Genomovars 11 to 18 of *Pseudomonas stutzeri*, identified among isolates from soil and marine sediment

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Amongst 440 strains of *Pseudomonas stutzeri* isolated from soil and marine sediment for a population genetic study, eight strains were each presumed to represent a novel genomic group and were compared with each other and to reference strains of *P. stutzeri* genomovars 1 to 10 and other *Pseudomonas* species by DNA–DNA hybridization, 16S rRNA and internally transcribed 16S–23S rRNA spacer region (ITS1) sequences and basic physiological properties defining the species. While 16S rRNA and ITS1 gene sequences positioned the eight strains within the phylogenetic branch of *P. stutzeri*, the DNA–DNA hybridizations with reference strains of the 10 described genomovars and among the novel strains were generally below 70 %, which is the threshold for species and genomovar differentiation. Since the physiological properties studied in the eight strains fitted the profile of *P. stutzeri*, eight new genomovars of *P. stutzeri*, numbered 11 to 18, are proposed, with strains 28a50, 28a39, 28a22, 28a3, 4C29, 24a13, 24a75 and MT-1 being the reference strains. The highly transformable reference strain 28a3 of genomovar 14 had a localized 16S rRNA gene sequence tag characteristic of genomovar strains 2 and 3, suggesting a possible horizontal gene transfer event involving part of the 16S rRNA gene.

The species *Pseudomonas stutzeri* is a non-fluorescent member of the genus *Pseudomonas* (γ-Proteobacteria) displaying high genetic (Rius et al., 2001; Cladera et al., 2004) and physiological diversity (Rosselló et al., 1991). Strains of *P. stutzeri* have been isolated from a variety of environmental and clinical habitats (Sikorski et al., 2002a and references therein). Some strains received attention as model organisms because of their specific metabolic properties (Musarrat & Hashsham, 2003; Obradors & Aguilar, 1991; Rosselló-Mora et al., 1994; Zumft, 1997) and their ability for natural genetic transformation (Berndt et al., 2003; Meier & Wackernagel, 2003; Sikorski et al., 1998, 2002b).

Taxonomically, *P. stutzeri* strains have been grouped into 10 genomovars by DNA–DNA hybridization (García-Valdés et al., 2003; Rossello et al., 1991; Rossello-Mora et al., 1996; Sepúlveda-Torres et al., 2001; Ursing et al., 1995). In a recent population-genetic study, approximately 440 strains from soil and marine environments were studied by random amplified polymorphic DNA-PCR (RAPD-PCR) and the 16S rRNA gene sequence was determined (>1450 bp) (Sikorski et al., 2002a) for 34 of the strains (several being representatives of the main RAPD groups). The 16S rRNA gene sequences suggested that seven representative strains (28a50, 28a39, 28a22 and 28a3, from soil close to Tel Aviv airport, Israel; 4C29, from marine sediment on the shore of the North Sea coast, Germany; 24a13 and 24a75, from a soil contaminated with mineral oil, Germany) and strain MT-1 (from Mariana Trench, Japan; Tamegai et al., 1997) were members of new genomovars. Based on their 16S rRNA gene sequence dissimilarity values, these eight strains were as different from each other and from reference strains of the established genomovars 1 to 10 as the genomovar reference strains differed from each other (Table 1). Moreover, the lowest dissimilarity value of any of these strains to each other or to a reference strain of an established genomovar (0·41 %; Table 1) was larger than the maximum dissimilarity value observed among nine strains within three established genomovars (0·31 %; Table 1). In the following,
we present further data to support the identification of eight new genomovars including the results of DNA–DNA hybridization studies, sequence analyses of 16S–23S rRNA internally transcribed spacer regions (ITS1) and comparisons of physiological properties. Additionally, two strains (28a18 and 28a69) which were suggested by RAPD-PCR to belong to the new genomovars represented by strains 28a3 (genomovar 14) and 28a22 (genomovar 13), respectively (Sikorski et al., 2002a), were included in part of the studies.

Genomic DNA from the eight new genomovar reference strains and from strains 28a18 and 28a69 was isolated using the Genomic DNA kit from QIAGEN and from the reference strains ATCC 17591, ATCC 14405, ATCC 17587; 3, genomovar 3 reference strains DSM 6084 (gv. 4), DSM 6082 (gv. 5), DSM 50238 (gv. 7), JM 300 (gv. 8), KC (gv. 9), CLN 100 (gv. 10).

The high DNA–DNA relatedness values of strain 28a18 with 28a3 (91 %) and 28a69 with 28a22 (93 %) indicate membership of the respective genomovar, as suggested by RAPD-PCR (Sikorski et al., 2002a). In contrast, the DNA–DNA relatedness values of the eight representative strains of the new genomovars to each other and to the reference strains of the genomovars 1 to 10 were at or below the threshold value of 70 % for species delineation (Table 2) except for two pairs (77 %, MT-1/28a22; 82 %, 28a3/24a13; Supplementary Figs S1 and S2 available in IJSEM Online), indicating their phylogenetic distinctness from all other genomovars.

### Table 1. 16S rRNA gene sequence dissimilarities

Strains: 1, Genomovar 1 reference strains CCUG 11256T, ATCC 17589, ATCC 17593; 2, genovar 2 reference strains ATCC 17591, ATCC 14405, ATCC 17587; 3, genovar 3 reference strains DSM 50227, AN10, AN11; 4, genomovar type strain CCUG 11256T (gv. 1) and reference strains ATCC 17591 (gv. 2), DSM 50227 (gv. 3), DSM 6084 (gv. 4), DSM 6082 (gv. 5), DSM 50238 (gv. 7), JM 300 (gv. 8), KC (gv. 9), CLN 100 (gv. 10).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Dissimilarity measures (%)</th>
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<tr>
<td></td>
<td>Mean</td>
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<tr>
<td>Within established genomovars</td>
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<tr>
<td>1*</td>
<td>0.20</td>
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<tr>
<td>2*</td>
<td>0.09</td>
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<tr>
<td>3*</td>
<td>0.25</td>
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<td>Among established genomovars 1 to 10†</td>
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<tr>
<td>4</td>
<td>1.34</td>
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<tr>
<td>Comparison of reference strains of new genomovars 11 to 18 to reference strains of established genomovars 1 to 10†</td>
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<tr>
<td>Gv. 11 28a50</td>
<td>0.99</td>
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<tr>
<td>Gv. 12 28a39</td>
<td>1.31</td>
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<tr>
<td>Gv. 13 28a22</td>
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<tr>
<td>Gv. 14 28a3</td>
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<tr>
<td>Gv. 15 4C29</td>
<td>1.22</td>
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<tr>
<td>Gv. 16 24a13</td>
<td>1.25</td>
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<tr>
<td>Gv. 17 24a75</td>
<td>2.89</td>
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<td>Gv. 18 MT-1</td>
<td>1.29</td>
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<tr>
<td>Among new genomovars 11 to 18</td>
<td>1.42</td>
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*Affiliation of three members per genomovar was previously shown by DNA–DNA hybridizations (Rosselló et al., 1991).
†Genomovar 6 was reclassified as Pseudomonas balearica (Bennasar et al., 1996).
genomic species, as indicated here by DNA–DNA hybridization for the eight strains, should not be classified as novel species unless differentiating phenotypes are found (Rosselló-Mora & Amann, 2001; Stackebrandt et al., 2002; Ursing et al., 1995), which is not the case here. Thus, we propose eight new genomovars, 11 to 18, of *P. stutzeri*, with strains 28a50, 28a39, 28a22, 28a3, 4C29, 24a13, 24a75 and strain MT-1, respectively, as the reference strains for each genomovar. The strains have been deposited as CCUG 50538–50545 (= DSM 17082–17089).

It is remarkable that strain 28a18 (Sikorski et al., 2002a) was found to be highly similar to the genomovar 14 representative 28a3, with a DNA–DNA relatedness value of 91% (Supplementary Table S1 in IJSEM Online), yet this strain had a position in the 16S rRNA tree different from that of 28a3 (Supplementary Fig. S1 available in IJSEM Online). The two strains were isolated from the same soil sample and are nearly identical in their ITS1 sequences (Supplementary Fig. S2 available in IJSEM Online), their RAPD and are nearly identical in their ITS1 sequences (Supplementary Fig. S2 available in IJSEM Online), yet this strain had a position in the 16S rRNA tree different from that of 28a3 (Supplementary Table S1 in IJSEM Online). The two strains were isolated from the same soil sample and are nearly identical in their ITS1 sequences (Supplementary Table S1 in IJSEM Online), their RAPD patterns (Sikorski et al., 2002a) and their partial rpoB sequences [as determined by restriction enzyme profiling of 1·5 kb PCR products (nucleotides 532 to 2034 of the *Escherichia coli* rpoB sequence); J. Sikorski and W. Wackernagel, unpublished]. The divergence of their 16S rRNA gene sequences results from five nucleotide changes within a stretch of 15 nucleotides (*E. coli* positions 74 to 92; *E. coli* has an insert of four nucleotides compared with all *P. stutzeri* strains), which makes the 16S rRNA gene sequence of strain 28a3 in this region identical to that of genomovars 2 and 3 strains. This rare specific local sequence identity between a single member of one genomovar and the members of two far-distant genomovars may be explained by a horizontal gene transfer event involving part of the 16S rRNA gene. Strain 28a3, as the putative recipient, was shown to be highly transformable, which is generally not the case for strains of the RAPD group of which 28a3 is the representative (Sikorski et al., 2002b).

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### References


