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## **Transfer of *Pseudomonas pictorum* Gray & Thornton 1928 to genus *Stenotrophomonas* as *Stenotrophomonas pictorum* comb. nov. and emended description of the genus *Stenotrophomonas*.**

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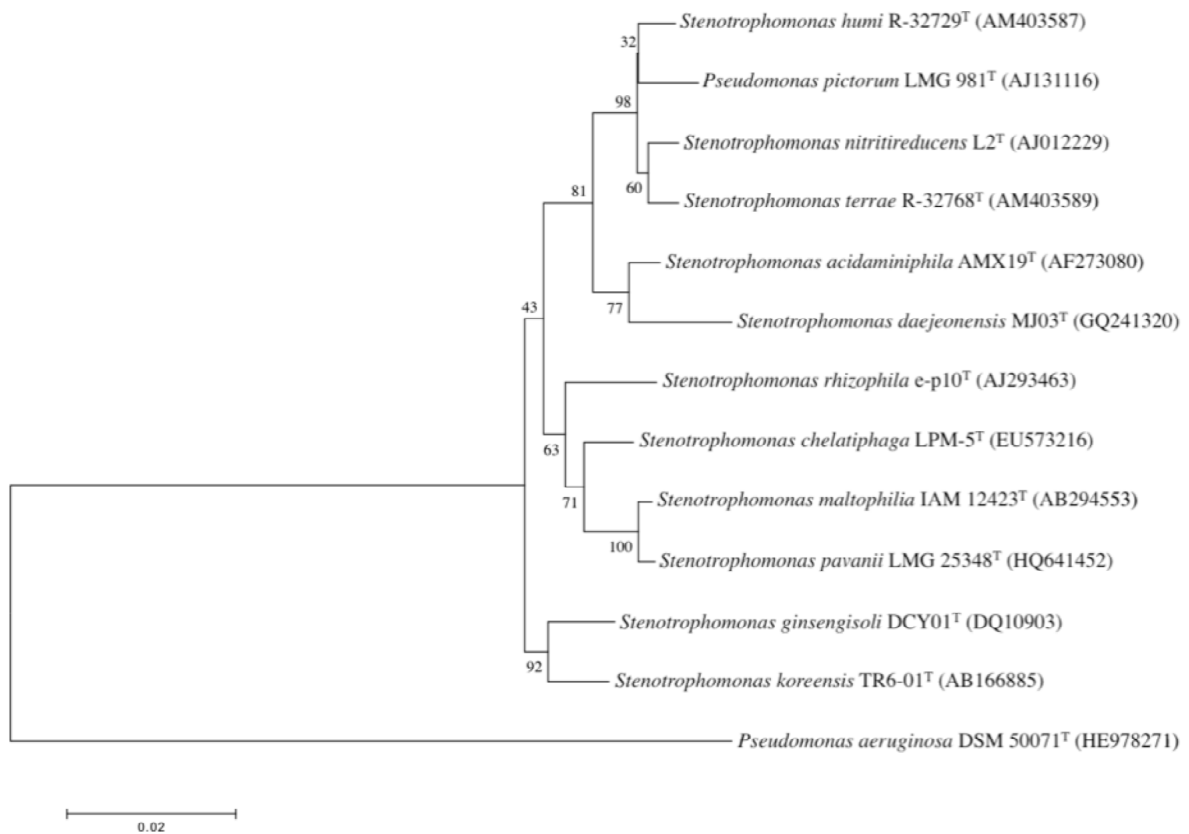
Accession number of 16S rRNA gene sequence of the type strain: AJ131116 (GenBank/EMBL/DDBJ)

### **Abstract**

A polyphasic taxonomic approach including analysis of phenotypic, physiologic and genotypic characteristics, 16S rRNA sequence and DNA-DNA hybridization analysis was used to determine the most consistent affiliation of *Pseudomonas pictorum*. *Pseudomonas pictorum* ATCC 23328<sup>T</sup> exhibited phenotypic traits of members of genus *Stenotrophomonas* including cellular fatty acid composition, quinone and limited range of substrate that could be used. Antibiotic susceptibility and physiological characteristics were determined. The DNA base composition was 65.7 mol % G+C. Phylogenetic analysis revealed that type strains of *Stenotrophomonas terrae*, *S. humi*, *S. nitritireducens* and *S. acidaminiphila* were the nearest relatives (16S rRNA similarity of 98.0 to 98.8 %). All the other type strains of *Stenotrophomonas* species showed high 16S rRNA sequence similarities (96.8 to 97.2 %). DNA-DNA hybridizations revealed 31.0, 32.0, 43.3 and 43.6 % reassociation between *Pseudomonas pictorum* ATCC 23328<sup>T</sup> and type strains of *Stenotrophomonas terrae*, *Stenotrophomonas humi*, *Stenotrophomonas nitritireducens* and *Stenotrophomonas acidaminiphila*, respectively. Our overall results indicate that *Pseudomonas pictorum* should be transferred in genus *Stenotrophomonas* as a new species of this genus, *Stenotrophomonas pictorum* comb. nov. Since the original description of the genus *Stenotrophomonas* with only one species (*S. maltophilia*), an emendation of the genus description is proposed in order to match better with the characteristics of the eleven new species assigned to this genus since then.

The first description of *Pseudomonas pictorum* proposed by Gray & Thornton (1928) was a report of the phenotypic characteristics of cells and morphology of colonies. Among the years, phenotypic studies (cellular fatty acid composition, quinone type content of cells, polyamine pattern and esterase polymorphism) and phylogenetic analysis (16S rRNA sequences, *gyrB* sequence, full genome sequence) clearly pointed out that *Pseudomonas pictorum* should rather be reclassified within the *Xanthomonadaceae* lineage and that its closest relatives were *Stenotrophomonas* species (Oyaizu & Komataga, 1983, Van den Mooter and Swings, 1990; Yang *et al.*, 1993a; Singer *et al.*, 1994; Anzai *et al.*, 2000; Assih *et al.*, 2002; Kaparullina *et al.*, 2009; Kim *et al.*, 2010; Ramos *et al.*, 2011; Svensson-Stadler *et al.*, 2012; Patil *et al.*, 2016). As a consequence, the type strain of *Pseudomonas pictorum* was included in several studies about the genomic and phenotypic diversity of the *Stenotrophomonas* genus in which this type strain was even sometimes presented as a member of *S. maltophilia* with which however it shows only 30% DNA-DNA reassociation (Hauben *et al.*, 1999; Yang *et al.*, 1993a; Coenye *et al.*, 2004). Despite of this body of evidence and wide acceptance, *Pseudomonas pictorum* has still not been formerly assigned to the genus *Stenotrophomonas*. The aim of the present paper is to determine the most consistent taxonomic position of *Pseudomonas pictorum* by a polyphasic taxonomic approach including analysis of phenotypic, physiologic and genotypic (16S rRNA sequence similarities and DNA-DNA hybridization) properties and characteristics of this species. Since no other *P. pictorum* strain than the type strain is presently available in public culture collections and all the accessible cultures of the type strain are derived from the strain deposited in the Czech collection of microorganisms under the number CCM 284<sup>T</sup>, our study was limited to the type strain.

Cultures of all the type strains were done using nutrient broth media (meat extract 3 g/l, peptone 5 g/l, yeast extract 5 g/l). Unless otherwise indicated, cultures of microorganisms to perform phenotypic tests were done under aerobic conditions at 35 °C, pH 7. All analyses were performed at least in duplicate. For phenotypic and biochemical characteristic determination as well as DNA-DNA hybridization experiments, we worked with *Pseudomonas pictorum* ATCC 23328<sup>T</sup>. The 16S rRNA sequence used for the phylogenetic analysis corresponds to that of *Pseudomonas pictorum* LMG 981<sup>T</sup> (GenBank accession number AJ131116; 1,502 positions used). All the other type strains of *Stenotrophomonas* species used during this study were obtained from public culture collections. The culture collection references of these type strains are listed in Table S1. The phylogenetic analysis was done as described by Assih *et al.* (2002) except that this time the MEGA software (version 5.2; <http://www.megasoftware.net>) and the Muscle algorithm were used to align the 16S rRNA sequences. This analysis revealed that the closest relatives were the type strains of *Stenotrophomonas humi*, *S. terrae*, *S. nitritireducens* and *S. acidaminiphila* with similarity levels of 98.8 %, 98.8 %, 98.6 % and 98.0 % respectively. All the other type strains of *Stenotrophomonas* species showed high 16S rRNA sequence similarities ranging from 96.8 to 97.2 % (Fig 1). *Pseudomonas pictorum* cannot be assigned to *Stenotrophomonas daejeonensis*, *S. koreensis*, *S. maltophilia* and *S. pavanii* since they share less



**Fig. 1.** 16S rRNA phylogenetic dendrogram showing the position of *Pseudomonas pictorum* LMG 981<sup>T</sup> among the representative members of the genus *Stenotrophomonas*. The scale bar indicates 2 nucleotides substitution per 100 nucleotides. Numbers (percentages) at the node corresponds to bootstrap values based on 1000 resampling. Sequence accession numbers are indicated.

than 97 % 16S rRNA similarity (Stackebrandt & Goebel 1994, Wayne *et al.*, 1987). Recently, a 16S rRNA similarity of 98.2 % has been proposed as the new cut off point above which DNA-DNA reassociation experiments should be necessary for testing the genomic uniqueness of a novel isolate (Stackebrandt & Ebers, 2006; Meier-Kolthoff *et al.*, 2013; Kim *et al.*, 2014). Based on this criteria, *P. pictorum* could not be assigned to *Stenotrophomonas chelatiphaga*, *S. rhizophila* or *S. ginsengisoli* with which it shares 97.1 to 97.2 % 16S rRNA sequence similarity. Therefore, DNA-DNA tests were performed only with the four type strains sharing at least 98% 16S rRNA sequence similarities. The DNA-DNA hybridizations with *S. acidaminiphila* CIP 106456<sup>T</sup> and *S. nitritireducens* DSM 12575<sup>T</sup> were done by DSMZ using the spectroscopic method as described elsewhere (De Ley *et al.*, 1970, Escara & Hutton 1980, Huss *et al.*, 1983, Jahnke & Bahnweg, 1986; Jahnke, 1992) while the DNA-DNA hybridizations with *S. humi* CCUG 54881<sup>T</sup> and *S. terrae* CCUG 54880<sup>T</sup> were determined by BCCM/LMG using the microplate method developed by Ezaki *et al.* (1989) with the modifications implemented by Goris *et al.* (1998) and Cleenwerck *et al.* (2002). These tests revealed that *P. pictorum* ATCC 23328<sup>T</sup> hybridized at  $31.0 \pm 3$  % with *S. humi* CCUG 54881<sup>T</sup>, at  $32.0 \pm 10$  % with *S. terrae* CCUG 54880<sup>T</sup> and at 43.6 % with *S. acidaminiphila* CIP 106456<sup>T</sup> and 43.3 % with *S. nitritireducens* DSM 12575<sup>T</sup>, all values significantly below the 70 % cut off limit for species delineation. The 16S rRNA analysis coupled to the DNA-DNA hybridization with the closest relatives showed that *Pseudomonas pictorum* belongs to *Stenotrophomonas* genus but that it cannot be assigned to any of the *Stenotrophomonas* species with valid standing in nomenclature described so far. As a consequence, the most consistent alternative seems to be the affiliation of *Pseudomonas pictorum* as a distinct new species of genus *Stenotrophomonas*. Such conclusion is in line with results pointed out by several authors (Anzai *et al.*, 2000, Svensson-Stadler *et al.*, 2012) including one based on the analysis of the full genome of *Pseudomonas pictorum* (Patil *et al.*, 2016).

On this last point it is interesting to note that contrarily to what was observed by Goris *et al.* (2007), no correlation could be found between the ANI (Average Nucleotide Identity) or dDDH (digital DNA-DNA hybridization) derived from the analysis of the full genome sequences of all *Stenotrophomonas* species by Patil *et al.* (2016) and the 46 experimental DNA-DNA hybridization values (expDDH) published in the descriptions of the different *Stenotrophomonas* species (Tables S4 and S5). It is important however not to forget that the 46 experimental DNA-DNA hybridization values available for these species are all well below 70 % since they were determined to confirm that close strains belonged to different species and that this may have created a statistical bias for such comparison. Although dDDH and experimental DNA-DNA hybridization did not gave identical values for the *Stenotrophomonas* species they were always in phase with respect to the 70% cutoff limit between species.

Additional biochemical characteristics provided by our study were the cellular fatty acid (CFA) composition and GC mole % of *P. pictorum* ATCC 23328<sup>T</sup>. The CFA composition of the type strains of all the validly published species assigned presently to the *Stenotrophomonas* genus was redetermined at DSMZ using the Sherlock Microbial Identification systems (MIDI) and MIDI standard procedures

for strain cultivation (24h at 28°C in trypticase soy broth supplemented with 15 g agar l<sup>-1</sup>). The CFA profiles obtained were compared by unweighted arithmetic average clustering to those of all the *Pseudomonas*, *Pseudoxanthomonas* and *Xanthomonas* species with valid standing in nomenclature present in MIDI TSBA6 library using MIDI proprietary software that disclose the results in the form of dendrogram. The CFA Profiles for each species of the MIDI TSBA6 library correspond to the average profile of several strains including the type strain ones. The major fatty acids of *Pseudomonas pictorum* ATCC 23328<sup>T</sup> were iso-C<sub>15:0</sub> (24.2 %), anteiso-C<sub>15:0</sub> (8.1 %), iso-C<sub>17:1ω9c</sub> (7.5 %), C<sub>16:0</sub> (6.7 %), iso-C<sub>14:0</sub> (6.1 %) and iso-C<sub>15:1</sub> F (5.5 %). Up to fifteen CFA were always present in the CFA profile determined for the type strains of all the *Stenotrophomonas* species and in the cell fatty acid pattern of *Pseudomonas pictorum* ATCC 23328<sup>T</sup> (Table S2 and genus description). The unresolved CFA mixture iso-C<sub>15:0</sub> 2OH/C<sub>16:1ω7c</sub> was also present in the CFA composition determined for all the strains included in our study (Table S2). The three CFA (iso-C<sub>11:0</sub>, iso-C<sub>11:0</sub>-3OH and iso-C<sub>13:0</sub>-3OH) identified by Yang *et al.* (1993b) as characteristic of genus *Stenotrophomonas* and *Xanthomonas* were detected in the cell fatty acid pattern of *Pseudomonas pictorum* ATCC 23328<sup>T</sup> (Table S2). Other fatty acids present at a level of more than 1 % in most of the type strains of *Stenotrophomonas* species tested were: iso-C<sub>11:0</sub> (3.4-6.4 %), iso-C<sub>11:0</sub> 3OH (1.3-3.3 %), C<sub>14:0</sub> (1.2-13.5 %), iso-C<sub>15:1</sub> F (1.1-20.0 %), iso-C<sub>15:0</sub> (17.1-38.1 %), anteiso-C<sub>15:0</sub> (1.8-18.1 %), iso-C<sub>16:0</sub> (1.2-11.9 %), C<sub>16:0</sub> (1.2-6.7 %) iso-C<sub>17:1ω9c</sub> (2.2-13.5 %). The comparison by unweighted arithmetic average clustering of the CFA profile of *Pseudomonas pictorum* ATCC 23328<sup>T</sup> showed that *Stenotrophomonas terrae* CCUG 54880<sup>T</sup> and *S. humi* CCUG 54881<sup>T</sup> were the two type strains with the closest CFA profiles (Fig. S1) which is in line with the 16S rRNA phylogeny (Fig. 1) and *gyrB* sequence analysis (Svensson-Stadler *et al.*, 2012). The CFA average clustering clearly showed also that *Pseudomonas pictorum* ATCC 23328<sup>T</sup> does not cluster with the *Pseudomonas* species but rather with those belonging to the *Xanthomonadaceae* lineage (Fig. S2). Within this lineage, the *Stenotrophomonas* species did not form however a homogenous separate cluster from the *Xanthomonas* and other related species (e.g. *Pseudoxanthomonas* spp). This indicates that CFA is not an adequate discrimination tool among the different genus of this lineage.

The guanine-plus-cytosine content (G+C%) of the bacterial DNA of *Pseudomonas pictorum* ATCC 23328<sup>T</sup> was determined by BCCM/LMG using the HPLC technique (Mesbah *et al.*, 1989). The value reported is the mean value of three independent analyses of the same DNA sample. The G+C mole % of *P. pictorum* ATCC 23328<sup>T</sup> found during this study was 65.7 which is within the range of values (64.0 – 69.2) reported for the other *Stenotrophomonas* species (Lee *et al.*, 2011, Table 1) and is identical to the value reported earlier by De Vos *et al.*, (1989). The two previous experimental values are in agreement with the G+C % (66.00 %) calculated from the full sequence of *Pseudomonas pictorum* JCM 9942<sup>T</sup> by Patil *et al.* (2016).

Procedures for determination of general phenotypic characteristics for *P. pictorum* were as described elsewhere (Assih *et al.*, 2002, Ouattara *et al.*, 2003, Thierry *et al.*, 2004). Our results of phenotypic characterization were partially consistent with those reported by Gray & Thornton (1928) and the general

**Table 1.** General phenotypic characters and G+C% of the type strain of *Pseudomonas pictorum* and that of type strains of *Stenotrophomonas* species with standing in nomenclature

1 = *Pseudomonas pictorum* ATCC 23328<sup>T</sup>, 2 = *Stenotrophomonas humi*, 3 = *Stenotrophomonas terrae*, 4 = *Stenotrophomonas nitritireducens*, 5 = *Stenotrophomonas acidaminiphila*, 6 = *Stenotrophomonas ginsengisoli*, 7 = *Stenotrophomonas chelatiphaga*, 8 = *Stenotrophomonas rhizophila*, 9 = *Stenotrophomonas daejeonensis*, 10 = *Stenotrophomonas maltophilia*, 11 = *Stenotrophomonas koreensis*, 12 = *Stenotrophomonas pavanii*. + : positive, - : negative, w : weak, v : variable, nr: not reported,. All the species : gram negative, oxidase positive, catalase positive. The following substrates were not utilized by *Pseudomonas pictorum* : D-galactose, D-trehalose, L-sorbose, D-melibiose, sucrose, D-raffinose, lactose, lactulose, 1-O-Methyl-β-galactoside, 1-O-Methyl-α-galactoside, D-cellobiose, 1-O-Methyl-β-D-glucoside, D-ribose, L-arabinose, D-xylose, palatinose, L-rhamnose, L-fucose, D-melizitose, D-arabitol, L-arabitol, xylitol, dulcitol, D-tagatose, glycerol, myo-inositol, D-mannitol, maltitol, D-turanose, D-sorbitol, adonitol, HQ-β-glucuronide, i-erythritol, 1-O-Methyl-α-D-glucoside, 3-O-methyl-D-glucose, D-saccharate, mucate, L-tartrate, D-tartrate, meso-tartrate, D-malate, L-malate, cis-aconitate, trans-aconitate, tricarballylate, citrate, D-glucuronate, D-galacturonate, 2-ketogluconate, 5-ketogluconate, tryptophan, D-gluconate, phenylacetate, protocatechuate, 4-hydroxybenzoate, quinate, gentisate, 3-hydroxybenzoate, benzoate, 3-phenylpropionate, m-coumarate, trigonelline, betaine, putrescine, 4-aminobutyrate, histamine, DL-lactate, caprate, caprylate, succinate, fumarate, glutarate, DL-glycerate, 5-aminovalerate, ethanolamine, tryptamine, D-glucosamine, Itaconate, 3-hydroxybutyrate, L-aspartate, L-glutamate, L-proline, D-alanine, L-serine, malonate, propionate, L-tyrosine, 2-ketoglutarate

Characteristics	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>b</sup>	4 <sup>c</sup>	5 <sup>d</sup>	6 <sup>e</sup>	7 <sup>f</sup>	8 <sup>g</sup>	9 <sup>h</sup>	10 <sup>i</sup>	11 <sup>j</sup>	12 <sup>k</sup>
Morphology of the colonies	<b>circular</b>	smooth irregular	smooth round	nr	circular	glossy circular	smooth, convex, shiny	nr	smooth, convex, shiny	smooth, glistening, entire margin white, grayish or pale yellow	smooth, convex, non glossy	nr
Color of the colonies	<b>Yellow-orange</b>	light yellow	beige	yellow	pale yellow	yellow	yellowish	yellowish	yellowish		yellowish	nr
Motility	+	+	+	+	+	-	+	nr	+	+	-	-
Acid production from glucose	+	+	+	-	+	-	+	nr	+	nr	-	nr
Tween 80 hydrolysis	+	-	+	+	+	-	-	+	+	+	+	+
Aesculin hydrolysis	+	-	w	v	+	-	-	+	+	+	-	+
Gelatin hydrolysis (Protease)	-	w	+	-	-	-	+	+	+	+	+	+
Amylase (starch hydrolysis)	v	-	-	+	-	-	+	-	-	-	-	-
Nitrate reduction to nitrite	+	+	+	-	+	-	-	+	-	+	-	nr
Nitrite reduction	-	-	-	+ (to N.O)	+	nr	nr	-	-	-	-	nr
Nutritional spectrum												
D-Glucose	+	-	+	v	+	+	+	+	+	+	-	-
D-Fructose	+	+	+	+	+	nr	+	+	+	+	-	-
D-Mannose	+	+	+	-	+	-	+	+	+	+	-	-
Maltose	+	+	+	-	+	+	+	+	+	+	-	-
Gentiobiose	+	-	-	-	-	nr	nr	v	nr	+	-	-
D-Lyxose	+	-	-	-	-	nr	nr	-	nr	-	-	-
L-histidine	+	-	v	v	+	-	nr	+	+	v	-	nr
L-proline	-	w	w	v	+	+	nr	+	+	v	+	nr
L-alanine	+	w	w	+	+	-	nr	v	+	+	+	nr
L-serine	-	w	+	+	+	-	nr	-	+	+	v	nr
Acetate	+	w	w	w	+	-	+	+	+	+	+	nr
Propionate	-	-	w	v	w	-	nr	v	+	v	v	nr
DL-Lactate	-	+	v	+	+	-	nr	v	-	v	v	nr
Citrate	-	+	+	+	-	-	+	+	+	+	-	+
D-Malate	-	+	-	-	-	-	+	+	-	+	-	nr
G + C content (mol%)	<b>65.7</b>	65.0	64.0	69.1	66.9	65.4-65.8	68.3	69.2	64.7	66.6	66.0	67.5

<sup>a</sup> data from this study, Gray and Thornton, 1928 and Lipski *et al.*, 1992; <sup>b</sup> data from Heylen *et al.*, 2007, Kim *et al.*, 2010, Kapparulina *et al.*, 2009 and Lee *et al.*, 2011; <sup>c</sup> data from Finkmann *et al.*, 2000, Heylen *et al.*, 2007, Kim *et al.*, 2010, Kapparulina *et al.*, 2009, Lee *et al.*, 2011, Palleroni 2005, Yang *et al.*, 2006 and Lipsky & Altendorf 1997; <sup>d</sup> data from Assih *et al.*, 2002 and Kim *et al.*, 2010; <sup>e</sup> data from Kim *et al.*, 2010, <sup>f</sup> data from Kapparulina *et al.*, 2009, <sup>g</sup> data from Wolf *et al.*, 2002, Heylen *et al.*, 2007, Kapparulina *et al.*, 2009, Wolf *et al.* 2002, Kim *et al.*, 2010 and Yang *et al.* 2006; <sup>h</sup> data from Lee *et al.*, 2011, <sup>i</sup> data from Palleroni 2005, Stanier *et al.*, 1966, Heylen *et al.*, 2007, Kim *et al.*, 2010, Yang *et al.* 2006, Lipsky & Altendorf 1997 and Lee *et al.*, 2011; <sup>j</sup> data from Yang *et al.*, 2006, Heylen *et al.*, 2007, Yang *et al.* 2006 and Kim *et al.*, 2010; <sup>k</sup> data from Ramos *et al.*, 2011.

characteristics given by Palleroni & Bradbury (1993) and Palleroni (2005) for the *Stenotrophomonas* genus.

The overall results of phenotypic characterization are given in the genus and species description and in Table 1. *P. pictorum* could be easily distinguished from the other *Stenotrophomonas* species by the color of its colonies on nutrient agar (data not shown) and from its closest relatives *S. humi*, *S. terrae*, *S. nitritireducens* or *S. acidaminiphila*, by its ability to assimilate gentiobiose, D-lyxose but not L-proline and L-serine. Motility and fructose utilization were the common properties shared by *P. pictorum* and its closest relatives pertaining to genus *Stenotrophomonas* (Table 1).

The disk diffusion technique as described by Thierry *et al.* (2004) was used to evaluate the susceptibility of the type strain of *Pseudomonas pictorum* towards a set of medical antibiotics. Our data showed that *P. pictorum* ATCC 23328<sup>T</sup> presented low MICs (Minimum Inhibitory Concentrations) to 14 of the 16 antibiotics tested and was only apparently resistant to cephalothin, a first generation cephem, and amoxicillin that both act on peptidoglycan synthesis. *P. pictorum* ATCC 23328<sup>T</sup> was however susceptible to the 6 other antibiotics tested working with the same mechanism (Table S3). These results suggest that *P. pictorum* is highly susceptible to antibiotics which is logical since it was isolated from soil in 1928 much before the start of the massive use of antibiotics that has resulted in an increase of the abundance of antibiotic resistance gene in soils since then (Knapp *et al.*, 2010). This observation is also in line with the analysis of the full genome of *P. pictorum* that has shown the absence of chromosomally encoded  $\beta$ -lactamases contrarily to *S. maltophilia*, *S. pavanii* and other related strains (Patil *et al.*, 2016).

Phylogenetic differences between the type strain of *Pseudomonas pictorum* and any of the type strains of genus *Stenotrophomonas* species are supported by phenotypic and biochemical differences. The overall present results together with past analyses (Anzai *et al.*, 2000, Assih *et al.*, 2002, De Vos *et al.*, 1989, Kersters *et al.*, 1996, Oyaizu & Komagata 1983, Patil *et al.*, 2016, Singer *et al.*, 1994, Svensson-Stadler *et al.*, 2012, Van den Mooter & Swings 1990, Yang *et al.*, 1993a) support that *Pseudomonas pictorum* should be transferred in genus *Stenotrophomonas* as a new species of this genus, *Stenotrophomonas pictorum* comb. nov. An emended description of the genus *Stenotrophomonas* is therefore proposed in order to take in account new taxonomic data available through the description of several new species assigned to this genus since its creation by Palleroni and Bradbury (1993).

### **Emended description of genus *Stenotrophomonas*.**

The etymology, morphology and biochemical properties are as indicated in the genus description (Palleroni and Bradbury 1993, Palleroni 2005). Additional or modified properties are: reduction of nitrate to nitrite is variable, oxidase reaction is variable, tween 80, gelatin and starch hydrolysis variable, species are non-motile or motile by means of a single polar flagellum or several polar flagella, some species may grow anoxically using nitrate as alternate electron acceptor, colonies are white, beige, grayish, yellowish, pale yellow, yellow light, orange-yellow or yellow on common solid media, colonies are smooth,



glistening and often circular, growth is not accompanied by odour on common solid media but odour could develop on some media. The cellular fatty acids are of the iso/anteiso type with iso-C<sub>15:0</sub> normally clearly predominating. The other predominating fatty acids present are iso-C<sub>11:0</sub>, anteiso-C<sub>15:0</sub> and iso-C<sub>17:1 $\omega$ 9c</sub>. Other CFA usually or always present in cells are: C<sub>10:0</sub>, iso-C<sub>11:0</sub> 3OH, iso-C<sub>13:0</sub>, C<sub>12:0</sub> 3OH, iso-C<sub>14:0</sub>, C<sub>14:0</sub>, iso-C<sub>13:0</sub> 3OH, iso-C<sub>15:1</sub> F, , C<sub>15:0</sub>, iso-C<sub>16:0</sub>, C<sub>16:0</sub>, and iso-C<sub>17:0</sub>. DNA G+C content is 64.0–69.2 mole %. Members of the genus are widely distributed in nature. The type species is *Stenotrophomonas maltophilia*.

#### **Description of *Stenotrophomonas pictorum* comb. nov.**

*Stenotrophomonas pictorum* (pic.to'rum). L. gen. pl. n. *pictorum*, of painters; here, intended to mean of the Picts, named after the Picts, a Scottish tribe.

Exhibits all of the characteristics of the members of the genus. Cells size: 0.5-0.8 x 1.5-3  $\mu$ m. Colonies are yellow and circular on Trypticase soy agar. On nutrient agar, colonies are orange-yellow or yellow. Cells are positive for catalase, aesculin, tween 80 esterase but negative for urease, indole, ONPG, Simmons citrate, lysine and ornithine decarboxylase, arginine dihydrolase, DNase and proteolysis. Oxidase and starch hydrolysis variable. Nitrate is reduced but not nitrite. Polyamines: spermidine (major), cadaverine and spermine (minor). Quinone type: Q8. A limited range of substrate can be utilized (11 on 99 tested) including D-glucose, D-fructose, D-mannose, maltotriose, maltose, gentiobiose, D-lyxose, N-Acetyl-D-glucosamine, L-histidine, L-alanine and phenol. Acid is produced from D-glucose (dextrose) and maltose. Cholesterol is depleted when grown on bovine calf serum but not used as sole carbon source in mineral medium. Substrates not used are listed in Table 1. No growth was observed at 4 °C or/and 41 °C. Antibiotics susceptibility: susceptible to Ticarcillin, Piperacillin, Piperacillin + Tazobactam, Imipenem, Cefotaxime, Ceftazidime, Tobramycin, Amikacin, Gentamicin, Netilmicin, Colistin, trimethoprim + Sulfamethoxazole, Ofloxacin and Ciprofloxacin; resistant to Amoxicillin and Cephalothin. All the CFA characteristic of genus *Stenotrophomonas* are present. Predominant fatty acids are by decreasing order of abundance iso-C<sub>15:0</sub>, anteiso-C<sub>15:0</sub>, iso-C<sub>17:1 $\omega$ 9c</sub>, C<sub>16:0</sub>, iso-C<sub>14:0</sub> and iso-C<sub>15:1</sub> F. The DNA G + C content is 65.7 mole %. Habitat: originally isolated from soil. Type strain : ATCC 23328<sup>T</sup>, CCM 284<sup>T</sup>, CCUG 1823<sup>T</sup>, CCUG 3368<sup>T</sup>, CIP 103273<sup>T</sup>, DSM 19282<sup>T</sup>, JCM 9942<sup>T</sup>, LMG 981<sup>T</sup>, NCIMB 9152<sup>T</sup>, VKM 1240<sup>T</sup>. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the type strain is AJ131116.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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## SUPPLEMENTARY MATERIAL

### Transfer of *Pseudomonas pictorum* Gray & Thornton 1928 to genus *Stenotrophomonas* as *Stenotrophomonas pictorum* comb. nov. and emended description of the genus *Stenotrophomonas*

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**Fig. S1.** Dendrogram generated from CFA compositions showing the relationship of *Pseudomonas pictorum* ATCC 23328<sup>T</sup> with the type strains of all *Stenotrophomonas* species as well as to the type species of *Pseudomonas* (*P. aeruginosa*), *Pseudoxanthomonas* (*P. broegbernensis*) and *Xanthomonas* (*X. campestris* pv. *campestris*) genus but also to *Pseudoxanthomonas mexicana*. The CFA of the species associated with strain code in microbial collections were determined in this study. The CFA of all other species corresponds to those present in MIDI TSBA6 library.

**Fig. S2.** Dendrogram generated from CFA compositions showing the relationship of *Pseudomonas pictorum* ATCC 23328<sup>T</sup> with the type strains of all *Stenotrophomonas* species (determined in this study) as well as all the *Pseudomonas*, *Pseudoxanthomonas* and *Xanthomonas* species with valid standing in nomenclature present in MIDI TSBA6 library. The references including two species signify that their CFA profile is indistinguishable by the MIDI system.

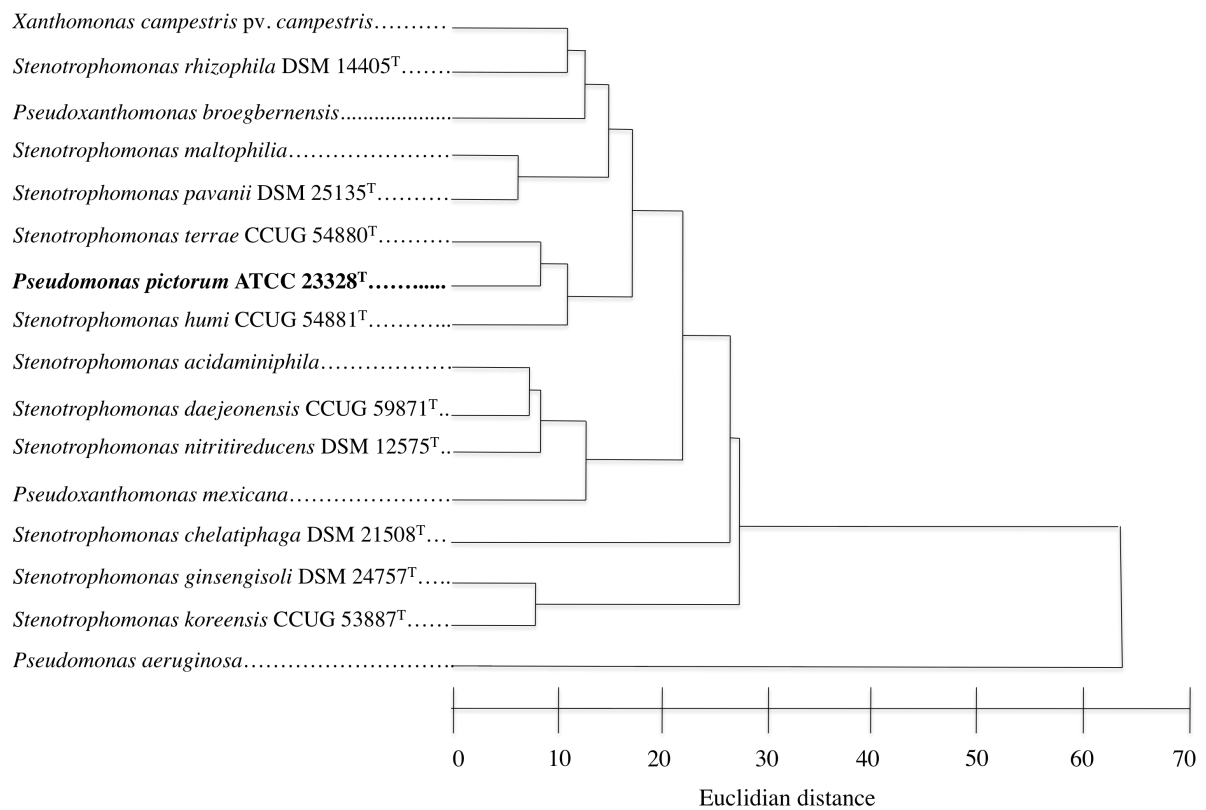
**Table S1.** Accession number in public culture collections of the type strains of the *Stenotrophomonas* species with standing in nomenclature used during the study.

**Table S2.** Fatty acid profile of *Pseudomonas pictorum* type strain compared to those of all type strains of *Stenotrophomonas* species with standing in nomenclature.

**Table S3.** Susceptibility of *Pseudomonas pictorum* ATCC 23328<sup>T</sup> towards selected antibiotics covering all mechanism of action.

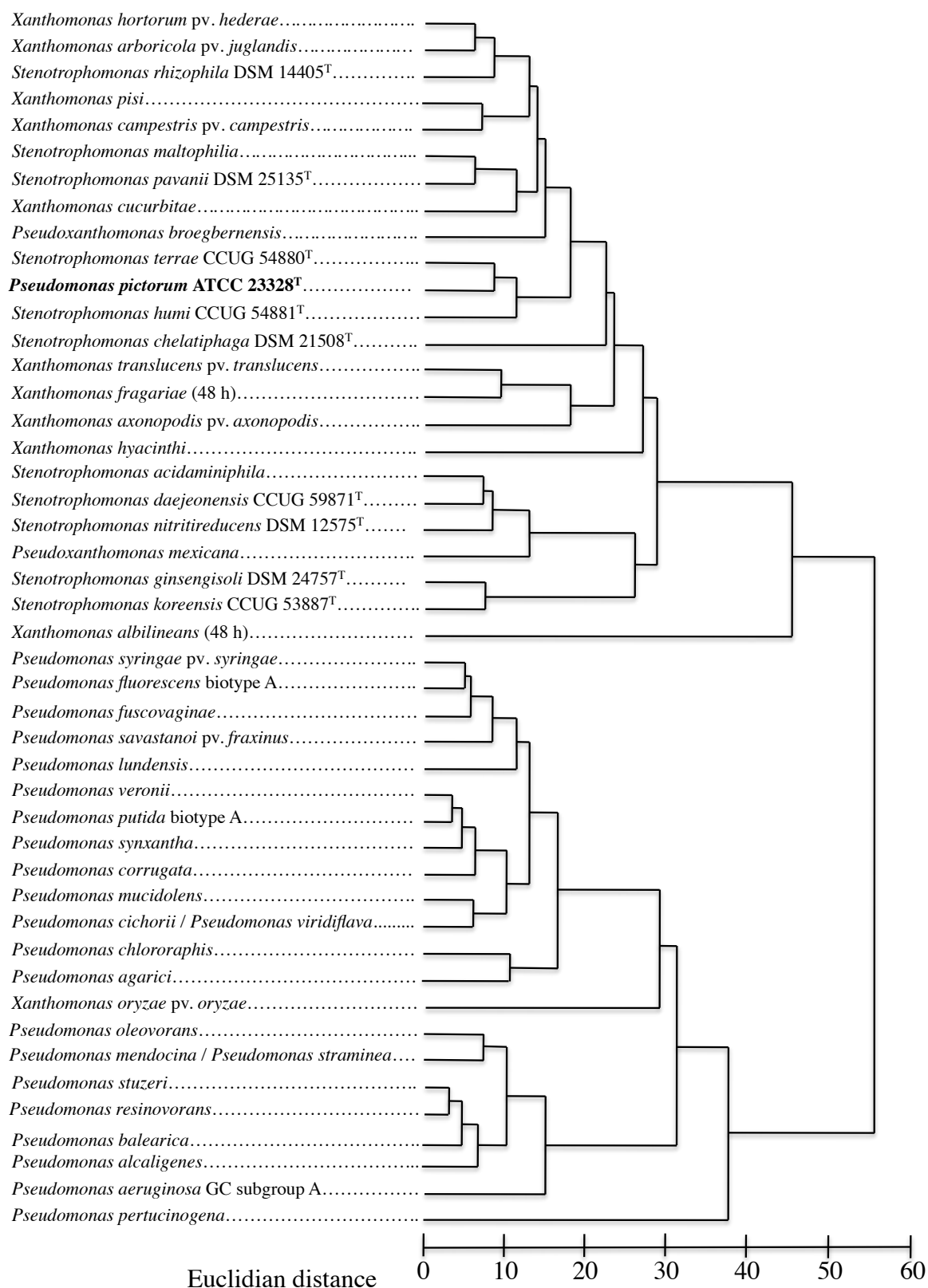
**Table S4.** Correlation between expDDH, dDDH (experimental and digital DNA-DNA hybridization respectively) and ANI (Average Nucleotide Identity) reported in literature for the type strains of the *Stenotrophomonas* species and related strains.

**Table S5.** Experimental DNA/DNA hybridization (expDDH) reported in literature between the type strains of the *Stenotrophomonas* species and related strains.



**Fig. S1.** Dendrogram generated from CFA compositions showing the relationship of *Pseudomonas pictorum* ATCC 23328<sup>T</sup> with the type strains of all *Stenotrophomonas* species as well as to the type species of *Pseudomonas* (*P. aeruginosa*), *Pseudoxanthomonas* (*P. broegbernensis*) and *Xanthomonas* (*X. campestris* pv. *campestris*) genus but also to *Pseudoxanthomonas mexicana*. The CFA of the species associated with strain code in microbial collections were determined in this study. The CFA of all other species corresponds to those present in MIDI TSBA6 library.





**Fig. S2.** Dendrogram generated from CFA compositions showing the relationship of *Pseudomonas pictorum* ATCC 23328<sup>T</sup> with the type strains of all *Stenotrophomonas* species (determined in this study) as well as all the *Pseudomonas*, *Pseudoxanthomonas* and *Xanthomonas* species with valid standing in nomenclature present in MIDI TSBA6 library. The references including two species signify that their CFA profile is indistinguishable by the MIDI system.

**Table S1.** Accession number in public culture collections of the type strains of the *Stenotrophomonas* species with standing in nomenclature used during the study.

<b>Species</b>	<b>Strain accession number in culture collections</b>	<b>Analysis performed</b>
<i>Stenotrophomonas acidaminiphila</i>	CIP 106456 <sup>T</sup>	DNA-DNA hybridization, phenotypic characterization, CFA
<i>Stenotrophomonas chelatiphaga</i>	DSM 21508 <sup>T</sup>	CFA
<i>Stenotrophomonas daejeonensis</i>	CCUG 59871 <sup>T</sup>	CFA
<i>Stenotrophomonas ginsengisoli</i>	DSM 24757 <sup>T</sup>	CFA
<i>Stenotrophomonas humi</i>	CCUG 54881 <sup>T</sup>	DNA-DNA hybridization, CFA
<i>Stenotrophomonas koreensis</i>	CCUG 53887 <sup>T</sup>	CFA
<i>Stenotrophomonas maltophilia</i>	CIP 60.77 <sup>T</sup>	CFA
<i>Stenotrophomonas nitritireducens</i>	DSM 12575 <sup>T</sup>	DNA-DNA hybridization, phenotypic characterization, CFA
<i>Stenotrophomonas pavanii</i>	DSM 25135 <sup>T</sup>	CFA
<i>Stenotrophomonas rhizophila</i>	DSM 14405 <sup>T</sup>	CFA
<i>Stenotrophomonas terrae</i>	CCUG 54880 <sup>T</sup>	DNA-DNA hybridization, CFA

**Table S2.** Fatty acid profile of *Pseudomonas pictorum* type strain compared to those of all type strains of *Stenotrophomonas* species with standing in nomenclature.

Species: 1, *P. pictorum*; 2, *S. acidaminiphila*; 3, *S. chelatiphaga*; 4, *S. daejeonensis*; 5, *S. ginsengisoli*; 6, *S. humi*; 7, *S. koreensis*; 8, *S. maltophilia*; 9, *S. nitritireducens*; 10, *S. pavanii*; 11, *S. rhizophila*; 12, *S. terrae*.

Values are percentages of total fatty acids. The fatty acids are listed in the order of elution obtained during analysis by gas chromatography (GC). The fatty acid names are given according to the carbon numbering from the carboxyl group ( $\alpha$ -end). When required, equivalence to the methyl ( $\omega$ -end) nomenclature numbering is given within parenthesis to easier the conversion between the two nomenclatures. \*Fatty acid is present in all tested strains. # Unresolved 15:0 iso 2OH / 16:1 cis 9, i.e. the two CFA 15:0 iso 2OH and 16:1 cis 9 (16:1  $\omega$ 7c) elute with the same retention time upon GC analysis.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12
10:0 iso	0.99	0.97		1.21	0.41	0.33	0.33		0.22			0.26
10:0*	0.64	0.24	1.02	0.21	0.54	0.38	0.58	0.70	0.19	0.54	0.55	0.35
11:0 iso*	3.84	4.06	3.74	3.99	6.44	4.92	5.23	3.43	5.47	3.35	3.85	5.63
11:0 anteiso	0.83	0.30	0.34	0.32	0.31	0.31	0.32		0.35		0.25	0.28
11:0					0.18		0.19					
10:0 3OH	0.20	0.04	0.24		0.14	0.08	0.13	0.22			0.16	
12:0 iso		0.14	0.19	0.17	0.17	0.19	0.12					
unknown 11.799			0.88			0.16		1.67		0.78	0.80	
12:0			0.83									
11:0 iso 3OH*	3.06	1.84	1.29	1.64	4.62	1.42	3.30	1.74	2.20	1.43	1.77	1.74
11:0 3OH	0.17	0.10		0.13	0.80	0.10	0.50		0.20		0.16	
13:0 iso*	1.74	0.60	3.40	0.92	2.65	0.73	4.52	0.73	0.96	0.36	0.40	0.83
13:0 anteiso	0.26	0.06	2.88		0.10	0.16					0.25	
13:0			0.17		0.22		0.50					
12:0 iso 3OH	1.06	1.85	0.34	1.78	0.68	2.38	0.38		1.44		0.40	1.52
12:0 3OH*	1.17	0.60	1.76	0.38	0.78	1.40	0.59	3.01	0.80	1.77	2.08	0.74
14:0 iso*	6.05	6.59	3.68	10.21	3.93	12.27	3.76	0.54	5.15	0.75	2.59	6.91
14:1 cis 9 (14:1 $\omega$ 5c)		0.16										
14:0*	4.01	0.92	13.51	1.30	1.57	2.94	2.56	4.44	1.24	2.87	3.12	3.05
13:0 iso 3OH*	1.61	1.26	0.79	1.56	2.50	1.04	1.37	3.54	1.70	3.38	1.89	1.25
13:0 2OH	0.45	0.23	0.43	0.28		0.36		0.25	0.39	0.56	0.61	0.39
15:1 iso F*	5.47	2.75	5.50	2.16	20.04	2.60	21.44	1.30	3.51	0.75	3.21	5.18
15:0 iso*	24.24	35.95	17.14	38.10	26.00	28.28	29.51	36.65	36.67	34.56	27.21	30.17
15:0 anteiso*	8.12	5.19	13.27	5.44	1.76	7.15	2.38	6.96	7.21	14.95	18.13	5.43
15:1 cis 7 (15:1 $\omega$ 8c)	0.28	0.10	0.37	0.15	0.95	0.14	1.73				0.20	0.28
15:1 cis 9 (15:1 $\omega$ 6c)		0.39		0.28	0.23	0.26	0.28				0.26	
15:0*	1.56	0.63	1.30	1.27	1.98	0.85	2.79	0.33	1.41	0.65	1.12	1.35
16:1 iso H		0.13										
16:0 iso*	3.71	10.20	1.20	8.54	2.21	9.50	1.50	0.62	5.89	1.61	2.57	5.25
16:1 cis 7 (16:1 $\omega$ 9c)	1.85	0.87	3.98	0.57		1.09		3.72	0.91	2.63	2.97	1.68
Sum in feature 3*#	12.82	5.79	13.86	3.39	5.22	10.98	5.57	12.88	2.11	10.45	12.54	11.22
16:0*	6.70	2.28	5.01	2.13	1.18	3.01	1.54	6.51	3.92	6.74	5.07	4.94
15:0 iso 3OH					0.19							
Iso 17:1 cis 7 (Iso 17:1 $\omega$ 9c)*	7.54	12.72	2.24	11.29	12.19	5.31	7.19	4.51	13.51	4.54	5.14	9.49
17:0 iso*	1.03	2.02	0.36	1.91	1.61	1.27	1.06	3.10	2.49	4.05	1.84	1.39
17:0 anteiso								0.17		0.52		
17:1 cis 9 (17:1 $\omega$ 8c)	0.61	0.47	0.26	0.66	0.41	0.40	0.63		0.59		0.61	0.66
17:1 cis 11 (17:1 $\omega$ 6c)											0.25	
17:0		0.11										
17:0 cyclo									1.47			
18:1 cis 9 (18:1 $\omega$ 9c)		0.11						1.43		1.68		
18:1 cis 11 (18:1 $\omega$ 7c)		0.10						0.77		1.09		
19:0 iso								0.40				

**Table S3.** Susceptibility of *Pseudomonas pictorum* ATCC 23328<sup>T</sup> towards selected antibiotics covering all mechanism of action.

Antibiotics are listed by mechanism of action and class. The values obtained in the present study correspond to MICs (Minimum inhibitory concentration) in mg per litre. The values within parenthesis after the name of the antibiotics correspond to the disk charge. S and R within parenthesis after the MIC values mean that the strains are respectively susceptible or resistant to the antibiotics. The S & R categories are based on the guidelines of the antibiogram committee of the French Society of Microbiology (CASFM, 2002).

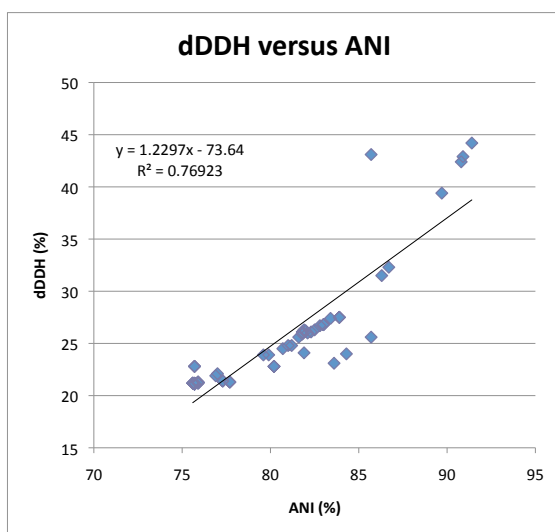
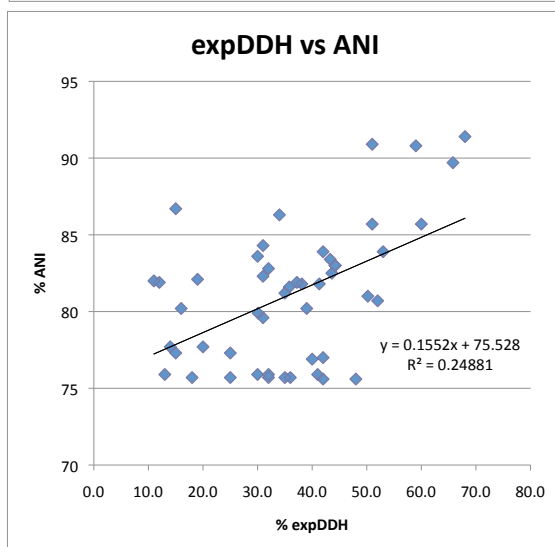
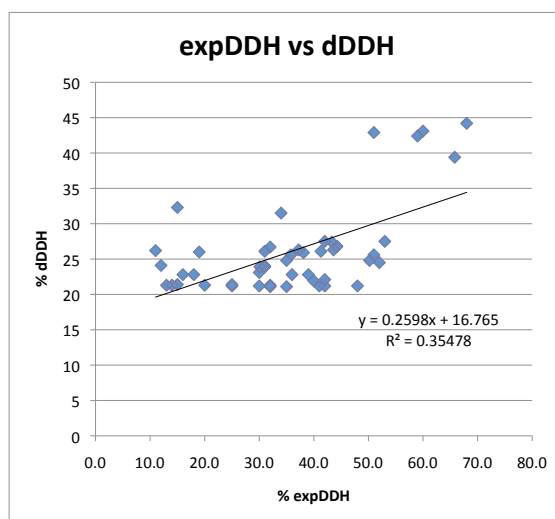
Antibiotic	MIC mg/l (sensitivity)
<b>Inhibition of protein synthesis</b>	
Aminoglycosides	
Amikacin (30 µg)	0.01 (S)
Gentamicin (15 µg)	0.03 (S)
Netilmicin (30 µg)	0.01 (S)
Tobramycin (10 µg)	0.06 (S)
<b>Inhibition of nucleic acid synthesis or DNA damage</b>	
Fluoroquinolones	
Ciprofloxacin (5 µg)	0.04 (S)
Ofloxacin (5 µg)	0.04 (S)
<b>Disruption of cell membrane</b>	
Polypeptides	
Colistin (50 µg)	< 2 (S)
<b>Inhibition of folate synthesis</b>	
Sulfonamides + di-aminopyrimidines (1.25 + 23.75 µg)	
Trimethoprim – Sulfamethoxazole	0.02 (S)
<b>Inhibition of peptidoglycan synthesis</b>	
Carbapenems	
Imipenem (10 µg)	0.57 (S)
Cephems	
Cefotaxime (30 µg)	0.71 (S)
Ceftazidime (30 µg)	0.02 (S)
Cephalothin (30 µg)	128 (R)
Penams	
Amoxicillin (25 µg)	> 16 (R)
Piperacillin (75 µg)	0.25 (S)
Piperacillin (75 µg) – Tazobactam (10 µg)	0.14 (S)
Ticarcillin (75 µg)	0.12 (S)

Reference.

**CASFM (2002).** Communiqué 2002. Special number SFM, pp. 1–47. Paris: Comité de l'Antibiogramme de la Société Française de Microbiologie (in French). <http://www.sfm.asso.fr>

**Table S4. Correlation between expDDH, dDDH (experimental and digital DNA-DNA hybridization respectively) and ANI (Average Nucleotide Identity) reported in literature for the type strains of the *Stenotrophomonas* species and related strains**

Couples of type strains	expDDH*	dDDH**	ANI**
1 <i>S. acidaminiphila</i> / <i>S. maltophilia</i>	31.0	24	84.3
2 <i>S. acidaminiphila</i> / <i>S. nitritireducens</i>	65.8	39.4	89.7
3 <i>S. acidaminiphila</i> / <i>P. pictorum</i>	43.6	26.3	82.5
4 <i>S. chelatiphaga</i> / <i>S. maltophilia</i>	51	25.6	85.7
5 <i>S. Chelatiphaga</i> / <i>S. rhizophila</i>	52	24.5	80.7
6 <i>S. daejeonensis</i> / <i>S. maltophilia</i>	12	24.1	81.9
7 <i>S. daejeonensis</i> / <i>S. acidaminiphila</i>	34	31.5	86.3
8 <i>S. daejeonensis</i> / <i>S. humi</i>	19	26	82.1
9 <i>S. daejeonensis</i> / <i>S. nitritireducens</i>	15	32.3	86.7
10 <i>S. daejeonensis</i> / <i>S. terrae</i>	11	26.2	82
11 <i>S. ginsengisoli</i> / <i>S. maltophilia</i>	20	21.3	77.7
12 <i>S. ginsengisoli</i> / <i>S. humi</i>	35	21.1	75.7
13 <i>S. ginsengisoli</i> / <i>S. koreensis</i>	53	27.5	83.9
14 <i>S. ginsengisoli</i> / <i>S. terrae</i>	30	21.2	75.9
15 <i>S. humi</i> / <i>S. acidaminiphila</i>	38.1	25.9	81.8
16 <i>S. humi</i> / <i>S. ginsengisoli</i>	32	21.1	75.7
17 <i>S. humi</i> / <i>S. koreensis</i>	42	21.2	75.6
18 <i>S. humi</i> / <i>S. maltophilia</i>	18	22.8	75.7
19 <i>S. humi</i> / <i>S. nitritireducens</i>	37.2	26.3	81.9
20 <i>S. humi</i> / <i>S. terrae</i>	44.2	26.8	83
21 <i>S. koreensis</i> / <i>S. maltophilia</i>	25	21.4	77.3
22 <i>S. koreensis</i> / <i>S. acidaminiphila</i>	40	21.9	76.9
23 <i>S. koreensis</i> / <i>S. ginsengisoli</i>	42	27.5	83.9
24 <i>S. koreensis</i> / <i>S. humi</i>	48	21.2	75.6
25 <i>S. koreensis</i> / <i>S. nitritireducens</i>	42	22.1	77
26 <i>S. koreensis</i> / <i>S. rhizophila</i>	25	21.2	75.7
27 <i>S. koreensis</i> / <i>S. terrae</i>	32	21.3	75.9
28 <i>S. maltophilia</i> / <i>S. ginsengisoli</i>	14	21.3	77.7
29 <i>S. maltophilia</i> / <i>S. humi</i>	36	22.8	75.7
30 <i>S. maltophilia</i> / <i>S. koreensis</i>	15	21.4	77.3
31 <i>S. maltophilia</i> / <i>S. terrae</i>	39	22.8	80.2
32 <i>S. pavanii</i> / <i>S. maltophilia</i>	60	43.1	85.7
33 <i>S. pavanii</i> / <i>S. nitritireducens</i>	31	23.9	79.6
34 <i>S. pavanii</i> / <i>S. rhizophila</i>	35	24.8	81.2
35 <i>S. pavanii</i> / <i>Pseudomonas geniculata</i>	59	42.4	90.8
36 <i>S. pavanii</i> / <i>Pseudomona beteli</i>	51	42.9	90.9
37 <i>S. pavanii</i> / <i>Pseudomonas hibiscola</i>	68	44.2	91.4
38 <i>S. rhizophila</i> / <i>S. maltophilia</i>	50.2	24.8	81
39 <i>S. rhizophila</i> / <i>S. nitritireducens</i>	30.1	23.9	79.9
40 <i>S. terrae</i> / <i>S. acidaminiphila</i>	35.8	25.6	81.6
41 <i>S. terrae</i> / <i>S. ginsengisoli</i>	41	21.2	75.9
42 <i>S. terrae</i> / <i>S. humi</i>	44.2	26.8	83
43 <i>S. terrae</i> / <i>S. koreensis</i>	13	21.3	75.9
44 <i>S. terrae</i> / <i>S. maltophilia</i>	16	22.8	80.2
45 <i>S. terrae</i> / <i>S. nitritireducens</i>	41.3	26.1	81.8
46 <i>P. pictorum</i> / <i>S. humi</i>	31	26.1	82.3
47 <i>P. pictorum</i> / <i>S. maltophilia</i>	30.0	23.1	83.6
48 <i>P. pictorum</i> / <i>S. terrae</i>	32	26.7	82.8
49 <i>P. pictorum</i> / <i>S. nitritireducens</i>	43.3	27.4	83.4



\*see Table S5

\*\* Patil, P. P., Midha, S., Kumar, S., & Patil, P. B. (2016). Genome sequence of type strains of genus *Stenotrophomonas*. *Front Microbiol* 7, 1-6.

**Table S5. Experimental DNA/DNA hybridization (expDDH) reported in literature between the type strains of the *Stenotrophomonas* species and related strains**

Species type strains	<i>S. acidaminiphila</i>												<i>S. chelatiphaga</i>			<i>S. daejeonensis</i>		<i>S. ginsengisoli</i>		<i>S. humi</i>		<i>S. koreensis</i>		<i>S. maltophilia</i>		<i>S. nitritireducens</i>		<i>S. pavanii</i>		<i>S. rhizophila</i>		<i>S. terrae</i>		<i>Pseudomonas pictorum</i>		<i>Pseudomonas geniculata</i>		<i>Pseudomonas betelii</i>		<i>Pseudomonas hibiscicola</i>		Reference	expDDH Method
	1	2	3	4	5	6	7	8	9	10	11	12																															
1 <i>Stenotrophomonas acidaminiphila</i> AMX19 <sup>T</sup>						31.0	65.8				43.6																									Assih <i>et al.</i> , 2002	Spectroscopic (DSMZ method)						
2 <i>Stenotrophomonas chelatiphaga</i> LPM-5 <sup>T</sup>								51		52																										Kaparullina <i>et al.</i> , 2009	Not specified**						
3 <i>Stenotrophomonas daejeonensis</i> MJ03 <sup>T</sup>	34								19		12	15																								Lee <i>et al.</i> , 2011	Microplate (LMG method)						
4 <i>Stenotrophomonas ginsengisoli</i> DCY01 <sup>T</sup>									35	53	20																									Kim <i>et al.</i> , 2010	Microplate (LMG method)						
5 <i>Stenotrophomonas humi</i> R-32729 <sup>T</sup>	38.1±5.1				32				42	18	37.2±6.6																									Heylen <i>et al.</i> 2007, Kim <i>et al.</i> , 2010	Microplate (LMG method)						
6 <i>Stenotrophomonas koreensis</i> TR6-01 <sup>T</sup>	40				42	48					25	42																								Yang <i>et al.</i> , 2006, Kim <i>et al.</i> , 2010	Microplate (LMG method)						
7 <i>Stenotrophomonas maltophilia</i> IAM 12423 <sup>T</sup>		51	12	14	36	15	-	34-38*	60±4.0	48.1-52.3	39	30																								Same as column 1, Hauben <i>et al.</i> 1999, Kim <i>et al.</i> , 2010							
8 <i>Stenotrophomonas nitritireducens</i> L2 <sup>T</sup>											34-38*																									Finkmann <i>et al.</i> , 2000	Spectroscopic (DSMZ method)						
9 <i>Stenotrophomonas pavanii</i> LMG 25348 <sup>T</sup>									60±4.0	31±0.0			35±3.0													59±5.0	51±10	68±3.0							Ramos <i>et al.</i> , 2011	Microplate (LMG method)							
10 <i>Stenotrophomonas rhizophila</i> e-p10 <sup>T</sup>											48.1-52.3	30.1																								Wolf <i>et al.</i> , 2002	Spectroscopic (DSMZ method)						
11 <i>Stenotrophomonas terrae</i> R-32768 <sup>T</sup>	35.8±4.7				41	44.2±2.8	13	16	41.3±7																											Heylen <i>et al.</i> 2007, Kim <i>et al.</i> , 2010	Microplate (LMG method)						
12 <i>Pseudomonas pictorum</i> ATCC 23328 <sup>T</sup>	43.6						31±3	30	43.3				32±10																							This work, Hauben <i>et al.</i> , 1999	Spectroscopic & microplate						

\**S. maltophilia* LMG11114 not the type strain, \*\*Kaparullina *et al.* (2009) make reference to a general article describing all the methods that can be used for DNA-DNA reassociation experiments without specifying which of them they used

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