Plasmid Occurrence and Diversity in the Genus Paracoccus

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Abstract

The results of screening for the occurrence of plasmids in several strains representing 11 out of
13 species of the genus Paracoccus are presented. We show that plasmids (ranging in size from
2.7 to above 450 kb) are widely distributed in this genus. Only one tested strain (P. alkenifer)
appears to be plasmid-free. The majority of the strains harbour at least two plasmids, one of which
usually fits into the class of megaplasmids.

The genus Paracoccus is a member of the α subdivision of Proteobacteria, which
is a group of bacteria very abundant in plasmid molecules. So far special attention has
been given to exceptionally big (megabase-size) rhizobial plasmids due to their role
in encoding for symbiosis and nitrogen fixation phenotype as well as to large
agrobacterial plasmids responsible for pathogenicity to the host plants. Such extensive
studies on plasmids have not been done for the genus Paracoccus, which comprises a growing number of species isolated from polluted soil, water or sewage purifi-
cation units. There are only a few reports on the presence of plasmids in paracocci.
The presence of extrachromosomal DNA in paracoccal species was first mentioned by Gerstenberg et al. (1982). The authors presented physical evidence that Paracoccus versutus (previously known as Thiobacillus A2 or Thiobacillus versutus), Paracoccus pantotrophus DSM 65 (formerly classified as Paracoccus denitrificans) and strains DSM 413 and DSM 415 of P. denitrificans carry extremely large plasmids of molecular mass more than 300×10^6 (> 450 kb). The same P. versutus and P. panto-
trophus strains were also found to harbour additional plasmids of molecular mass of
about 70×10^6 (~ 100 kb) and 50×10^6 (~ 75 kb), respectively. The above findings were
confirmed for P. versutus in our laboratory several years ago (Bednarska et al.,
1983; Włodarczyk and Piechucka, 1995). The results presented by Chandra and Friedrich (1986), Steinrücke and Ludwig (1993), Jordan et al. (1997) and Winterstein and Ludwig (1998) documented the presence of mega-
plasmids (from 500 kb to above 700 kb) in all the tested strains currently allocated by Rainey et al. (1999) to P. pantotrophus (DSM 65, LMD 82.5, DSM 11072, DSM
11073, DSM 11104) and P. denitrificans (DSM 413, DSM 415, Pd 1222, ATCC 13543). Plasmids in the size range from 70 kb to about 100 kb were identified only in the representatives of P. pantotrophus. The smallest (2.7 kb) plasmid was found in P. pantotrophus DSM 11072 (Jordan et al., 1997). No further studies on the structure or function of identified paracoccal plasmids were done except for pTA1 from P. versutus, whose characterisation at the molecular level was undertaken in our laboratory. Paracoccus versutus is quite a new addition to the genus Paracoccus (Katayama et al., 1995) and a comparison of its plasmids with other paracoccal plasmids may be of additional value for phylogenetic studies.

pTA1 is a large (~107 kb) cryptic plasmid in which two distinct replicator regions have been identified. These have been cloned in the form of mini-replicons pTAV202 and pTAV320 and characterised (Barbosik et al., 1995, 1997, 1998). Both replication systems show similarity of their main replication initiation protein sequences but differ in overall genetic organisation. The most interesting feature of pTAV320 is the finding that it belongs to the family of repABC-type replicons widely distributed in plasmids identified in the genera Agrobacterium and Rhizobium. Replicons of the repABC-type demonstrate a unique, close structural association of two of the most important maintenance mechanisms for plasmids — replication and partition.

As shown in the accompanying review paper (Baj, 2000) the genus Paracoccus currently includes 13 species (see also Table I). The data summarised above on plasmids in paracocci cover only several strains falling into three species: P. denitrificans, P. pantotrophus and P. versutus. The majority of the paracoccal species have never been tested for the presence of plasmids. Obtaining information on the occurrence of plasmids in other species of the genus would open a possibility for comparative studies (including a search for distribution of repABC-type replicons among paracoccal plasmids).

We have collected strains representing all the species of the genus Paracoccus except for the patented P. marcusii and P. carotinifaciens. Some species were represented by several independent but well characterised isolates deposited in culture collections. The assignment of individual strains to P. denitrificans or P. pantotrophus species was accepted after the most recent proposal of Rainey et al. (1999). All the strains used for plasmid isolation were cultivated in optimal conditions using the growth media, pH and temperature given in corresponding references (Table I).

Screening for the presence of plasmids was done with the use of a wide range of classical, commonly known procedures. For the majority of P. pantotrophus strains (DSM 65, DSM 11072, DSM 11073 and DSM 11104) the identification of plasmids of up to about 100 kb was satisfactorily accomplished with the use of the original alkaline lysis procedure (Birnboim and Doly, 1979), followed by electrophoresis in 0.8% agarose in TAE buffer under standard conditions. In some cases (for P. pantotrophus LMD 82.5 and P. thiocyanatus) the addition of lysozyme to the lysis solution and increasing the culture volume was necessary. For some strains plasmid bands could be seen only by following the procedure recommended for Rhodococcus fascidus (Desomer et al., 1993). The method involves pre-treating cells with lysozyme and polyethylene glycol (PEG) to improve the sensitivity of bacteria to detergent (this applied to P. denitrificans LMD 22.21, P. alcaliphilus, P. aminophilus
<table>
<thead>
<tr>
<th>Nr</th>
<th>Species</th>
<th>Strain designation</th>
<th>Strain source</th>
<th>References</th>
<th>PLASMIDS</th>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>small (located below chrom. band)</td>
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<tr>
<td>1</td>
<td><em>P. alcophilus</em></td>
<td>JCM 7364</td>
<td>K. Suzuki (Japan)</td>
<td>Urakami <em>et al.</em> 1989</td>
<td>--</td>
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<tr>
<td>2</td>
<td><em>P. alkenifer</em></td>
<td>DSM 11593</td>
<td>A. Lipski (Germany)</td>
<td>Lipski <em>et al.</em> 1998</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td><em>P. aminophilus</em></td>
<td>JCM 7686</td>
<td>K. Suzuki (Japan)</td>
<td>Urakami <em>et al.</em> 1990</td>
<td>pAML2 (17 kb)</td>
</tr>
<tr>
<td>4</td>
<td><em>P. aminovorans</em></td>
<td>JCM 7685</td>
<td>K. Suzuki (Japan)</td>
<td>Urakami <em>et al.</em> 1990</td>
<td>pAMV2 (4 kb)</td>
</tr>
<tr>
<td>5a</td>
<td><em>P. denitrificans</em></td>
<td>DSM 413</td>
<td>R. van Spanning (Holland)</td>
<td>Rainey <em>et al.</em> 1999</td>
<td>--</td>
</tr>
<tr>
<td>5b</td>
<td><em>P. denitrificans</em></td>
<td>LMD 22.21</td>
<td>C. Goodhew (UK)</td>
<td>Rainey <em>et al.</em> 1999</td>
<td>--</td>
</tr>
<tr>
<td>6a</td>
<td><em>P. pantotrophus</em></td>
<td>DSM 65</td>
<td>R. van Spanning (Holland)</td>
<td>Rainey <em>et al.</em> 1999</td>
<td>--</td>
</tr>
<tr>
<td>6b</td>
<td><em>P. pantotrophus</em></td>
<td>DSM 11072</td>
<td>A. Wood (UK)</td>
<td>Jordan <em>et al.</em> 1997</td>
<td>pWKS1 (2.7 kb)</td>
</tr>
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<td>6c</td>
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<td>DSM 11073</td>
<td>A. Wood (UK)</td>
<td>Jordan <em>et al.</em> 1997</td>
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</tr>
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<td>6d</td>
<td><em>P. pantotrophus</em></td>
<td>DSM 11104</td>
<td>A. Wood (UK)</td>
<td>Jordan <em>et al.</em> 1997</td>
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<tr>
<td>6e</td>
<td><em>P. pantotrophus</em></td>
<td>LMD 82.5</td>
<td>B. Ludwig (Germany)</td>
<td>Jordan <em>et al.</em> 1997</td>
<td>--</td>
</tr>
<tr>
<td>7a</td>
<td><em>P. kocurii</em></td>
<td>CCM 4331</td>
<td>CCM (Czech Republic)</td>
<td>Ohara <em>et al.</em> 1990</td>
<td>--</td>
</tr>
<tr>
<td>7b</td>
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<td>CCM 4332</td>
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<td>CCM 4333T</td>
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<td>--</td>
</tr>
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<td>8</td>
<td><em>P. methylutens</em></td>
<td>DM12 (VKM B–2164)</td>
<td>Y. Trotsenko (Russia)</td>
<td>Doronina <em>et al.</em> 1998</td>
<td>--</td>
</tr>
<tr>
<td>9a</td>
<td><em>P. solventivorans</em></td>
<td>DSM 11592</td>
<td>A. Lipski (Germany)</td>
<td>Siller <em>et al.</em> 1996</td>
<td>pSOV1 (~5 kb)</td>
</tr>
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<td>9b</td>
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<td>DSM 6637</td>
<td>A. Lipski (Germany)</td>
<td>Siller <em>et al.</em> 1998</td>
<td>pSOS1 (~70 kb)</td>
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<td>10</td>
<td><em>P. thiocyantus</em></td>
<td>IAM 12816</td>
<td>A. Lipski (Germany)</td>
<td>Katayama <em>et al.</em> 1995</td>
<td>--</td>
</tr>
<tr>
<td>11a</td>
<td><em>P. versutus</em></td>
<td>A2 strain of Taylor &amp; Hoare</td>
<td>U. Tuovinen (USA)</td>
<td>Harrison, 1983</td>
<td>--</td>
</tr>
<tr>
<td>11b</td>
<td><em>P. versutus</em></td>
<td>CCM 2505</td>
<td>CCM (Czech Republic)</td>
<td>Harrison, 1983</td>
<td>--</td>
</tr>
</tbody>
</table>

+ plasmid present; -- plasmid absent; ? non-reproducible results obtained; 1 plasmid of size corresponding to pTAV1; 2 plasmid of size corresponding to pTAV3
and *P. methylutens*). It was most difficult to establish growth and lysis conditions for *P. kocurii*. Because of poor growth in liquid media, bacterial cells collected from the surface of one plate were used for a single plasmid isolation. When the existence of plasmid in a particular strain after the use of small scale preparation raised doubts, large scale preparation including the ultracentrifugation in CsCl+EтBr (Sambrook et al., 1989) was applied (e.g. *P. kocurii*, *P. alkenifer*). The search for megaplasmids, because of their large size, was done with application of the “Eckhardt in gel lysis” method (Eckhardt, 1978) with some modifications or its simplified version (Wheatcroft et al., 1990).

For some strains visualisation of plasmids was very difficult and only one of several attempts was successful, so further improvement of the procedures is necessary to obtain reproducible and reliable results. We found that all tested strains except for *P. alkenifer* DSM 11593 harbour one or more plasmids ranging in size from 2.7 to above 450 kb. The upper limit was established by comparison to the previously identified plasmid pHG16-b (~450 kb) in the strain DSM 65 (Gerstenberg et al., 1982; Winterstein and Ludwig, 1998). The majority of plasmid symbols included in Table I was introduced by us, except for pHG series (Gerstenberg et al., 1982). The designations of plasmids present in the strains DSM11072, DSM11073 and DSM 11104 were changed (with the permission of Dr. Ann Wood) compared to those in Jordan et al. (1997) in order to avoid repetitions of the symbols already existing in the literature.

The results of our studies are compiled in Table I. We distinguished three classes of plasmids according to their size: (1) small plasmids – located below band comprising fragments of the chromosomal DNA under applied electrophoretic conditions; (2) large plasmids (from 40 to about 100 kb) visible as distinct bands above chromosomal DNA after standard alkaline lysis and, (3) megaplasmids (> 450 kb) visible only in “Eckhardt gel”. As can be seen, the last class (megaplasmids) is widespread among paracoccal species. Only two strains of *P. solventivorans* may be positively considered megaplasmid free. The use of pulsed-field gel electrophoresis is required to see if the megaplasmid band visible in the Eckhardt gel contains more than one such large DNA species, as observed by Winterstein and Ludwig (1998) for two *P. pantotrophus* strains.

As shown in Table I, small plasmids are in minority. The smallest is a high copy number plasmid pWKSI (2.7 kb) from *P. pantotrophus* DSM 11072. Four other small plasmids are: pAMV2 (~4 kb) of *P. aminovorans* JCM 7685, pSOV1 (~5 kb) of *P. solventivorans* DSM 6637, pAMI2 (~17 kb) and pAMI3 (~6 kb) of *P. aminophilus* JCM 7686. pSOV1 is the only plasmid molecule in DSM 11592 strain while pWKSI in DSM 11072 is accompanied by a megaplasmid.

At least 15 identified plasmids fall in the range from 40 to about 100 kb. Only three; out of several tested strains (*P. denitrificans* DSM 413, *P. pantotrophus* DSM 11072 and *P. solventivorans* DSM 11592) do not carry plasmids of this type. The type strain of the genus *Paracoccus* – *P. denitrificans* is represented in our survey only by two strains (DSM 413 and LMD 22.21) while *P. pantotrophus* has five representatives which results from the correction of the assignment of the strains already being under study to a new species. In view of the above, a conclusion concerning the possible prevalence of large (approx. 40–100 kb) plasmids in *P. pantotrophus* compared to
P. denitrificans cannot be drawn at present although such a tendency could be noticed when looking at the results of Winterstein and Ludwig (1998) and considering the actual allocation of the strains they used for their studies.

In conclusion, we can state that the occurrence of plasmids is common in Paracoccus spp. We have identified a number of novel plasmids. The replicator region of one of them (pALC1) has been recently analysed (Bartosik et al., 2000). Hybridization analysis with pALC100 (mini-replicon of pALC1) as a probe, revealed that none of the tested strains (listed in Table I) carried related sequences. The replicator region of pALC1 thus seems to be unique among paracoccal plasmids.

Other large paracoccal plasmids are being analysed in our laboratory. We are especially interested in studying the distribution of repABC replicons among them. It was previously observed that pTAV320 (the mini-replicon of pTAV1) showed strong incompatibility towards pKLW1 (100 kb) of P. pantotrophus DSM 11073, suggesting that this plasmid also carries a repABC-type replicon (Jordan et al., 1997; Bartosik et al., 1998). Application of the hybridization analysis of the identified paracoccal plasmids with pTAV320 (as a probe) as well as incompatibility testing will enable to distinguish those belonging to the repABC family.

So far all identified paracoccal plasmids remain cryptic. The only exception is the finding that the gene encoding for subunit I of the cytochrome oxidase in P. denitrificans Pd1222 is duplicated on the megaplasmid (Steinrücke and Ludwig, 1993), which according to Winterstein and Ludwig (1998) justifies the use of the term chromosome (chromosome III) for this structure although the other criterion, the presence of rRNA genes, could not be so far provided (missing data). It can be just a matter of time to connect the common appearance of plasmids to the great metabolic versatility of the paracoccal hosts.

Some newer species were isolated from environments containing a range of toxic components: e.g. acetone (P. solventivorans), dimethyloformamide (P. aminovorans and P. aminophilus), carbon disulfide (some strains of P. panthotrophus). This raises the possibility for the application of some of the Paracoccus species for bioremediation and justifies extending the search on the function of plasmids in these bacteria.

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Literature


