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Microbial diversity in contaminated soils along the T22 trench of the Chernobyl experimental platform

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ABSTRACT

The diversity of bacterial communities exposed to radioactive contamination in Chernobyl soils was examined by a combination of molecular and culture-based approaches. A set of six radioactive soil samples, exhibiting high levels of ^{137}Cs contamination, were collected from the T22 trench. Three samples were also collected in nearby soils with low contamination. Complex bacterial community structures were observed in both highly and weakly contaminated samples, using a molecular approach targeting the 16S rRNA gene. However, the presence of specific populations within samples from highly contaminated soils could not be revealed by statistical analysis of the DGGE profiles. More than 200 culturable isolates, representative of dominant morphotypes, were grouped into 83 Operational Taxonomic Units (OTUs) and affiliated to *Firmicutes*, *Actinobacteria*, *Alpha-*, *Beta-*, *Gamma-Proteobacteria* and *Bacteroïdetes*. No specific pattern linked to contamination was observed for these culturable bacteria. The results show that both highly and weakly contaminated soils host a wide diversity of bacteria, suggesting that long term exposure to radionuclides does not lead to the extinction of bacterial diversity.

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1. Introduction

Shortly after the Chernobyl accident in April 1986, radioactively contaminated soil and other debris (including vegetation from the Red Forest) were buried in several trenches in an area approximately 2.5 km from the Chernobyl Nuclear Power Plant (CNPP) and then covered with a layer of fine sand. In this area, trench no. 22 (T22) has been a pilot site for the study of radionuclide (RN) migration behavior since 1999 (Bugai et al., 2005). Different RN are present in this trench, including ^{137}Cs, ^{90}Sr, ^{60}Co, ^{154}Eu, $^{238, 239, 240}$Pu, ^{241}Am and ^{235,238}U.

Although the consequences of the accident, which include chronic radiation exposure to eukaryotic organisms, have been extensively studied (Kovalchuk et al., 2000; Møller and Mousseau, 2006; Dighton et al., 2008; Yablokov et al., 2009), very few studies have examined the effect of RN contamination on bacterial communities inhabiting Chernobyl soils (Yablokov, 2009). In one such study, Romanovskaya et al. (1998) worked with soil surface samples collected within a 10 km zone of the CNPP. Their results showed that radioactive contamination still had a negative impact on the abundance of N-fixing, cellulolytic, heterotrophic Fe-oxidizing, nitrifying and SO_4-reducing culturable bacteria, 7–9 years after the accident. They also reported that spore-formers were retrieved from all contaminated soil samples. They concluded that chronic radiation exerts selective pressure on bacteria, altering both their abundance and diversity. Some other data have been obtained on bacterial isolates retrieved from the Chernobyl soils. Zavilgesky et al. (1998) showed that several Chernobyl isolates related to *Bacillus* were resistant to DNA-damaging conditions (X-rays, UV-radiation, 4-nitroquinoline 1-oxide). In addition, resistance to UV- and γ-radiation, desiccation, and hydrogen peroxide has been documented for *Methylobacterium* and *Bacillus* strains. However, this resistance phenotype was observed irrespective of the zone from which the bacteria were isolated (either around or outside

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the CNPP zone) (Romanovskaya et al., 1998, 2002). These data suggest that survival of bacteria in the RN-contaminated soils of Chernobyl relies on effective DNA repair mechanisms.

The aim of this work was to analyze the diversity of bacterial communities which have been evolving in the T22 Chernobyl environment over the past 25 years, by a combination of molecular and culture-based approaches. Furthermore, the potential impact of long-term radioactive contamination on bacterial communities has been assessed through a comparative study of soil samples with high and low RN contamination.

2. Materials and methods

2.1. Experimental site and sample collection

Trench T22 has previously been described in detail by Bugai et al. (2005). Briefly, the site is located 2.5 km from the CNPP, and contains clean-up waste from the Red Forest and other radioactively contaminated debris dating since 1987. Nine samples from sandy soils (numbered 1, 3, 4, 8, 10, 12, 13, 14 and 20) were collected in and outside the trench with a soil core sampler at a depth of 50–60 cm during three successive campaigns in October 2008, April 2009, and October 2009 (exceptionally, sample 20 was only sampled in 2009; see Fig. 1). The internal part of the core was removed with a sterile spatula and stored in sterile tubes for subsequent DNA extraction, bacteria isolation, and measurements of pH, water content, ^{137}Cs and organic C.

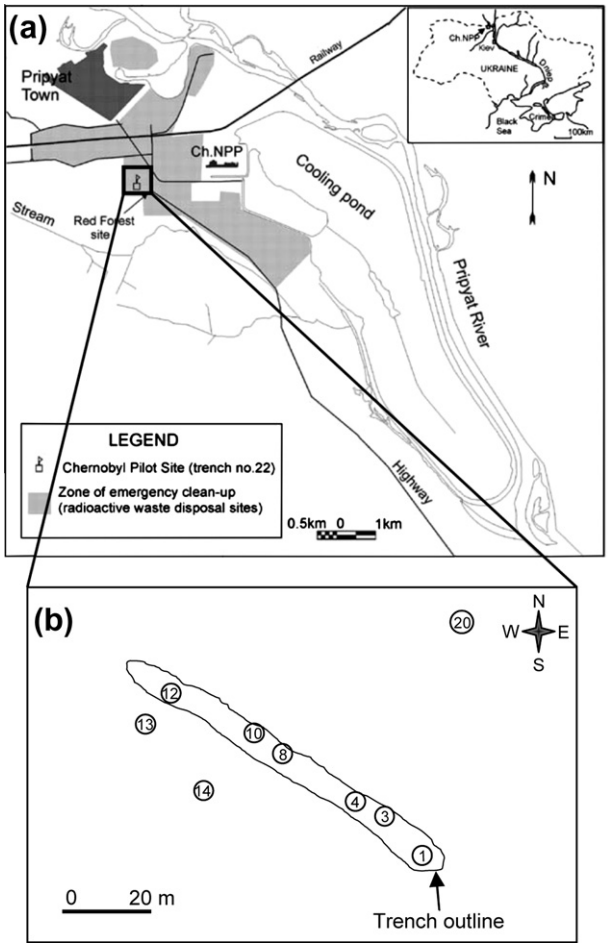

Fig. 1. General map of the Chernobyl trench 22 (a) (Bugai et al., 2005) and a focus on the location of the sampling points (b).

2.2. pH, water content, organic carbon and RN measurements

The pH was measured on a 1:5 soil:water suspension. Two g soil samples were combined in a polypropylene tube with 10 mL of 18 MΩ water. Suspensions were shaken for 30 min, and after decantation and 2 h atmosphere equilibration, the pH of the supernatant was measured. To determine water content (%), a 10 g soil sample was subjected to oven-drying at 105 °C until the weight remained constant, and the change in weight was calculated. Soil NaOH-extractable organic C ($C_{org(NaOH)}$) was measured, based on the International Humic Substances Society (IHSS) protocol (as described in Coppin et al., 2006). 0.15 g of soil sample was mixed with 1.5 mL of NaOH (0.1 mol L^{-1}) and shaken overnight. After centrifugation (10 min at 10,000*g*) the solution was removed and three appropriate dilutions were made to measure the absorbance at 280 nm (A_{280}) (BioPhotometer®, Eppendorf). The A_{280}/total organic C (mg L^{-1}) calibration was made with humic acid solutions of known concentrations (3–60 mg L^{-1}).

The ^{137}Cs specific activity in the samples was measured under laboratory conditions, using a gamma-spectrometer ADCAM-300 (ORTEC, USA) equipped with a high purity germanium GEM-30185 detector. For each sample, 50 g of soil were measured for 1 h.

The U content and U isotopic ratio (^{235}U/^{238}U) of soil samples were measured by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, Agilent 7500 Cx) after a dry "ashing" procedure, followed by a mineralization step. Ten g of oven-dried soil samples (105 °C) were placed in a muffle furnace (550 °C) for 20 h to destroy the combustible organic portion of the sample by thermal decomposition. After cooling in a desiccator, 100 mL of concentrated HNO_3 (8 mol L^{-1}) were added and heated to near-boiling temperature, while mixing for 1 h. The supernatant was then filtered and analyzed by ICP-MS after a 1:20 dilution to obtain a 2% HNO_3 solution. Radiofrequency power was set to 1600 W, and samples were introduced using a CETAC ASX 500 autosampler with a peristaltic pump (flow rate 1 mL min^{-1}). Signal optimization and mass calibration were performed using a 1 μg L^{-1} multielemental reference solution (Analab, in 2% HNO3). Signal acquisition was performed in continuous signal mode. Bismuth and Thallium were used as internal standards at 1 μg L^{-1} for each sample. Determination of U was performed by external calibration on each U isotope. The limit of quantification (LOQ) was 2–2.5 ng ^{238}U L^{-1}.

2.3. DNA extraction and PCR-DGGE analysis

The soil samples were processed at Chernobyl within 18 h of collection. DNA was extracted with the PowerSoil™ DNA Isolation Kit (MO Bio, USA) in several replicates (two from October 2008, one from April 2009, and three from October 2009; 1 g of soil per extraction). For DGGE analysis, total DNA extracted from soil samples was used as a template for the PCR amplification of a fragment of the 16S rRNA gene, using appropriate primers. For *Bacteria* community analysis, the universal primers set P1-GC and COM2 was used (Table S1, Supplementary material). A nested approach was used for group analysis: 16S rRNA genes were first amplified using specific primers for *Alphaproteobacteria*, *Betaproteobacteria*, *Gamma-Proteobacteria*, *Actinobacteria* and *Firmicutes* and re-amplified to generate DGGE-compatible fragments. Experimental conditions for PCR reactions and DGGE are detailed in the Supplementary material. DGGE profiles were analyzed by the Genetools software (SYNGENE, England) to obtain densitometric curves and subsequently similarity indices using the Bray–Curtis distance measure. Dendrograms were created using the unweighted pair group method with arithmetic averages (UPGMA) at 1% tolerance.

2.4. Isolation of culturable bacteria and subsequent 16s rRNA gene sequencing

Samples were processed within 18 h of collection, and culturable bacteria were recovered by two distinct methods to improve the yield. Thus for one method, soil (1 g) was resuspended in 1 mL of 0.1× tryptic soy broth (TSB, Difco Laboratories), and homogenized for 5 min under strong agitation. Dilutions of the resulting supernatant were spread on 0.1× TSB-agar plates. For the second method, an aerobic medium (AEM1) for aerobic organotrophic microorganisms, was prepared according to the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) recommendations (http://www.dsmz.de/). After 1 h agitation of soil in contact with physiological water 1/5 (v/v), the dilutions were spread on agar plates. To isolate sporulating bacteria, the soil suspension was then incubated for 10 min at 80 °C and dilutions were spread on agar plates. Plates were incubated at room temperature for 2 days (in Chernobyl), then at 30 °C in the laboratory. One isolate representative of each dominant morphotype was selected.

For the 229 isolates recovered from 0.1 × TSB agar plates, a 1500 bp fragment of the 16S rRNA gene was amplified with the universal primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and S17 (5′-GTTACCTTGTTACGACTT-3′) and partially sequenced using the fD1 primer. Alternatively, for the 74 isolates cultured on AEM1 plates, the universal primers fD1 and 1492r (5′-TACGGYTACCTTGT TACGACTT-3′) were used and the PCR product was partially sequenced with the primer 27f (5′-AGAGTTTGATCMTGGCTCAG-3′).

2.5. Phylogenetic analyses of 16S rRNA gene sequences

Each sequence (about 650 bp) was compared to reference sequences of the Silva ribosomal RNA database (version 102, released in February 2010) by BLAST, from which the five most similar sequences were retrieved. These sequences and the clone sequences were then aligned using Muscle (Edgar, 2004), followed by a final manual check using SeaView (Gouy et al., 2010). For each phylogenetic analysis, distances were calculated using the Kimura 2-parameters distance correction as implemented in SeaView. Only domains that were present in each sequence of the data set were used; positions with obvious homoplasy or that were difficult to align were excluded. Trees were computed using BIONJ (Gascuel, 1997) as implemented in SeaView, and were used for taxonomic assignation of the new sequences.

For OTUs (operational taxonomic units), uclust 3.0.617 was used to cluster sequences as indicated in the uclust manual and using a distance of 97%. The representative (seed) sequence of each cluster was used to build the final tree.

2.6. Statistical analyses

The dependence between ^{137}Cs and $C_{org(NaOH)}$ contents was assessed using a linear mixed model. The dependant variable was "^{137}Cs concentration", whereas "$C_{org(NaOH)}$ concentration" and "time" were the explanatory variables. The fixed effect variable was "concentration", whereas "time" was a random effect variable. The effect of the contamination (i.e. ^{137}Cs content) on the number of OTU and CFU was assessed by Poisson Generalized Linear Mixed Models (Poisson GLMMs). Regarding the number of CFU, the initial model used the "condition" variable (contaminated/control) as a fixed effect, and "sampling site" and "time" (2009/2008) as random effect variables. As for the number of OTU, the initial model contained three fixed effect variables: the "phylum" (*Bacteroidetes*/*Actinobacteria*/*Proteobacteria*/*Firmicutes*), the "condition" (contaminated/control) and the interaction "phylum × condition", and one random effect variable: the sampling site.

In all cases, the significance of the fixed effect was assessed with a nested modelling approach, where significance level (α risk) was fixed at 5%. Statistical analyses were calculated with R software (R Development Core Team, 2009). GLMM was performed with the "lme4" library (Bates and Maechler, 2009), and linear mixed models used the "nlme" library (Pinheiro et al., 2009).

3. Results and discussion

3.1. Sampling and physicochemical analysis of soils

Bugai et al. (2005) have previously characterized the distribution of radioactivity in trench 22. Based on their report, soil samples with high RN contamination were collected all along trench 22, at positions indicated in Fig. 1. A set of weakly contaminated control samples was also collected outside of the trench.

Various parameters that could influence bacterial diversity were then measured in these samples (Table 1). The pH values ranged from 4.4 to 6.1 and soil water content ranged from 2.2% to 7.3%. The NaOH extractable organic C content ($C_{org(NaOH)}$) (measured only in 2009) was within the same range for all samples (average = 2.25 mg g^{-1}), except for samples 4, 13, and 14 which were lower in content (0.1–0.88 mg g^{-1}).

As expected, high ^{137}Cs content was observed in the six samples collected from the trench (1, 3, 4, 8, 10 and 12), with activities ranging from 61 to 750 Bq g^{-1}. The ^{137}Cs content showed variability between the different campaigns, likely due to soil heterogeneity. This heterogeneity is probably linked to the presence of radioactive debris. For these six samples, a significant linear dependence between the ^{137}Cs concentration and $C_{org(NaOH)}$ was observed (p-value = 0.03). By comparison, the three samples collected outside the trench showed much lower ^{137}Cs activity (with values ranging from 0.35 to 1.5 Bq g^{-1}). Several analyses of the U content also reveal a clear difference between soil samples collected within and outside the trench. First, the level of ^{238}U was higher for soils sampled from within the trench (Table 1). Second, the ^{235}U/^{238}U ratio is elevated for trench samples (with values ranging from 0.98% to 1.17%), while it is low (0.72%) for samples collected outside of the trench. The high ratio for trench samples signifies soil contamination by radioactive fuel particles disseminated during the accident, while the value measured outside the trench corresponds to the natural U ratio. These results show that the samples collected outside the trench constitute suitable control soil samples.

3.2. Molecular diversity of bacterial communities

Bacterial community diversity was analyzed in the six highly contaminated samples and three weakly contaminated controls collected in October 2008, April 2009, and October 2009 (one of the controls was only sampled in 2009). The PCR-DGGE reproducibility was assessed for each soil sample by a comparative analysis of DGGE profiles (Fig. 2). DNA templates were obtained from two independent extractions in October 2008 and three in October 2009. PCR-DGGE was then performed with a universal primer set targeting the V3–V5 region of the bacterial 16S rRNA gene. As shown in Fig. 2, the DGGE profiles of replicates were similar, demonstrating the reproducibility of the method (complete results are provided in Fig. S1).

To assess the impact of RN contamination on bacterial community structure, a comparative study was performed of DGGE profiles from high and low contaminated soils collected in April and October 2009. Only a single DNA sample was processed for each series, in order to load all samples on the same DGGE gel. A cluster analysis of DGGE profiles was performed by the construction of a

Table 1
^{137}Cs activity, ^{238}U content and uranium (^{235}U/^{238}U) isotopic ratio, physico-chemical parameters and abundance of culturable bacteria of soil samples collected in and outside the trench 22 in Chernobyl. Contaminated samples are indicated with an asterisk.

Soil samples	^{137}Cs (Bq g^{-1})			^{238}U (μg g^{-1} of soil)	^{235}U/^{238}U (%)	$C_{org(NaOH)}$ (mg g^{-1} of soil)		pH			Water content (%)			CFU (g^{-1} of soil sample) (0.1 × TSB agar)	
Nos.	October 2008	April 2009	October 2009	April 2009	April 2009	October 2008	April 2009	October 2008	April 2009	October 2009	October 2008	April 2009	October 2009	October 2008	April 2009
1*	290	420	420	43	1.17	nd	2.56	5.6	5.1	4.8	7.2	5.8	7.3	9×10^5	2.6×10^5
3*	340	300	750	18	1.07	nd	1.48	5.5	5.6	5.0	5	5.7	5.1	6×10^5	2×10^5
4*	170	61	140	11	1.08	nd	0.49	5.6	5.4	4.8	2.2	3.5	5.1	10^5	4×10^4
8*	410	640	670	81	1.26	nd	4.12	5.0	5.2	4.5	4.1	6.2	3.6	1.5×10^5	8×10^4
10*	450	290	710	26	0.98	nd	2.15	5.2	4.7	4.9	2.9	4.9	5.2	8×10^4	5×10^4
12*	150	260	370	29	1.15	nd	1.16	5.4	5.4	4.4	4.6	3.7	4.8	1.2×10^5	7×10^5
13	0.95	0.37	1.5	5	0.73	nd	0.16	5.0	6.1	4.3	3.4	2.2	2.7	10^4	2×10^4
14	0.65	0.91	0.79	5	0.72	nd	0.10	5.2	6.1	4.8	3.7	2.5	4.1	6×10^3	3×10^4
20	–	0.35	0.54	5	0.72	–	1.99	–	5.4	4.5	–	4.7	5.1	–	6×10^4

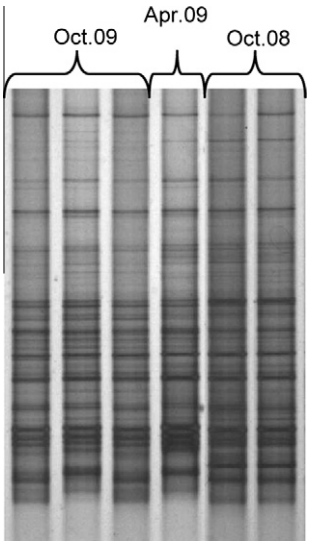

Fig. 2. DGGE profiles of bacterial communities retrieved from soil sample no. 4. Total DNA was extracted from two independent samples in October 2008, from one sample in April 2009 and from three samples in October 2009. Universal primers P1-GC and Com2, targeting bacterial 16S rRNA gene were used.

similarity dendrogram (Fig. 3). Bacterial communities exhibited high apparent diversity, even in the most contaminated samples (demonstrated by the presence of 24–36 discernible bands and a high number of unresolved bands in the separation profiles). There were slight variations in the community structure of samples 1, 4, 8, 13 and 14 between April and October, as observed in the corresponding DGGE profiles with similarity values ranging from 65% to 73%. In contrast, samples 3, 10, 12 and 20 exhibited greater variations between April and October, with similarity values varying from 51% to 35%. The similarity dendrogram revealed two major clusters at the 27% similarity level (Fig. 3). The first cluster consists of the highly contaminated samples 1, 3, 4, 8, 10 grouping at 35%, whereas the other cluster includes the control soils 13, 14, 20 and the contaminated sample 12, grouping at 30%. The bacterial patterns from contaminated samples exhibited low similarity and were not grouped in the same cluster, indicating a high degree of phylogenetic heterogeneity among the communities. The DGGE analysis did not reveal the presence of any specific bacterial community in RN-rich samples.

To complement these results, a DGGE analysis of several taxonomic groups was performed on the samples collected in October 2009 using *Actinobacteria*, *Alpha-*, *Beta-*, *Gamma-Proteobacteria* and *Firmicutes* specific primers. For each primer set, the PCR products were separated on the same gel using an appropriate denaturing gradient and similarity dendrograms were constructed to compare the profiles (Fig. 4). As observed for *Bacterial* communities, the apparent diversity of *Alpha-*, *Beta-*, *Gamma-proteobacteria*, and *Firmicutes* was high in all profiles, with an average of 28, 25, 20 and 30 visible bands, respectively, whereas the *Actinobacteria* profiles were less diverse with an average of 13 bands (Fig. 4). Compared to controls, the apparent diversity was not reduced in the highly contaminated samples (Fig. S2). The dendrograms exhibited various topologies; however none of them revealed any obvious associations between community structure and RN content.

Taken together, these results demonstrate that long term RN exposure at activities ranging from 61 to 750 Bq g^{-1} did not lead to extinction of bacterial diversity. In fact, the Chernobyl T22 trench hosts a wide diversity of bacteria, after 25 years of contamination with RN. Furthermore, profile comparison by the construction of similarity dendrograms did not indicate the presence of specific community fingerprints related to contamination

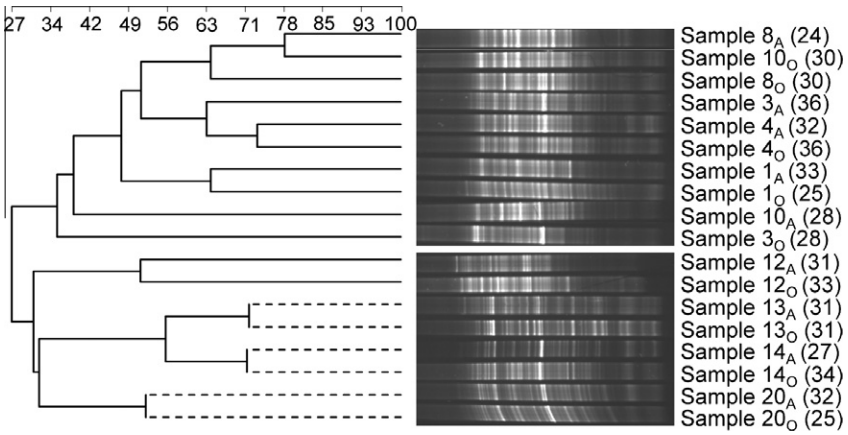

Fig. 3. DGGE analysis of bacterial communities from Chernobyl soils. DGGE profiles of total bacterial communities in soil samples collected in April and October 2009 and the corresponding similarity dendrogram are shown. Universal primers targeting bacterial 16S rRNA genes were used (see Supplementary material for details). The number of discernible bands on the DGGE profiles is indicated in parentheses for each sample. Dotted lines correspond to control soil samples (nos. 13, 14 and 20). Scale bar indicates percent similarity. Sample$_A$: collected in April 2009; sample$_O$: collected in October 2009.

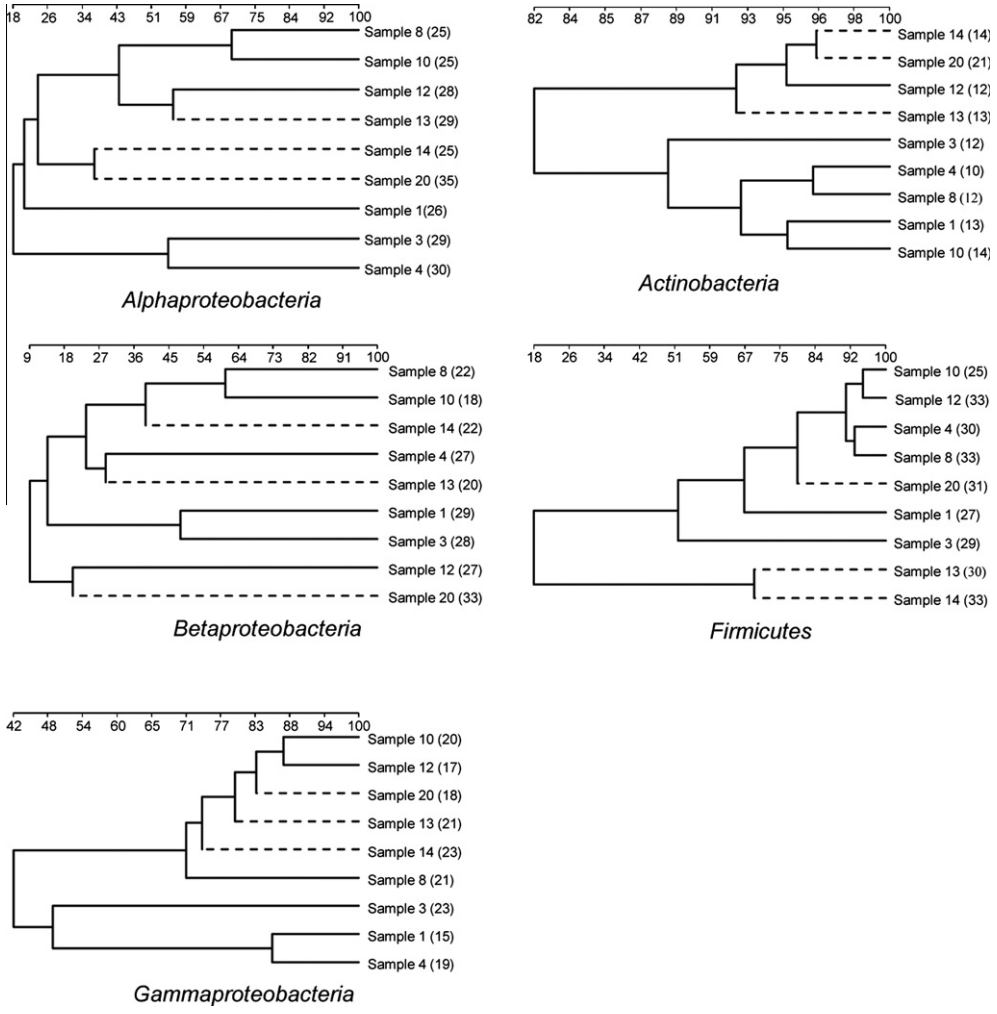

Fig. 4. Clustering analysis of DGGE profiles of different taxonomic groups. Clustering analysis of *Alpha-*, *Beta-*, *Gamma-Proteobacteria*, *Firmicutes*, *Actinobacteria* in soil samples collected in October 2009. Primers targeting these different taxonomic groups were used (for details, see Supplementary material). The number of discernible bands on the profiles is indicated in parentheses. Dotted lines correspond to control soil samples (nos. 13, 14 and 20). Scale bar indicates percent similarity.

level. These results suggest that a diverse set of microorganisms could potentially respond to RN stress and that RN content is likely not the sole factor influencing bacterial community structure.

3.3. Phylogeny of culturable bacteria

Aerobic heterotrophs inhabiting the Chernobyl soils were isolated by cultivation-based methods. The statistical analysis of

colony forming units (CFU) revealed a significant condition (contaminated/control) effect (p-value = 0.008), indicating that highly contaminated samples contained significantly higher amounts of culturable bacteria than the controls (Table 1). Importantly, this demonstrates that the presence of contamination does not decrease the abundance of culturable bacteria. This observed abundance is likely linked to the high level of organic C found in these samples (Table 1) indicating that organic C content in the soil is a determinant factor controlling this parameter. However, this result does not refute Romanovskaya et al. (1998), who reported a decrease in culturable bacteria in the years immediately following the accident. Indeed, in their conditions, only natural soils were considered while a large amount of organic matter was buried in the trench.

To estimate phylogenetic diversity, the 16S rRNA gene was partially sequenced from 303 isolates representative of dominant morphotypes retrieved on plates, and phylogenetic trees were constructed (Figs. 5 and 6). These sequences were affiliated with four bacterial phyla, which cluster in 83 operational taxonomic units (OTUs) at 97% sequence identity: *Firmicutes* (197 isolates, including 15 retrieved with a protocol for selection of sporulating bacteria; see Materials and methods), *Proteobacteria* (62 isolates), *Actinobacteria* (39 isolates) and *Bacteroidetes* (5 isolates). Seventy OTUs were represented in the RN contaminated soils, whereas 37 OTUs were detected in the controls (including 24 OTUs in common). The statistical analysis of OTU distribution according to these four phyla revealed that only the "phylum" effect was significant (p-value < 1e−5); neither the interaction "phylum × condition"

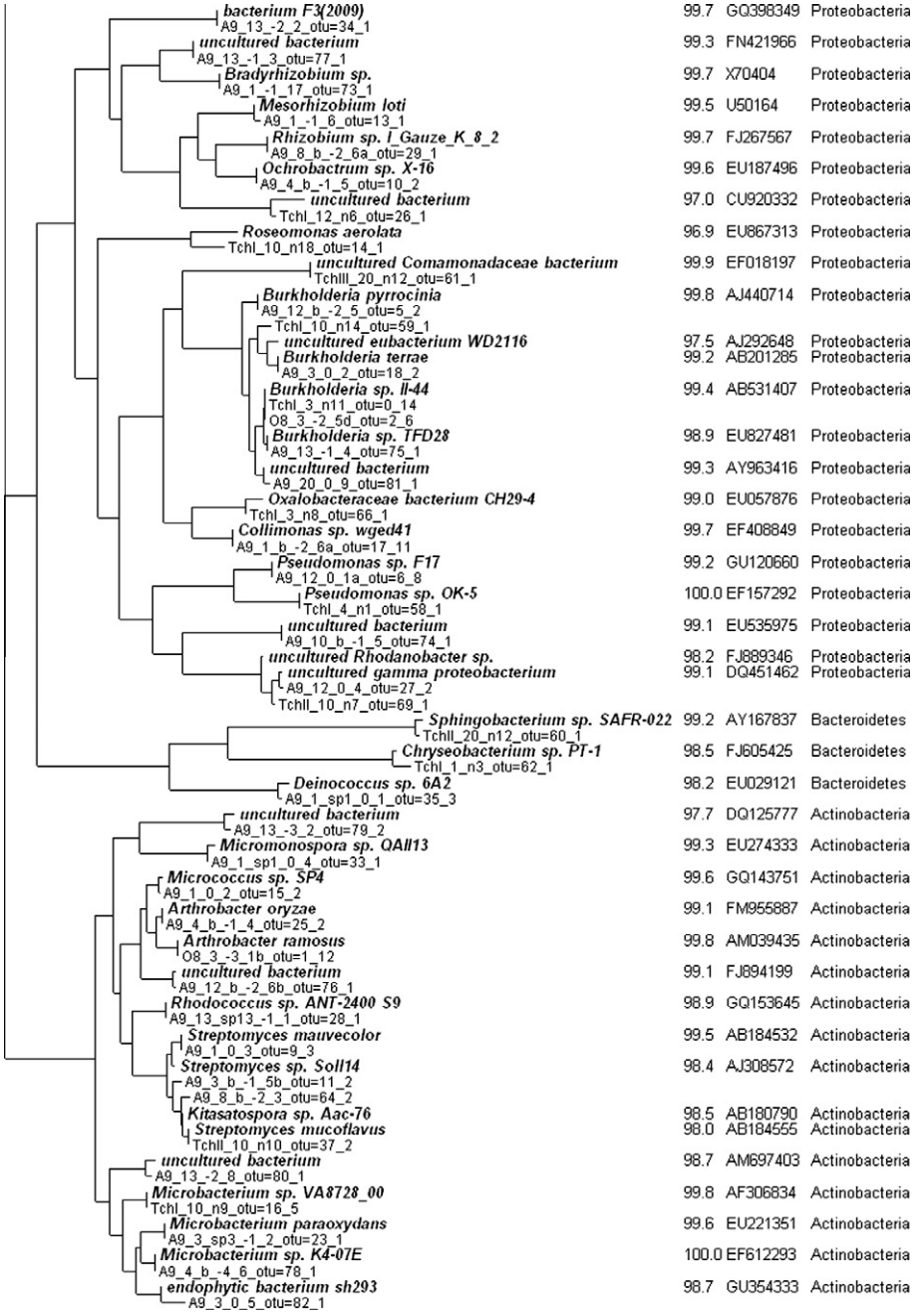

Fig. 5. Unrooted phylogenetic tree of 16S rRNA gene sequences from *Firmicutes*-related isolates. The tree includes one sequence per OTU and the most similar sequence retrieved from public databases.

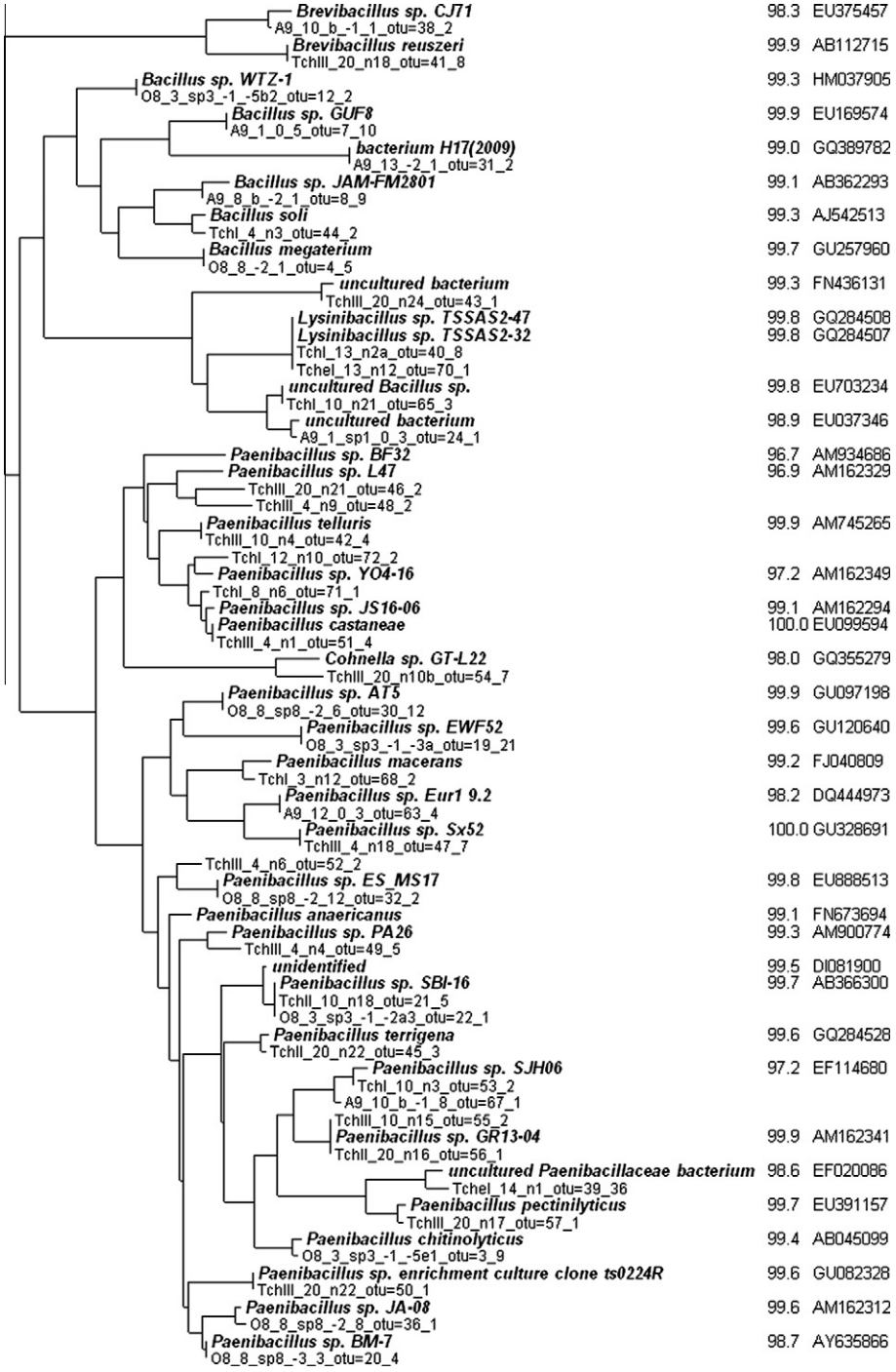

Fig. 6. Unrooted phylogenetic tree of 16S rRNA gene sequences from *Actinobacteria*-, *Bacteroidetes* and *Proteobacteria*-related isolates. The tree includes one sequence per OTU and the most similar sequence retrieved from public databases.

(p-value = 0.89) nor the "condition" effect (p-value = 0.12) were significant (Fig. 7). Thus, the number of OTU according to the four phyla differed globally, although they did not correlate with the contamination level of the soil samples. By using two different culture media, the isolation of diverse bacteria was enhanced: 35 OTUs were recovered from TSB agar plates, 26 OTUs were retrieved from AEM1 plates, and 22 OTUS were recovered from both. *Firmicutes* is the dominant group, representing 65% of the culturable population, and clustered in 41 OTUs with isolates related to the genera *Bacillus*, *Brevibacillus*, *Cohnella*, *Lysinibacillus*, *Paenibacillus*, *Sporosarcina* and *Staphylococcus* (Fig. 5). The presence of *Bacillus* as an important population in Chernobyl soils has previously been described (Romanovskaya et al., 1998; Zavilgesky et al., 1998). The distribution of the different OTUs related to *Firmicutes* in soil samples from Chernobyl was heterogeneous, and did not correlate with contamination (Fig. S3). Members of *Paenibacillus*, *Bacillus*, *Brevibacillus*, and *Lysinibacillus* are common inhabitants of soil, in which their key role in the degradation of organic matter is linked to the production of extracellular hydrolytic enzymes such as cellulases and xylanases. They are also able to form spores, a cellular structure that provides considerable protection from drastic environmental conditions. In Chernobyl, this population of

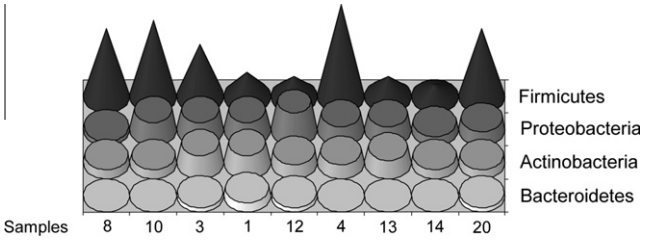

Fig. 7. Repartition of the OTUs (97% cut-off value) in the different phyla retrieved from high- and low-contaminated soil samples of Chernobyl. Soil samples are ordered from left to right according to decreasing ^{137}Cs content.

spore-forming bacteria was largely dominated by *Paenibacillus* (144 isolates), which were retrieved from every soil sample. Previously, a high frequency of *Paenibacillus* has been reported in various contaminated environments such as heavy metal- and RN-waste areas (Petrie et al., 2003; Gao et al., 2010). Furthermore, *Paenibacillus jamilae* and *Paenibacillus polymyxa* have been shown to produce either Pb- or Cd-binding exopolysaccharides, respectively (Morillo et al., 2006; Mokaddem et al., 2009). Other *Firmicutes* which have been isolated from a U mine site and RN-contaminated soil were shown to chelate RN (Selenska-Pobell et al., 1999), or bioprecipitate U (Martinez et al., 2007). Thus, *Firmicutes* isolated from Chernobyl likely possess similar features to handle the challenges of high RN concentration in the soils, and so may represent an interesting source of new metal-binding molecules.

Proteobacteria was the second most represented phylum, with 20% of the isolates clustered into 23 OTUs, which were affiliated to the alpha (9 isolates), beta (40 isolates) and gamma (13 isolates) subdivisions. As observed for *Firmicutes*, there was no correlation between the abundance of the different genera of *Proteobacteria* and the level of soil contamination (Fig. S4). Sequences belonging to OTUs 5, 6, 18, 29, 58 and 66 exhibited high identity (97–100%) to sequences retrieved from various heavy-metal contaminated environments, or corresponding to metal-resistant bacteria (see Table S2). The *Betaproteobacteria* group was largely dominated by *Burkholderia*-related isolates (27) while the representation of *Gammaproteobacteria* was dominated by *Pseudomonas* (9 isolates). These two genera constitute ubiquitous groups of bacteria which are able to colonize a wide range of habitats, and can exhibit extreme nutritional versatility. In particular, *Pseudomonas* and *Burkholderia* are common in metal-contaminated environments (Akob et al., 2008; Fredrickson et al., 2004; Lazzaro et al., 2008; Palmroth et al., 2007; Radeva and Selenska-Pobell, 2005; Zhang and Min, 2010), and heavy metal/RN resistant/accumulating species have also been described (Jones et al., 1997; Jiang et al., 2003; Ruggiero et al., 2005; Li et al., 2007; Choi et al., 2009; Kazy et al., 2009; Choudhary and Sar, 2011). Here, nine *Alphaproteobacteria*-related species were isolated, which belong to six genera including *Methylobacterium* and *Rhodobacter*. *Methylobacterium* species were previously detected in Chernobyl soils, and exhibited a γ-radiation resistant phenotype (Romanovskaya et al., 1998).

Actinobacteria were represented by 39 isolates clustered in 16 OTUs and affiliated to the genera *Arthrobacter*, *Kitasatospora*, *Microbacterium*, *Micrococcus*, *Micromonospora*, *Rhodococcus*, and *Streptomyces*. OTU sequences 15, 23, 25, 28, 33, 78 and 79 exhibited high similarity with sequences originating from heavy-metal contaminated environments, or from metal or oxidative-stress resistant species (Table S2). *Actinobacteria* (formerly called high-GC Gram-positive bacteria) are common soil inhabitants and have been detected as a major group in radioactive waste contaminated sediments (Fredrickson et al., 2004). Actinobacterial species related to the genera *Arthrobacter*, *Streptomyces* and *Microbacterium* are heavy-metal tolerant (Martinez et al., 2006; Nedelkova et al., 2007; Schmidt et al., 2007) or radioresistant (Mao et al., 2007).

Finally, three OTUs related to *Bacteroidetes* were detected. They were affiliated to *Sphingobacterium*, *Chryseobacterium* and *Hymenobacter*. Interestingly, the closest Genbank relative of the *Hymenobacter*-related isolates was retrieved by Fredrickson et al. (2008) from irradiated soils (accession number: EU029121 mistakenly annotated as a *Deinococcus* strain in the Genbank database).

4. Conclusions

Based on the analysis of bacterial diversity using both molecular- and culture-based approaches, the results demonstrate that RN contaminated soils from the Chernobyl T22 trench host a wide diversity of bacteria which is dominated by *Firmicutes*, and similar to that observed in nearby control soils bearing a much lower RN content. These data illustrate that 25 years exposure to RN did not lead to extinction of bacterial diversity. No dominant community fingerprint related to RN content was found, suggesting that a diverse set of microorganisms is potentially responding to the contamination. This does not preclude potential effects of RN on bacteria. Additional factors, such as organic C content linked to the presence of vegetable debris buried in the trench, could have an important impact on community structure. Moreover, it is possible that phenotypic adaptations not observed in the diversity studies could have occurred in response to RN contamination. In line with this hypothesis, the large collection of culturable isolates from Chernobyl that were constructed may be a promising source of RN-accumulating species and RN-chelating molecules. Analysis of bacteria–RN interactions using this collection should provide a better understanding of mechanisms controlling RN migration in the trench.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.apgeochem.2011.08.011.

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