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ORIGINAL ARTICLE

The physicochemical conditions of isolation source determine the occurrence of *Pseudomonas fluorescens* group species

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Abstract *Pseudomonas fluorescens* group species are indigenous bacteria in many different environments. The physicochemical conditions related to their selection to different surface and subsurface environments are poorly understood. We isolated 35 *P. fluorescens* group strains under the pesticide stress from mesocosms of surface soils and groundwater-monitoring pipe deposit slurries, and from a few of their initially sterilized counterparts, which were colonized during 599–625 days of laboratory incubation. In addition, strains were isolated from mesocosms of drilling sediment slurries. Based on the 16S rDNA analysis, strains belonged to five species. The statistical analysis of relationships between the isolates and physicochemical conditions in their isolation mesocosms revealed the typical habitats of five species. *Pseudomonas* IC038 and *Pseudomonas* LAB-23-like strains were common in mesocosms of deposit/sediment slurries low in growth substrates. *Pseudomonas marginalis*-like isolates preferred surface soil mesocosms rich in nutrients. *Pseudomonas veronii* and *Pseudomonas mandelii*-like strains grew in mesocosms of surface soils and subsurface deposit/sediment slurries, which indicated that they either had a versatile metabolism to adapt to different environments, or differed in substrate specificities. The initially sterilized mesocosms were mainly colonized with the same species as their non-sterilized counterparts, while all *P. fluorescens* group species tolerated atrazine and/or terbutryn. Our results on habitats were in agreement with the isolation sources of strains with identical 16S rDNA sequences in databases. The observed differences in the distribution patterns of the *P. fluorescens* group species indicate that species selection should be carefully done for man-made ecosystem applications, such as stable bioremediation.

Keywords *Pseudomonas fluorescens* group · 16S rDNA phylogeny · Surface soil · Subsurface deposits/sediments · Physicochemical conditions

Introduction

The *Pseudomonas fluorescens* group is an important subgroup within the genus *Pseudomonas*, based on the 16S rDNA phylogeny. *P. fluorecens* group members are rod-shaped *Gammaproteobacteria*. They are metabolically versatile, important in the carbon cycle, and can be facultative chemolithotrophs. The classification of the genus *Pseudomonas* has involved a lot of arguing, due to the lack of conserved phenotypic differences (Moore et al. 1996; Anzai et al. 2000; Silby et al. 2011). The initially homogenous *P. fluorescens* population developed three different phenotypes in the spatially heterogenous environment, while one phenotype only appeared in the spatially homogenous environment (Rainey and Travisano 1998). Strains of the *P. fluorescens* group are found in large numbers in all of the major natural environments. They occur in association with diseased plants or mushrooms, and saprophytic strains have been isolated from surface soil, subsurface layers, vegetation, contaminated sites, water and on plants (Moore et al. 1996; Shapir et al. 2000; Silby et al. 2011). The occurrence of *P. fluorescens* group strains as a major cultivable population in many different environments results from the interactions

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between ecological and genetic factors. However, the causes and ecological significance of the diversity have been largely ignored (Spiers et al. 2000; Siggins et al. 2012). The accumulating data on isolation sources of strains has not been assembled to elucidate the ranges of habitats in which species of the *P. fluorescens* group can colonize.

The pesticides atrazine (6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine) and terbutryn (*N*-(1,1-dimethylethyl)-*N'*-ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine) are typically degraded in a few months by microorganisms in surface soils. The biodegradation rate decreases with increasing depth, and pesticides can persist in deep sediments and groundwater for years (Kruger et al. 1993; Vanderheyden et al. 1997; Tomlin 2000; Houot et al. 2000; Talja et al. 2008). *Pseudomonas* spp. can degrade numerous organic compounds, including aromatic and halogenated molecules. They carry catabolic plasmids that encode enzymes involved in the degradation of aromatic structures such as atrazine (Kersters et al. 1996; Spiers et al. 2000). Several *Pseudomonas* strains have been related to atrazine degradation (Behki and Khan 1986; Yanze-Kontchou and Gschwind 1994; Mandelbaum et al. 1995; de Souza et al. 1998). Therefore, *Pseudomonas* spp. have the potential for the remediation of persistent organic pollutants in the environment. However, in many cases, the indigenous microbial population of soil has overgrown the microbe(s) used in bioaugmentation. The microorganisms isolated from environments similar to the contaminated sites have been the most stable in the remediation (Feakin et al. 1995; Newcombe and Crowley 1999; Shapir et al. 2000; Silva et al. 2004). The selective factors for microbial species in the environment should be better understood.

Despite of the persistence of s-triazine pesticides, their long-term degradation and concomitant selection of microbial community composition are insufficiently understood. Therefore, we studied atrazine and terbutryn dissipation (Talja et al. 2008) and the related selection of *P. fluorescens* group community composition in environmental samples from the boreal region (Finland), and compared the results to those from other regions. We did 599–625 day-long degradation experiments in mesocosms of surface soils and subsurface groundwater-monitoring pipe deposits. Sterilized counterparts of the soils and deposits were included, to separate microbial and chemical degradation. In addition, short-term pesticide dissipation was studied in subsurface drilling sediments. During the degradation experiments, a collection of microorganisms was isolated, and at the end of long experiments, a few strains were isolated during sterility checks of sterilized controls. The aim was to gain insight into differences in the microbial community compositions between pesticide-contaminated surface soil and subsurface deposit mesocosms in relation to the strains colonizing some of the sterilized controls. The dominating group of isolates consisted of 35 *P. fluorescens* group strains representing five species. The species composition was statistically related to the physico-chemical conditions of isolation mesocosms. The results obtained were then compared with the isolation environments of strains having the same partial 16S rDNA sequence in the gene library. The hypothesis of the study was that natural selection of *P. fluorescens* group species in the mesocosms/environments contaminated with pesticides atrazine and terbutryn can be related to physicochemical conditions.

Materials and methods

Soils, deposits, and sediments The surface soils from three gardens (depth 0–20 cm; drill diameter 3 cm), drilling sediments from 13.6 m (groundwater table 14.0 m; drill-diameter 4.8 cm), and deposits from four groundwater monitoring pipes and one well were collected in Lahti (60° 58′ 0″ N / 25° 40′ 0″ E, Finland), as presented by Talja et al. (2008). The dry weight of samples was measured after heating at 105 °C for 16 h (SFS-EN 13040). Then the organic matter was determined by heating at 550 °C for 4 h (SFS-EN 13039). Total carbon was analysed using LECO model 2000 CNS analyser (St. Joseph, MI, USA), according to the instructions of LECO. NH_4-N, NO_3-N and elements were analysed as a purchased service in Ramboll Analytics (Lahti, Finland), using methods that were accredited according to the guidelines of Finnish Accreditation Service T039 (SFS-EN ISO/IEC 17025; Talja et al. 2008).

Mesocosms In a 100 ml flask, the 15 g (dry weight) sample or sterilized counterpart was supplemented with atrazine (100 μg g^{-1}) and terbutryn (67 μg g^{-1}, not in drilling sediments), in triplicate (Talja et al. 2008). The sterilized soils/deposits (not drilling sediment due to the short incubation time) were autoclaved on three successive days (1 h; 121 °C, 101 kPa). The moisture of the soils was adjusted to 60 % of saturated, and the deposits/sediments were supplemented with 50 ml of water. Flasks with a hole (diameter 5 mm) in the screw cap were covered with aluminium foil, and shaken (120 rpm) at 21±2 °C; the groundwater deposits at 16 ±2 °C. Samplings were conducted aseptically in laboratory, flasks were handled in a laminar flow cabinet (Class II, NuAire, USA), and all the sampling supplies were sterile. Evaporated water was replaced with sterile water before the samplings. For the microbial isolations, samples of about 100 mg were collected from the surface soil mesocosms after 47 and 189 days. A volume of 100 μl was taken from the groundwater deposit slurry mesocosms after 176 and 409 days, and from the drilling sediment slurry mesocosms after 11 days. Samples from the sterilized counterparts of the soils and deposits were

taken after 599–625 days of incubation (Table 1). In the samplings, 100 mg or 100 μl samples were also collected to follow the dissipation of atrazine and terbutryn (Talja et al. 2008).

Isolation of *Pseudomonas* spp. and 16S rDNA sequencing To isolate *Pseudomonas* spp., 100 μl of the liquid was cultivated on mineral agar (atrazine, 33 mg l^{-1} or terbutryn, 20 mg l^{-1}) (Pukkila et al. 2009) from the deposit slurry, from tenfold dilution series of the drilling sediment slurry, and from 100 mg of the soil in 1.2 ml of water. The sterility of the autoclaved soils and deposits was checked after 599–625 days of incubation in the laboratory. The soils/deposits were touched with an inoculation loop, followed by plating on tryptone-yeast extract-glucose (TYG) agar (Suutari et al. 2002). The isolates of a few flasks were re-plated on mineral agar until they were pure cultures by microscopy (Olympus SZ40, Hatagaya, Japan). DNA was isolated after cultivation of the isolates on mineral agar (21±2 °C, 14 days), and16S rDNA was amplified and sequenced as presented by Pukkila et al. (2009). The sequences were compared to those in the EMBL database using the FASTA algorithm. The partial 16S rDNA sequences were aligned using ClustalW2. The phylogenetic analysis on the basis of 16S rDNA sequence alignment was performed with Phylogeny Inference Package PHYLIP 3.67. The evolutionary distances were calculated with the Jukes and Cantor (1969) algorithm of the DNADIST, and a phylogenetic tree was constructed with the neighbour joining method. Bootstrap analyses of 1000 replicates were conducted using the SEQBOOT program of the PHYLIP.

Fatty acid analysis The cellular fatty acid profiles of selected *Pseudomonas* spp. were determined in duplicate after cultivation on TYG agar at the temperature of 21±2 °C for 2 days. The cells were collected from the plates, and fatty acids were saponified, methylated, and extracted as methyl esters, as presented by Suutari et al. (1990). The fatty acid methyl esters were analysed by a Shimazu model GC-17A gas chromatograph (GC) (Duisburg, Germany) equipped with a mass-selective detector (model GCMC-QP5000), automatic sampler (model AOC-17, long), and column Zebron ZB-5 (30 m×0.25 mm×0.25 μm; Shimadzu). The GC conditions were as follows: splitless injection; carrier gas, helium (1 ml min^{-1}); injector temperature, 280 °C, detector temperature, 270 °C. The oven temperature was held at 50 °C for 1 min, followed by an increase of 30 °C min^{-1} to 160, and 5 °C min^{-1} to 270 °C. In the mass selective detector, the electron energy was 70 eV, emission current was 75 mA, and ion source temperature was 215 °C. The fatty acid methyl esters were identified by comparing the mass spectra and retention times to those of standards.

Calculations The average (± S.D.) of physicochemical conditions in the isolation sources of five *P. fluorescens* group species was calculated from these data, and is presented in Table 2. The physicochemical conditions of isolation mesocosms of strains, presented in Table 2 and by Talja et al. (2008), were compared by the principal component analysis (PCA), which transforms the set of observations into a set of linearly uncorrelated variables called principal components (SPSS Inc., Chicago, IL, USA). The values of physicochemical conditions were standardised before the PCA. The first principal component (PC1) accounts for the greatest percentage of variation in the data, while the PC2 accounts the second greatest percentage of variation, etc. Kruskal-Wallis test (K-W) followed by Mann–Whitney test (M-W) for pairwise comparisons were used to determine the statistical significances between the physicochemical conditions of isolation mesocosms (program SPSS for Windows).

Results and discussion

Characterization of *Pseudomonas* isolates Altogether, 35 *P. fluorescens* group strains were isolated from study mesocosms on mineral agar supplemented with atrazine or terbutryn as a nitrogen source (Table 1). The partial 16S rDNA sequences of strains were typical for the genus *Pseudomonas*. The similarity of the 16S rDNA sequences varied between 93.5 and 99.1 % within the overlapping region at nt 68–498 (*E. coli* numbering) (Gutell et al. 1985), and 35 strains represented five different species of the *P. fluorescens* group: *Pseudomonas veronii*, *Pseudomonas mandelii*, *Pseudomonas marginalis*, *Pseudomonas* IC038 and *Pseudomonas* sp. LAB-23 (Tables 1 and 3; Fig. 1).

The 16S rDNA sequences of the isolates included hypervariable regions 1 and 2 at nt 71–95 and 455–475, respectively, which are powerful in species identification (Moore et al. 1996; Anzai et al. 2000) (Table 4). It had been suggested that *P. fluorescens* group strains possess the characteristic sequence of 5′-TAG - - - AGA GAA GCT TGC TTC TCT TGA - - - G-3′ in the 16S rDNA between nt 70 and 100, with the exception of *Pseudomonas chlororaphis*, which has a two-nucleotide difference (Moore et al. 1996). According to the results of this study, *P. mandelii*-like strains also differ from other *P. fluorescens* group strains within this hypervariable region 1 (Table 4). *P. mandelii*-like strains have in this position the 16S rDNA sequence of 5′- CAG C- - ACG GGT ACT TGT ACC TGG TGG C - - G-3′. Interestingly, 16S rDNA sequences of *P. mandelii* and *Pseudomonas* LAB-23-like strains were identical outside the hypervariable region 1. It has been possible to distinguish the other *P. fluorescens* group strains from each other

Table 1 *Pseudomonas fluorescens* group strains were isolated from mesocosms of surface soils, and subsurface deposit or sediment slurries after incubation times presented. Sample numbers indicate the numbers of mesocosm flasks used in isolation of strains on mineral medium supplemented with atrazine (ATR) or terbutryn (TER) as a nitrogen source. Strains were identified by partial 16S rDNA sequencing between nt positions presented according to *E. coli* numbering (Gutell et al. 1985)

Strain	16S rDNA sequence	Accession number	Isolation time(d)	Sample number	Isolates from mesocosms of surface soils	Isolates from mesocosms of subsurface deposits/sediments
Pseudomonas veronii CIP 104663 T like strains (AF064460)						
ATR81	60–875	KM501427	47	20 (ATR)	Garden E	
TER41/3	56–887	KM501404	47	120 (TER)	Garden E	
TER45/1	60–882	KM501407	47	120 (TER)	Garden E	
ATR80	51–499	KM501420	189	105 (ATR)	Garden E	
ATR206	56–875	KM501408	606	7 (ATR)	Garden E (sterilized)	
TER146	62–548	KM501421	606	8 (TER)	Garden E (sterilized)	
ATR202	60–877	KM501406	606	3 (ATR)	Garden E (sterilized)	
ATR203	62–512	KM501422	606	4 (ATR)	Flowerbed G (sterilized)	
ATR120	48–920	KM501405	625	47 (ATR)		Groundwater well W (sterilized)
ATR18/2	296–875	KM501426	176	18 (ATR)		Groundwater monitoring pipe A
Pseudomonas mandelii CIP 105273^T like strains (AF058286)						
TER41/1	61–888	KM501397	47	120 (TER)	Garden F	
TER43/1	65–551	KM501417	189	120 (TER)	Garden F	
TER43/2	55–550	KM501416	189	120 (TER)	Garden F	
TER45/2	62–842	KM501393	47	120 (TER)	Garden F	
ATR14	56–874	KM501395	47	93 (ATR)	Flowerbed G	
ATR18	61–540	KM501419	47	93 (ATR)	Flowerbed G	
ATR59	60–770	KM501418	189	93 (ATR)	Flowerbed G	
TER35	52–508	KM501410	189	93 (TER)	Flowerbed G	
TER65	65–498	KM501411	189	93 (TER)	Flowerbed G	
ATR85[a]	60–770	KM501394	599	22 (ATR)	Flowerbed G (sterilized)	
TER106	60–659	KM501424	599	31 (TER)		Groundwater monitoring pipe B (sterilized)
Pseudomonas marginalis ATCC 10844^T like strains (AB021401)						
ATR13	54–882	KM501400	47	93 (ATR)	Flowerbed G	
ATR79	54–882	KM501399	189	105 (ATR)	Garden F	
Pseudomonas sp. IC038 like strains (U85869)						
TER17/5b	194–697	KM501409	409	17 (TER)		Groundwater monitoring pipe A
TER17/5c	38–548	KM501413	409	17 (TER)		Groundwater monitoring pipe A
TER161	54–513	KM501414	625	38 (TER)		Groundwater monitoring pipe C (sterilized)
TER175	60–887	KM501401	625	41 (TER)		Groundwater monitoring pipe D (sterilized)
ATR119/1	61–560	KM501415	625	47 (ATR)		Groundwater well W (sterilized)
ATR119/2	100–789	KM501425	625	47 (ATR)		Groundwater well W (sterilized)
ATR122	61–535	KM501412	625	48 (ATR)		Groundwater well W (sterilized)
Pseudomonas sp. LAB-23 like strains (AB051699)						
ATR41	53–507	KM501423	11	6 (ATR)		Drilling sediment H
ATR45	54–886	KM501402	11	1 (ATR)		Drilling sediment H
ATR51	61–883	KM501396	11	4 (ATR)		Drilling sediment H
ATR54	68–890	KM501403	11	7 (ATR)		Drilling sediment H
TER39/5[b]	37–887	KM501398	409	39 (TER)		Drilling sediment H

[a] Identical 16S rDNA sequence with *Pseudomonas* sp. 11/20CMC control (AY263482) (nt 68–498 same as AF058286)

[b] The sequence similarity of 99.645 % with *Pseudomonas* sp. EM0527 (FJ392836) (nt 68–498 same as AB051699)

on the basis of the hypervariable region 2 (Moore et al. 1996).

The fatty acid profiles of selected strains were typical for the genus *Pseudomonas* (Table 5). *Pseudomonas mandelii*-

Table 2 The physicochemical conditions in drilling sediment H, and in soils from garden E, garden F and flowerbed G (see Table 1), while those in deposits from well W, and pipes A, B, C and D are presented in Talja et al. (2008). The average physicochemical conditions (± S.D.) and ranges of variation in isolation mesocosms of *P. fluorescens* group strains. Letters a (*P. veronii*), b (*P. mandelii*), c (*P. marginalis*), d (*Pseudomonas* IC038) and e (*Pseudomonas* LAB-23) in superscripts indicate statistically significant differences in physicochemical conditions between isolation mesocosms (Kruskal-Wallis test, $p<0.05$; Mann-Whitney's test, $p<0.05$)

	Drilling sediment H	Garden E	Garden F	Flower-bed G	(a) *P. veronii*		(b) *P. mandelii*		(c) *P. marginalis*		(d) *Pseudomonas* IC038		(e) *Pseudomonas* LAB-23	
Number of isolates					Average ± S.D. 8 soils/2 slurries^{d,e}	Range	Average ± S.D. 10 soils/1 slurry^{d,e}	Range	Average ± S.D. 2 soils^{d,e}	Range	Average ± S.D. 7 slurries^{a,b,c}	Range	Average ± S.D. 5 slurries^{a,b,c}	Range
Organic matter (mg g^{-1})	4.3±0.2	97.6±1.9	31.6±4.9	110.2±2.5	75.4±35.4^{d,e}	5–110	92.1±26.1^{d,e}	25–110	96.0±19.8^{d,e}	82–110	10.6±3.8^{a,b,c,e}	5–14	5.8±3.4^{a,b,c,d}	4.3–12
Total carbon (mg g^{-1})	0.3±0.1	38.0±9.8	31.6±3.2	47.6±2.0	29.3±15.5^{d,e}	0.5–48	37.9±14.4^{d,e}	1.4–48	40.0±11.3^{d,e}	32–48	1.2±0.32^{a,b,c}	e 0.5–1.4	0.5±0.4^{a,b,c,d}	0.3–1.1
NH4-N (μg g^{-1})	<0.39	130.0±14.0	85.0±12.7	80.0±0.1	81.8±46.1^{d,e}	1.8–130	75.5±21.5^{d,e}	11–85	82.5±3.5^{d,e}	80–85	3.8±2.4^{a,b,c}	1.8–7	1.4±3.1^{a,b,c}	0–7
NO3-N (μg g^{-1})	<0.1	56.5±0.7	93.0±0.1	60.0±0.1	60.3±35.9^{d,e}	0–93	66.5±27.4^{d,e}	0–93	76.5±23.3^{d,e}	60–93	0^{a,b,c}	0	0^{a,b,c}	0
Mn (μg g^{-1})	96±24	475±7.1	455±7.1	325±4.9	392.5±121.6^{d,e}	165–475	365.0±75.1^{d,e}	245–455	390.0±91.9^{d,e}	325–445	190.7±38.1^{a,b,c}	165–275	131.8±80.1^{a,b,c}	96–275
Zn (μg g^{-1})	27±9	97±1.4	130±0.1	74.5±0.7	97.2±34.4^{d,e}	46–130	93.4±29.5^{d,e}	57–130	102.5±38.9^{d,e}	75–130	46.3±8.5^{a,b,c}	41–65	34.6±17.0^{a,b,c}	27–65
Co (μg g^{-1})	3±1	14.0±0.1	14.0±0.1	9.5±0.7	12.3±2.8^{d,e}	8–14	11.3±2.2^{d,e}	8–14	12.0±2.8	10–14	8.1±1.8^{a,b}	7–12	4.8±4.0^{a,b}	3–12
Cr (μg g^{-1})	12±4	51.0±0.1	47.0±0.1	33.0±0.1	41.5±12.4^{d,e}	21–51	38.2±7.0^{d,e}	33–47	40.0±9.9	33–47	22.9±9.0^{a,b}	16–41	17.8±13.0^{a,b}	12–41
Pb (μg g^{-1})	3±1	21.5±0.7	33.0±1.4	13.5±0.7	24.1±8.4^{d,e}	10–33	21.5±9.3^{e}	14–33	23.5±13.4^{e}	14–33	14.0±2.9^{a,e}	10–17	5.0±4.5^{a,b,c,d}	3–13
Ni (μg g^{-1})	6±2	26.5±0.7	23.0±0.1	16.0±0.1	22.2±4.5^{d,e}	12–27	18.7±3.4^{e}	16–23	19.5±4.9	5–20	16.3±2.9^{a}	12–21	9.0±6.7^{a,b}	6–21
Cu (μg g^{-1})	15±5	29.0±1.4	37.5±4.9	25.0±0.1	32.1±6.9	19–38	30.3±6.4	25–38	31.5±9.2	9–32	28.9±9.4	19–38	18.0±6.7	15–30
Fe (mg g^{-1})	12±3	28.5±0.7	3.0±0.1	22.5±0.7	16.8±12.7	3–29	14.7±9.8	3–23	13.0±14.1	3–23	23.6±8.1	15–31	15.0±6.7	12–27
Pesticide	Atr	Atr, Ter	Atr, Ter	Atr, Ter	Atr, Ter^{e}		Atr, Ter^{e}		Atr, Ter^{e}		Atr, Ter^{e}		Atr, Ter^{a,b,c,d}	
Temperature (°C)	12±3	21±2	21±2	21±2	20.0±2.1^{d}	16–21	20.5±2^{d}	16–21	21.0±2^{e}	21	16.0±2^{a,b,c,e}	16	20.0±2^{d}	16–21

Table 3 Homology and number of nucleotide differences in the 16S rDNA sequences (nt 68–498) of *Pseudomonas* species. Values on the lower left are percentage homology, and those on the upper right are the number of nucleotide differences

	P. veronii	*P. mandelii*	*P. marginalis*	*P.* sp. IC038	*P.* sp. LAB-23
P. veronii		19	4	14	4
P. mandelii	95.6		23	29	15
P. marginalis	99.1	94.7		14	8
P. sp. IC038	96.8	93.3	96.8		14
P. sp. LAB-23	99.1	96.5	98.1	96.8	

like strain TER41/1, *Pseudomonas marginalis*-like strains ATR13 and ATR79, and *Pseudomonas veronii*-like strains TER45/1 and ATR202 had significant percentages of iso- and anteiso-branched acids, occasionally detected in *Pseudomonas* spp. (Ratledge and Wilkinson 1988), including i-15:0, i-17:0, a-15:0 and a-17:0. All the strains with iso-branched and anteiso-branched fatty acids were isolated from garden soils, mainly from the garden F soil (Tables 1 and 5). The rest of strains had 16:0, 18:0, 16:1ω7c and/or 18:1ω7c among the major fatty acids. They are characteristic fatty acids of Gram-negative bacteria (Ratledge and Wilkinson 1988). In addition, the strains had low percentages of several other fatty acids (Table 5). Although the strains formed five clusters in the 16S rDNA phylogeny, this clustering was not seen in the fatty acid profiles, as has also been concluded, e.g., by Kuske et al. (1999). The differences in the fatty acids indicate variation within species.

Conditions in mesocosms selective for *Pseudomonas* spp. The *P. fluorescens* group strains were isolated from mesocosms of the surface soils and groundwater deposit slurries, and from their initially sterilized counterparts, some of which were colonized during the 599–625 days of incubation (Table 1). The autoclave sterilization for 1 h (121 °C, 101 kPa) on three successive days was powerful enough to destroy all vegetative forms of non-sporulating *Pseudomonas* strains. In addition, *Pseudomonas* strains were isolated from mesocosms of drilling sediment slurries. In the PCA of physicochemical conditions of isolation mesocosms, *Pseudomonas* strains isolated from the surface soil mesocosms were separated from those isolated from mesocosms of the groundwater deposit or drilling sediment slurries, independent of the initial sterilization (Table 1; Fig. 2). The statistical pairwise comparisons (M-W, $p>0.05$) confirmed that the physicochemical conditions in isolation mesocosms did not differ between the soils (15 isolates) / deposits (four isolates), and their initially sterilized counterparts followed by colonization (soils, five isolates; deposits, seven isolates). Therefore, *Pseudomonas* strains isolated from mesocosms of the surface soils, groundwater deposit slurries, and drilling sediment slurries are compared in the following text.

In the PCA, *Pseudomonas* isolates from the surface soil mesocosms were positioned to the right, and those from mesocosms of the subsurface groundwater deposit and drilling sediment slurries were positioned to the left along the PC1 axis, which explained as much as 65.7 % of the variance in conditions of mesocosms (Fig. 2). Altogether, 20 *P. fluorescens* group strains were isolated from the surface soil mesocosms. They consisted of eight *P. veronii*, ten *P. mandelii*, and two *P. marginalis*-like strains (Table 1; Fig. 2). The 15 isolates from mesocosms of the subsurface drilling sediment and groundwater deposit slurries were mainly *Pseudomonas* sp. IC038 (7 strains) and *Pseudomonas* sp. LAB-23 (five strains)-like strains. In exception, one *P. mandelii*-like strain, and two *P. veronii*-like strains were isolated from mesocosms of the groundwater deposit slurries. In conclusion, some of the initially sterilized mesocosms were mainly colonised by the same *Pseudomonas* species as their unsterilised counterparts, which indicates that the conditions of mesocosms were selective for *Pseudomonas* species.

Pseudomonas veronii, P. mandelii and *P. marginalis*-like isolates from the surface soil mesocosms had positive scores along the PC1 axis, and the loadings of organic matter, total-C, nitrogen compounds, elements and pesticides were positive along the PC1 axis (Fig. 2). *Pseudomonas* sp. IC038 and *Pseudomonas* sp. LAB-23-like isolates from mesocosms of the subsurface deposit/sediment slurries had negative scores along the PC1 axis, and the loading of water (soil/slurry) was negative. The variance analysis (K-W, $p<0.05$) confirmed that the distribution of *Pseudomonas* species between mesocosms of the surface soils and subsurface deposit/sediment slurries was associated with the concentrations of carbon (organic matter, total-C), nitrogen (NH_4-N, NO_3-N), elements (Mn, Zn), and water (soil/slurry) (Table 2). The sources of *P. veronii, P. mandelii* and *P. marginalis* like-strains, that is the surface soil mesocosms were rich in carbon, nitrogen, Mn, and Zn, while the water volume was low. The sources of *Pseudomonas* sp. IC038 and *Pseudomonas* sp. LAB-23-like strains, that is mesocosms of the subsurface deposit/sediment slurries had a low concentration of carbon, nitrogen, Mn and Zn, while water was present in excess. The elements Co, Cr, Pb and Ni could also affect the distribution of *Pseudomonas* strains between the surface and subsurface mesocosms, but not Cu and Fe (see statistical significances in Table 2). The Cu and Fe concentrations did not differ statistically significantly (K-W, $p>0.05$) between isolation mesocosms of

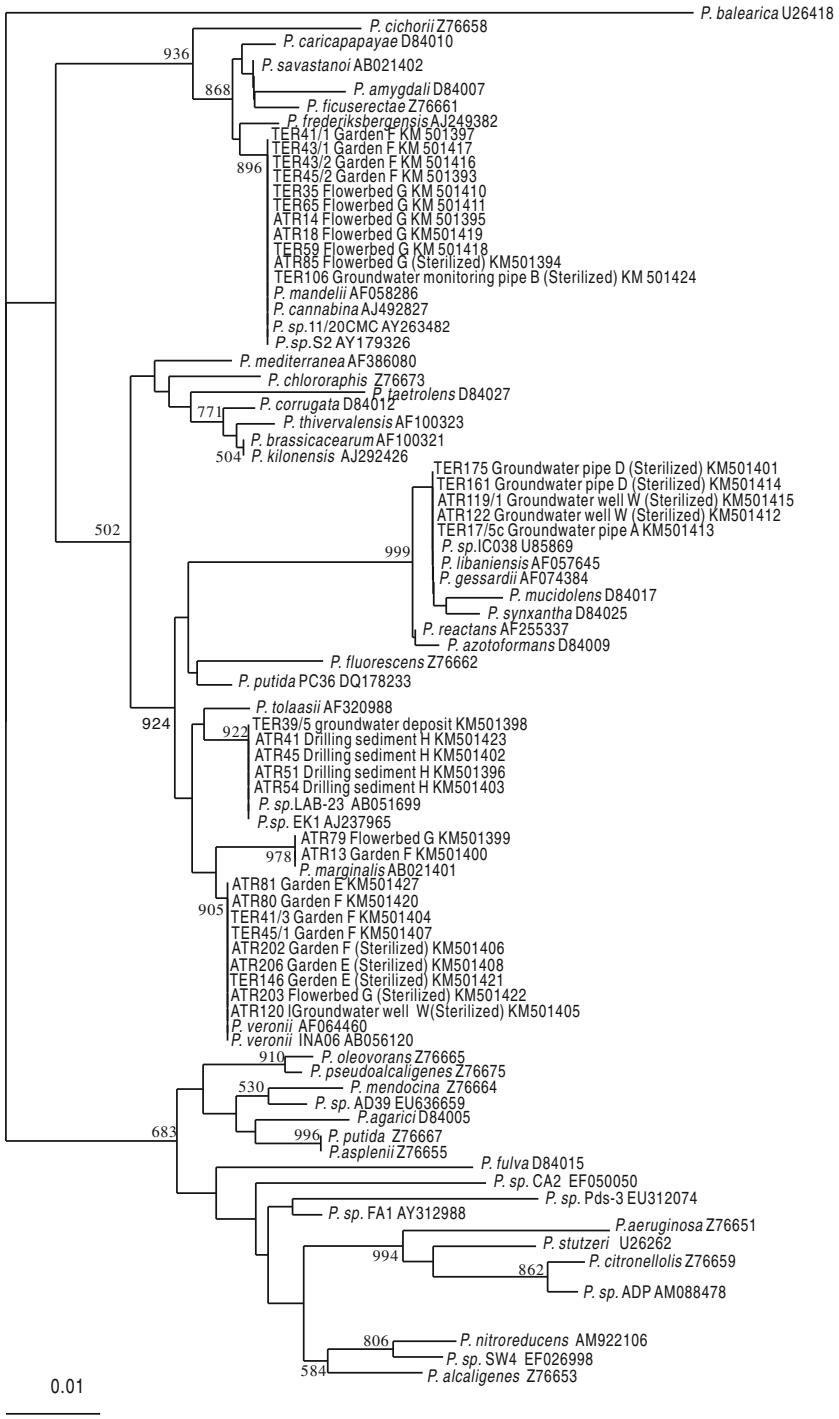

Fig. 1 The neighbour-joining phylogenetic tree derived from the 16S rDNA sequences between nt 68–498 of *Pseudomonas fluorescens* group isolates, and reference *Pseudomonas* strains with the sequence accession numbers presented. The numbers at the nodes indicate the levels of bootstrap support based on the analysis of 1000 resampled data sets. *Pseudomonas balearica* (U26418) is used as the root organism

P. fluorescens group strains. In conclusion, the concentration of organic matter, nitrogen, elements and water were statistically related to the selection of *Pseudomonas* isolates between the surface and subsurface mesocosms.

In the PCA, *Pseudomonas* sp. IC038-like strains from mesocosms of the deposit slurries, and *Pseudomonas* sp. LAB-23-like strains mainly from mesocosms of the drilling sediment slurries, were separated along the PC2 axis, which explained 16.1 % of the variance in results (Fig. 2). The pairwise comparisons (M-W, $p<0.05$) revealed that concentrations of organic matter, total-C, Pb and terbutryn in isolation mesocosms of *Pseudomonas* sp. IC038-like strains were greater than in those of *Pseudomonas* sp. LAB-23-like strains (Tables 1 and 2). In addition, *Pseudomonas* sp. IC038-like strains were isolated after a long, 409–625 day incubation at 16 °C, while *Pseudomonas* sp. LAB-23-like strains were mainly isolated after a short 11-day incubation at 21 °C. The changes in the bacterial community composition occurred as

Table 4 The 16S rDNA alignment of variable regions between nt 70–490 of *Pseudomonas fluorescens* group isolates and *Escherichia coli*

Closest neighbour / nt	70	155	166	199	216	253	258	273	456	470	490
P. veronii CIP 104663^T like isolates (AF064460)	T·G–AG·····C.TGA–·	TTC·	A	CA	·TG	T	A	A	CAGTT·CC	G·GATTGTT	G
P. mandelii CIP 105273^T like isolates (AF058286)	C·G··ACG·GTA····TAC·TGGTGGC–·	CTC·	A	CA	·TG	T	A	A	CAGTT·CC	G·A·CTGTT	G
P. marginalis ATCC 10844^T like isolates (AB021401)	T·G–AG·····C.TGA–·	TTC·	A	CA	·TG	T	·	A	CCATT·CC	G·GATGGTT	G
Pseudomonas sp. IC038 like isolates (U85869)	T·G–AG·····C·TGA–·	TTC·	A	CA	·TG	T	·	A	TTGT·GA·	TC·GCAATT	G
Pseudomonas sp. LAB-23 like isolates (AB051699)	T·G–AG·····C·TGA–·	CTC·	A	CA	·TG	T	A	A	CAGTT·CC	G·A·CTGTT	G
E. coli (J01695)	TAACAGGAAGAAGCTTGCTTCTT TGCTGACG	ACTG	T	AG	TCT	A	G	T	AGTAAAGT	CTTTGCTCA	C

soon as 14 days after colonization (Wertz et al. 2007). Therefore, *Pseudomonas* LAB-23-like strains could be original isolates from the deposits/sediments, while *Pseudomonas* IC038-like strains had adapted to the indoor incubation conditions of the mesocosms. The selection of *Pseudomonas* spp. between mesocosms of the subsurface groundwater deposit (*Pseudomonas* IC038-like strains) and drilling sediment (*Pseudomonas* LAB-23-like strains) slurries was also apparent, although it was not as clear as between the surface and subsurface mesocosms.

Isolation sources of *P. fluorescens* group strains The isolation mesocosms of isolates were compared to those of published strains, which were isolated by cultivation and have the same 16S rDNA sequences in the gene library (Tables 1 and 6). Based on the results presented above, the isolation sources were grouped as follows: water-related environments, environments rich in organic matter, sandy environments (low organic matter content), and polluted environments.

Pseudomonas veronii CIP104663^T-like strains were isolated from mesocosms of the garden surface soils (eight strains) and subsurface deposit slurries (two strains; Table 1). Similarly, *P. mandelii* CIP 105273^T-like strains were isolated from mesocosms of the garden surface soils (nine strains) and subsurface deposit slurry (one strain). According to the literature, *P. veronii* and *P. mandelii*-like strains were also isolated earlier from water environments; for example, from natural mineral water and lake sediments often low in organic matter (Elomari et al. 1996; Verhille et al. 1999; Francis and Tebo 2001) (Table 6). In addition, they have been isolated from garden and surface soils rich in organic matter, and from polluted environments (Yamamoto and Harayama 1998; Soares et al. 2003; Meyer et al. 2004; Hendrickx et al. 2006). Altogether, the strains with *P. veronii* and *P. mandelii*-like 16S rDNA sequences are living in a great variety of natural and contaminated environments varying a great deal in concentrations of carbon and nitrogen compounds, elements, and water.

Pseudomonas marginalis ATCC 10844^T-like strains ATR13 and ATR79 were isolated from the surface soil mesocosms (Table 1). Other strains with the 16S rDNA sequence of *P. marginalis* have also been isolated from sources rich in organic matter, like from plant and plant rhizosphere soil (Anzai et al. 2000; Ghyselinck et al. 2013) (Table 6). Interestingly, the reference strain is phytopathogenic, not saprophytic (Moore et al. 1996; Anzai et al. 2000). These findings indicate that *P. marginalis*-like strains are typical in sources rich in organic matter.

Seven *Pseudomonas* sp. IC038-like strains and five *Pseudomonas* LAB-23-like strains were isolated from mesocosms of subsurface deposit slurries (Table 1). The strains with the same 16S rDNA sequences were also isolated earlier from water and environments low in organic matter, like from Antarctic sea ice, lake water, sea water, paddy soil, trichloroethylene contaminated sandy aquifer sediment, and

Table 5 Fatty acid profiles of selected isolates (average ± standard deviation, n=2)

Fatty acid	*P. marginalis*		*P. mandelii*										*P. veronii*
	ATR13	ATR79	ATR18	TER41/1	TER43/1	TER43/2	TER45/2	TER35	ATR59	TER65	ATR85	TER106	TER41/3
12:0	tr	5.4±0.1	5.1±0.1	tr	12.2±8.1	8.7±0.8	6.3±0.1	2.3±0.1	12.2±0.1	8.2±5.2	5.6±0.1	tr	tr
i13:0	0.5±0.1	0	0	1.6±0.1	0	0	0	0	0	0	0	0	0
a13:0	0.8±0.2	0	0	tr	0	0	0	0	0	0	0	0	0
i14:0	9.3±1.0	1.5±0.1	0	0.7±0.1	0	0	0	0	0	0	0	0	tr
14:0	0.5±0.1	2.3±0.1	0.6±0.1	2.7±0.1	1.2±0.3	0.9±0.4	0.5±0.1	0.9±0.1	1.4±0.1	0.8±0.1	1.4±0.1	5.5±0.1	8.0±0.2
i15:0	10.2±0.1	3.6±0.1	tr	12.7±0.1	0	0	0	tr	0	tr	tr	tr	0,5±0.1
a15:0	17.8±3.4	18.7±0.2	tr	3.9±0.2	0	0	0	0.7±0.1	0	5.8±8.2	tr	tr	tr
15:0	tr	tr	0.6±0.1	0.6±0.1	0.6±0.1	0.5±0.1	1.0±0.1	0.6±0.2	0.7±0.1	1.0±0.3	0.9±0.1	tr	0,5±0.1
br16:1	6.5±0.1	0.8±0.1	0	tr	0	0	0	0	0	0	0	0	0
i16:0	21.2±0.9	1.2±0.1	tr	1.0±0.1	0	tr	0	tr	0	1.0±1.2	tr	tr	tr
16:1w7c	10.3±0.7	0.8±0.1	16.6±0.1	0.3±0.1	23.3±3.0	15.7±7.0	33.3±0.2	32.8±0.1	17.5±0.1	24.4±5.9	21.1±0.2	24.9±0.1	25.9±0.4
16:1	0	0	tr	2.8±0.1	0	tr	0	0	tr	0	2.5±0.2	tr	0.7±0.1
16:0	3.2±1.4	16.9±0.8	32.0±0.2	31.2±0.1	25.7±0.8	30.2±2.7	31.0±0.1	40.7±0.1	22.0±0.1	26.5±0.5	30.7±0.1	32.5±0.1	24.2±0.1
br17:1	tr	0	0	0	0.8±0.1	2.1±0.2	1.1±0.1	0	2.0±0.1	1.6±0.4	1.9±0.2	1.1±0.1	1.5±0.1
i17:1	5.2±0.6	0	0	1.1±0.1	0	0	0	0	0	0	0	0	0
a17:1	4.1±0.3	0	0	tr	0	0	0	0	0		0	0	0
i17:0	1.4±0.3	tr	0	2.8±0.1	0.5±0.4	0	0	tr	0	0	0	tr	tr
a17:0	3.5±0.7	2.6±0.2	0	1.1±0.1	tr	0	0	tr	0	0	0	tr	tr
cy17:0	0.7±0.1	0	2.2±0.2	0	2.4±2.5	1.2±0.2	0.8±0.1	0	0 1.4±0.1	1.0±0.4	1.3±0.1	0.7±0.1	0.8±0.1
17:1	1.2±0.1	0	2.8±0.1	0	1.7±0.4	6.4±0.2	tr	0	5.9±0.1	4.4±0.3	2.1±0.1	1.0±0.1	3.7±0.1
17:0	tr	tr	0.5±0.1	1.0±0.1	0.6±0.2	tr	tr	0.6±0.1	0.5±0.1	0.6±0.4	0.6±0.1	0.6±0.1	1.3±0.2
18:2w6c	0	3.7±0.2	0	0.5±0.1	0	0	0	0	1.0±0.1	0	tr	1.0±0.1	tr
18:2w9c	tr	5.1±0.1	0	1.5±0.1	1.0±0.3	1.0±0.3	0	0	3.0±0.1	0	1.9±0.1	1.0±0.1	tr
18:1w7c	tr	21.9±0.1	23.5±0.1	0.9±0.1	11.7±3.4	18.1±1.0	20.9±0.1	11.1±0.3	15.7±0.1	16.2±1.2	18.0±0.2	19.9±0.1	19.7±0.5
18:0	0.7±0.8	13.9±0.8	1.5±0.1	31.9±0.3	12.1±4.6	1.7±1.0	tr	8.4±0.2	2.6±0.1	2.3±2.1	2.0±0.1	2.3±0.1	1.5±0.1
br19:1a	tr	0	0.7±0.1	0	0.7±0.1	0.5±0.2	1.3±0.1	1.2±0.2	tr	tr	1.3±0.1	2.5±0.1	1.0±0.1
br19a	tr	0	1.7±0.1 0	0.8±0.6	3.2±2.3	tr	0	3.7±0.1	0.8±0.3	0.5±0.1	0.7±0.1	2.1±0.1	0
br19b	0.7±0.2	0	6.5±0.1	0	2.1±0.1	4.6±0.4	1.1±0.1	0	4.7±0.3	2.7±1.2	3.6±0.1	2.3±0.1	2.9±0.2
cy19:0	0	0	tr	0	tr	0.8±0.1	tr	0	tr	tr	1.1±0.1	0.5±0.1	1.7±0.1
19:1	tr	0	0.8±0.1	0	0.6±0.5	1.2±0.5	tr	0	1.7±0.2	0.7±0.1	0.6±0.1	tr	0.9±0.1
9:0	tr	0	tr	tr	tr	tr	0	0	tr	tr	tr	0	tr
br20:1	0.5±0.1	0	4.1±0.1	0	1.1±0.2	2.5±0.5	0.7±0.1	0	2.9±0.3	1.5±0.7	2.2±0.1	1.4±0.1	1.7±0.1
20:0	0	0.8±0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	0	tr

Fatty acid	*P. veronii*				*Pseudomonas*sp. IC038						*P.* LAB23
	TER45/1	TER146	ATR202	ATR203	TER17/5b	TER17/5c	ATR119/1	ATR119/2	ATR122	TER161	TER39.5
12:0	2.7±1.0	5.0±0.1	tr	2.4±0.1	2.7±0.	3.6±0.1	2.3±0.1	1.3±0.1	1.7±0.1	tr	0.3±0.1
i13:0	3.4±0.3	0	tr	0	0	0	0	0	0	0	0
a13:0	1.6±0.9	0	tr	0	0	0	0	0	0	0	0
i14:0	1.9±0.1	0	0.8±0.1	0	0	tr	0	0	0	0	0

Table 5 (continued)

Fatty acid	*P. veronii*				*Pseudomonas*sp. IC038						*P.* LAB23
	TER45/1	TER146	ATR202	ATR203	TER17/5b	TER17/5c	ATR119/1	ATR119/2	ATR122	TER161	TER39.5
14:0	4.2±0.1	2.4±0.1	1.3±0.2	0.5±0.1	0.8±0.1	1.7±0.1	2.2±0.1	0.7±0.1	1.1±0.1	7.2±0.1	5.5±0.1
i15:0	15.4±0.1	tr	15.7±2.0	0	0	tr	tr	0	tr	tr	0
a15:0	5.5±0.6	tr	55.3±5.7	0	0	tr	tr	0	tr	0	0
15:0	6.6±0.9	tr	tr	tr	1.3±0.2	tr	1.0±0.1	tr	tr	tr	2.9±0.1
br16:1	0	0	1.2±0.2	0	0	0	0	0	0	0	0
i16:0	1.9±0.7	tr	3.7±0.5	0	0	tr	tr	0	tr	tr	0
16:1w7c	5.8±2.5	0.8±0.1	3.3±0.4	33.9±0.1	11.5±0.3	25.5±0.1	6.7±0.1	32.6±0.3	24.9±0.1	29.5±0.2	18.7±0.1
16:1	0	0.3±0.1	0	0	0.7±0.1	tr	tr	3.8±0.1	0	0	0
16:0	20.2±2.7	24.0±0.1	1.8±0.2	39.9±0.5	33.8±0.2	31.9±0.4	32.8±0.1	37.8±0.1	29.6±0.4	31.8±0.3	31.3±0.1
br17:1	0	0	tr	0.9±0.1	1.4±0.1	0.5±0.2	0.8±0.2	0.6±0.1	0.8±0.1	1.3±0.4	0.9±0.1
i17:1	0	0	1.3±0.2	0	0	0	0	0	0	0	0
a17:1	0	6.6±0.8	0	0	0	0	0	0	0	0	
i17:0	4.9±0.5	tr	0.9±0.1	0	0	tr	tr	0	tr	0	0
a17:0	1.7±0.3	tr	6.6±0.8	0	0	tr	tr	0	tr	0	0
cy17:0	0	0	tr	tr	1.9±0.1	tr	tr	tr	tr	1.1±0.1	2.9±0.1
17:1	0	tr	tr	tr	2.9±0.1	0.9±0.1	4.5±0.1	tr	1.4±0.1	2.1±0.1	0.5±0.1
17:0	0.5±0.1	tr	tr	tr	1.2±0.1	tr	1.3±0.1	tr	0.6±0.1	0.6±0.1	0.8±0.1
18:2w6c	0	3.4±0.1	0	0	0	3.3±0.1	2.0±0.1	0	0.6±0.1	0	0
18:2w9c	0.9±0.3	4.7±0.1	tr	0	0.8±0.1	4.4±0.1	2.7±0.1	0	1.2±0.1	0	0
18:1w7c	5.0±0.3	37.7±0.1	tr	16.0±0.7	21.1±0.3	15.9±0.2	11.8±0.2	18.3±0.2	21.8±0.1	19.1±0.2	23.5±0.7
18:0	17.0±0.3	14.7±0.1	tr	0.8±0.1	5.7±0.1	7.6±0.1	17.7±0.1	1.0±0.1	11.9±0.1	1.3±0.1	1.9±0.1
br19:1a	0	3.7±0.1	tr	0.9±0.1	1.5±0.3	0.7±0.1	0.7±0.1	1.0±0.1	0.7±0.1	0.8±0.1	0.7±0.1
br19a	0	0	tr	1.3±0.1	0	0	2.0±0.1	0	tr	0	9.6±0.2
br19b	0	0	tr	1.3±0.1	5.3±0.4	1.1±0.1	3.8±0.1	0.8±0.1	1.2±0.1	2.0±0.1	0.5±0.1
cy19:0	0	tr	tr	tr	1.7±0.1	tr	1.8±0.1	tr	tr	1.0±0.1 0	0
19:1	0	0	0	tr	1.1±0.1	tr	1.0±0.2	tr	tr	0	0
9:0	0.7±0.5	tr	0	0	tr	tr	tr	0	tr	tr	0
br20:1	0	0	0	0.9±0.1	3.3±0.1	0.5±0.1	2.1±0.1	0.5±0.1	0.6±0.1	1.3±0.1	tr
20:0	0	1.6±0.1	0	0	tr	tr	tr	0	tr	0	tr

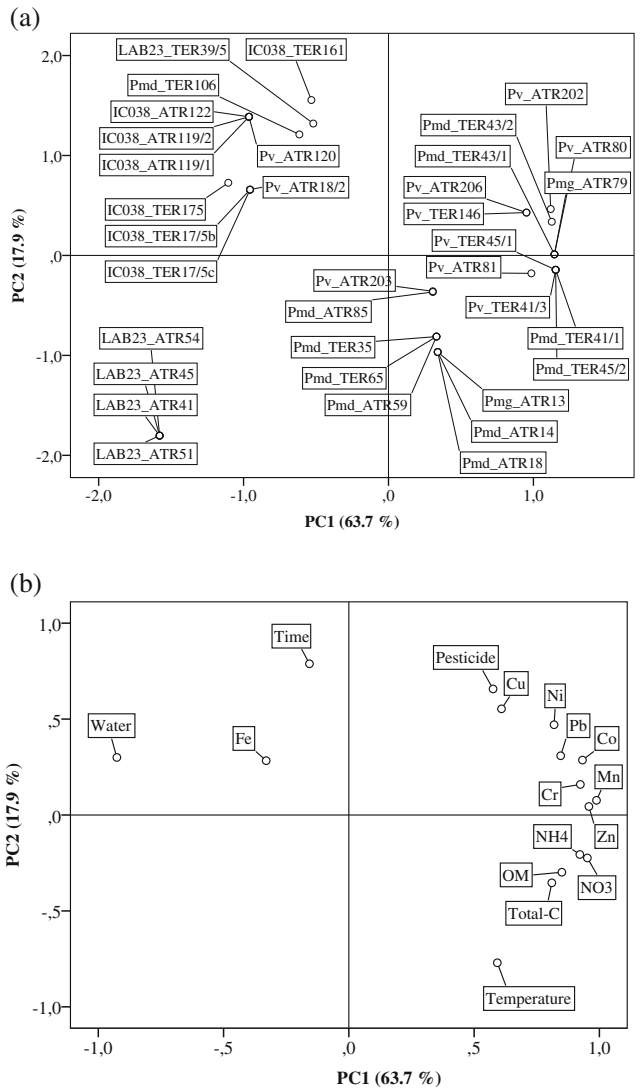

Fig. 2 **a** Score plot of PCA showing separation of isolates from surface soil mesocosms to the right, and isolates from subsurface deposit (*up*) and sediment (*down*) slurry mesocosms to the left along PC1 and PC2 axes (Pv, *P. veronii*; Pmd, *P. mandelii*, Pmg, *P. marginalis*; LAB23, *Pseudomonas* sp. LAB-23; IC038, *Pseudomonas* sp. IC038; Strain names as in Table 1). **b** Loading values for physicochemical conditions of isolation environments along PC1 and PC2 axes

heavy metal contaminated soil (Gorlach et al. 1994; Bowman et al. 1997; Futamata et al. 2001; Voolaid et al. 2013; Prasad et al. 2014) (Table 6). Therefore, *Pseudomonas* sp. IC038 and *Pseudomonas* LAB-23-like strains appear to be common in water, sandy and polluted environment.

Pseudomonas fluorescens **group strains in environment** The results showed that *P. veronii* and *P. mandelii*-like strains belong to the major cultivable microbial population in a great variety of environments, including water, garden soil, subsurface sediments and polluted environment (Tables 1 and 6). *P. marginalis*-like strains were common in environments rich in organic matter, and in this study, they were detected under pesticide contamination. *Pseudomonas* sp. IC038-like strains and *Pseudomonas* sp. LAB-23-like strains were typically isolated from water, sandy and contaminated environment. As a part of the indigenous major microbial community in these environments specified, the *Pseudomonas* species of this study could be appropriate for the remediation of contaminated sites. Alternatively, they could overgrow the added remediating microorganisms, which generally has been a problem in bioaugmentation. Microorganisms isolated from environments similar to the contaminated sites have survived best in remediation (Newcombe and Crowley 1999; Feakin et al. 1995). Therefore, microorganisms used in remediation should maintain stability and functionality, i.e., the genes for the degradation of xenobiotics, in the presence of *P. fluorescens* group strains found in this study to be common in environments specified, even though remediating microbes would not belong to the major cultivable strains.

The selection of *P. fluorescens* group strains was clearly driven by the physicochemical conditions of mesocosms. The sterilized mesocosms of the surface soils and subsurface deposit slurries were mainly colonized during the long incubation by the same species as their non-sterilized counterparts (Table 1). Generally, the microbial population in laboratory could have developed indoors, or originate from the outdoor environment, due to the easy passage of microorganisms between outdoor and indoor habitats (Lee et al. 2007; Tsai and Macher 2005). Several species of the *P. fluorescens* group have been reported as airborne bacteria from the indoor environment in central and eastern European countries (Górny and Dutkiewicz 2002). Thus, indoor species of *Pseudomonas* likely were the origin of our isolates in sterilized mesocosms. The overlap in species between the surface soil mesocosms and the subsurface deposit/sediment mesocosms was minor. Similarly, the viability of *P. fluorescens* has been restricted in the transport toward groundwater (Langenbach et al. 2006). According to the statistical analysis, the selective pressure between *Pseudomonas* strains from surface and subsurface environments was caused by carbon and nitrogen compounds, and elements (Table 2). The strong influence of soil characteristics on *Pseudomonas* spp. abundances has also been observed previously (Kuske et al. 1999; Langenbach et al. 2006). Generally, elements are essential in low amounts, affect ionic strength, and form complexes with nitrogen compounds and organic matter (Bååth 1989; Bradl 2004; Ros et al. 2009). The sorption to organic matter and water solubility affect bioavailability of nutrients, and all these factors are related to the development of microbial community.

Especially *P. veronii* and *P. mandelii*-like strains had the ability to live in a wide variety of environmental conditions,

Table 6 The 16S rDNA sequences in database identical to those of *Pseudomonas fluorescens*group strains of this study. Isolation sources of strains, and related references

Accession number	Isolation source	Reference
*P. veronii*CIP 104663^{T}like strains ATR81, TER41/3, TER45/1, ATR80, ATR206, TER146, ATR202, ATR203, ATR120, ATR18/2		
AF064460 (*P. veronii*CIP 104663^{T}or CFML 92–134^T)	Natural mineral water, France	Elomari et al. 1996
D86003	Garden soil, United Kingdom	Yamamoto and Harayama 1998
AY51261520, AY512643, AY364085	Benzene and derivatives contaminated subsurface soil, Czech Republic	Hendrickx et al. 2006
AY179328	Nonylphenol contaminated soil, Sweden	Soares et al. 2003
*P. mandelii*CIP 105273^{T}like strains TER41/1, TER45/2, ATR14, ATR18, TER43/1, TER43/2, TER35, TER65, ATR59, TER106		
AF058286 (*P. mandelii*CIP 105273^T)	Natural mineral water, France	Verhille et al. 1999
AF326374	Lake sediments, USA	Francis and Tebo 2001
AY179326	Nonylphenol contaminated soil, Sweden	Soares et al. 2003
*Pseudomonas*sp. 11/20CMC control like strain ATR85a		
AY263482 (*Pseudomonas*sp. 11/20CMC)	Alpine soil, USA	Meyer et al. 2004
*P. marginalis*ATCC 10844^{T}like strains ATR13, ATR79		
AB021401 (*P. marginalis*ATCC 10844 T)	Plant, USA	Anzai et al. 2000
HE603508	Potato rhizosphere soil, Andean	Ghyselinck et al. 2013
*Pseudomonas*sp. IC038 like strains TER17/5b, TER17/5c, TER119/1, TER119/2, ATR122, TER161, TER175		
U85869 (*Pseudomonas*sp. IC038)	Antarctic sea ice	Bowman et al. 1997
KC195897	Heavy metal contaminated soil, France	Colinon et al. 2013
*Pseudomonas*sp. LAB23 like strains ATR41, ATR45, ATR51, ATR54, TER39/5[b]		
AB051699 (*Pseudomonas*sp. LAB23)	Trichloroethylene contaminated sediment, Japan	Futamata et al. 2001
AB649011, D84568	Paddy soil, Japan	Gorlach et al. 1994
JX899629	Lake Vortsjaerv water, Estonia	Voolaid et al. 2013
JQ800176	Sea water, Kongsfjorden, Norway	Prasad et al. 2014

[a] Identical 16S rDNA sequence with *Pseudomonas*sp. 11/20CMC control (AY263482) (nt 68–498 same as AF058286)

[b] The sequence similarity of 99.645 % with *Pseudomonas*sp. EM0527 (FJ392836) (nt 68–498 same as AB051699)

while *P. marginalis*, *Pseudomonas* LAB-23 and *Pseudomonas* IC038-like strains appeared to have narrower substrate specificity. The key question is to what extent individual strains inside *P. veronii* or *P mandelii* vary even though they have the same 16S rDNA sequence, or whether one strain has the genetic capacity to adapt to the wide variety of environments. On the basis of the fatty acid analyses of this study, the variation occurs especially in strains inside *P. veronii, P. mandelii* and *P. marginalis* (Table 5). The concentrations of iso-branched and anteiso-branched fatty acids (i-15:0, i-16:0, i-17:0, i-17:1, a-15:0, a-17:0, and/or a-17:1) in lipids of *P. veronii*-like strains TER45/1 and ATR202, *P. mandelii*-like strain TER41/1 and *P. marginalis*-like strain ATR13 were significantly greater than in other strains inside these species. The biosynthesis of fatty acids bound to cellular membranes is under strict control, even though bacteria can to some extent shift between fatty acids used in the regulation of membrane fluidity (Suutari and Laakso 1994). The differences in fatty acid profiles of strains within the five species could be related to differences in the ability to adapt different environments.

According to the genome sequencing of three species, as much as 54 % of the *P. fluorescens* genome has been heterogenic pangenome, while the core genome represents only 45–51 %. The genetic variation between *P. fluorescens* strains has been so great that they could constitute different species. However, different *P. aeruginosa* strains have been closely related (Loper et al. 2012; Silby et al. 2009; Silby et al. 2011). Although *P. veronii* and *P. mandelii*-like stains seemed to adapt to a wide variety of environments, a single isolate may have a narrower ability to adapt to different environments. There is a risk that a strain isolated from surface soil is not able to survive in subsurface environment, and vice versa. The species identification in this study was based on the 16S rDNA sequencing. The other genes, like *atp*D, *car*A, *cum*A, *gyr*B, *opr*I, *rec*A, *rpo*B and *rpo*D have been proposed

to provide a resolution higher than that based on 16S rDNA sequencing (De Vos et al. 1998; Francis and Tebo 2001; Yamamoto et al. 2000; Hilario et al. 2004; Tayeb et al. 2005; Mulet et al. 2010).

Conclusions

Pseudomonas fluorescens group isolates consisted of 35 strains from five species. Based on the statistical analysis, concentrations of carbon (organic matter, total-C) and nitrogen (NH_4-N, NO_3-N) compounds, elements (Mn, Zn, Co, Cr, Ni, Pb) and water were associated with the almost complete differentiation of *Pseudomonas* strains between surface and subsurface mesocosms. *P. veronii* and *P. mandelii*-like strains were colonizing mesocosms of surface soils and subsurface deposit slurries that varied greatly in the concentration of carbon, nitrogen, elements, and water. These strains either had a versatile metabolism to adapt to various environments, or differed in genetic capacity for substrate utilization, despite of the same 16S rDNA sequences. The latter possibility was supported by differences in cellular fatty acid profiles. *P. marginalis*-like strains were living in surface soil mesocosms rich in organic matter. *Pseudomonas* IC038 and *Pseudomonas* LAB-23-like strains were colonizing mesocosms of subsurface deposit/sediment slurries. In the isolation mesocosms of *Pseudomonas* IC038-like strains, the concentrations of organic matter and Pb were higher and the incubation time was longer than in the isolation sources of *Pseudomonas* LAB-23-like strains. A few of the initially sterilized mesocosms were colonized after 599–625 days of incubation by the same strains as their non-sterilized counterparts, indicating strong species selection, although all strains tolerated atrazine and/or terbutryn. The habitats found in this study for *P. fluorescens* group strains were in agreement with those reported in the literature for strains having the identical 16S rDNA sequence. In conclusion, to avoid the overgrowth of remediating *Pseudomonas* strains by indigenous microorganisms, species selection should be done carefully for environmental applications, such as the remediation of contaminated sites.

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