</td>
<td>Sm Su Hg Te Sn Tra+</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>pBS33</td>
<td>P2</td>
<td>Sm Hg Cr Te Uv Tra+</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>pBS31</td>
<td>P2</td>
<td>Sm Te Cm Su Hg Te Tra+</td>
</tr>
<tr>
<td><em>Pseudomonas sp.</em></td>
<td>pBS218</td>
<td>P7 (P2) b</td>
<td>Nah Sal Te Tra+</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>pBS219</td>
<td>P7 (P2) b</td>
<td>Nah Sal Te Tra+</td>
</tr>
<tr>
<td><em>Pseudomonas sp.</em></td>
<td>pBS266</td>
<td>P2</td>
<td>Cap Te Sn Tra+</td>
</tr>
<tr>
<td><em>Pseudomonas sp.</em></td>
<td>pBS271</td>
<td>P2</td>
<td>Cap Te Hg Cr Tra+</td>
</tr>
</tbody>
</table>

a Abbreviations not given in footnotes to Tables 1 and 2: Cap, ability to degrade e-caprolactam; Nah, ability to degrade naphthalene; Sal, ability to degrade salicylate.

b Plasmids pBS218 and pBS219 exhibit a partial incompatibility with IncP2 plasmids.
inhibited by the presence of plasmids belonging
to the IncP2 group [12,31]. Plasmids in this group
differed in the spectrum of bacteriophages whose
growth they inhibited. The IncP2 CAP plasmid
pBS271 (Table 5) is unique because it suppresses
the growth of a broad range of \( \textit{P. aeruginosa} \) and
\( \textit{P. putida} \) phage.

The maintenance of the plasmid and the ex-
pression of the plasmid genes is the energy price
that the bacteria must pay for harboring the
plasmids. This price, to a certain extent, increases
with the size of the plasmid. Indeed, this ten-
dency was revealed in the growth rate studies of
\( \textit{Escherichia coli} \) strains carrying various \( \textit{R} \) plas-
mids [32]. Interestingly, despite their enormous
sizes, the IncP2 plasmids that we investigated are
very stable under various growth conditions.
These data suggest that these plasmids have
evolved mechanisms that ensure their stable
maintenance in the bacterial population despite
the metabolic burden imposed upon their host
cell.

6. PLASMIDS OF \( \textit{P. syringae} \) AND RHIZO-
SPHERE \( \textit{PSEUDOMONAS} \)

One of the most widespread phytopathogenic
bacterial species is \( \textit{P. syringae} \), which is classified
into 41 pathovars. We studied the distribution of
plasmids in 49 \( \textit{P. syringae} \) strains of 17 pathovars.
The plasmids range in size from 3 to 135 kb. In
the \( \textit{P. syringae} \) strains studied here and also by
other investigators [33,34] no plasmids of over 150
kb were found. Apparently, large plasmids char-
acteristic of the IncP2 group of \( \textit{Pseudomonas} \)
have not been found in \( \textit{P. syringae} \) strains. In six
strains of the pathovars \textit{holci, astrosecienis, pisi, lachrymans, phaseolicola} \) and \textit{glycinea}, three or
four plasmids were found. The functions of the
plasmids that we investigated, as well as those of
most of the other \( \textit{P. syringae} \) plasmids, are not
known.

In rhizosphere pseudomonads, including plant
growth-promoting rhizobacteria, we found only a
minor amount of cryptic plasmids with molecular
sizes of 60 to 100 MDa. Some plasmids are in-
compatible with IncP7 plasmids.

7. CONCLUSIONS

Plasmids are ubiquitous in \( \textit{Pseudomonas} \). On
the other hand, the frequency of occurrence of
plasmids greatly varies in particular species or
groups of species of these bacteria. Virtually all
strains of \( \textit{P. syringae} \) studied contained plasmid
DNA. The frequency of occurrence also depends
on the ecological niche from which the bacteria
were isolated. For instance, the frequency of oc-
currence of \( \textit{R} \) plasmids in \( \textit{P. aeruginosa} \) was
significantly higher in hospital strains than in the
bacteria isolated from water or soil. The same is
true for the appearance of \( \textit{D} \) plasmids in the
strains of the \( \textit{P. putida-P. fluorescens} \) group: their
frequency of occurrence was far greater in bacte-
ria isolated from habitats polluted with xenobi-
otics than in those isolated from the plant rhizo-
sphere.

The plasmids found in various \( \textit{Pseudomonas} \)
species reveal a diversity in their incompatibility
groups that characterize them as replicons. Strains
of \( \textit{P. aeruginosa} \) contain a greater diversity of
plasmids than the \( \textit{P. putida-P. fluorescens} \) strains.
On the basis of their incompatibility, all the \( \textit{D} \)
plasmids of the \( \textit{P. putida-P. fluorescens} \) group can
be classified into three groups: IncP2, IncP7 and
IncP9.

Although plasmids are prevalent in \( \textit{Pseu-
domonas} \) and differ in many characteristics, it can
be predicted that the number of replicons carry-
ning antibiotic resistance or degradative genes is
likely to be limited, especially in any one particu-
lar species or group of species. Many of these
replicons have already been discussed. New types
of replicons will, most probably, be broad-host-
range plasmids or fused replicons.

Thus, natural bacterial populations of \( \textit{Pseu-
domonas} \) consist of plasmids which determine the
same or similar phenotypes differing in some
features. Our preliminary data support the sug-
gestion that in nature the \( \textit{D} \) plasmid-host combi-
nations give rise to greater diversity of bacterial
strains capable of degrading particular xenobi-
otics than generally thought. In part this is due to
the fact that a genetic system which controls, for
instance, the degradation of naphthalene and
salicylate also allows some catabolism of their
numerous chloro- or methyl derivatives [27,35,36]. That is why the bacteria have to modify this genetic system to adjust it to the environmental conditions by turning on or off particular genes or blocks of genes [37–42].

Studies of this type of microbial diversity are closely associated with the problem of the release of genetically modified microorganisms into the natural environment. Attention should be paid not only to the fate of the genetically modified microorganisms in the environment, but also to the diversity of the indigenous microbial community which already contains various naturally occurring D plasmids. We should be aware that these plasmids are also the products of genetic engineering performed in vivo by the microorganisms themselves.

ACKNOWLEDGEMENTS

I am grateful to the staff of the Laboratory of Plasmid Biology; our collaborative work formed the basis for this mini-review. I also thank V.D. Selivanov for technical assistance. This study was supported by the Russian Academy of Sciences.

REFERENCES