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ORIGINAL PAPER

Archaeal diversity in a Fe–As rich acid mine drainage at Carnoulès (France)

O. Bruneel · N. Pascault · M. Egal · C. Bancon-Montigny · M. S. Goñi-Urriza · F. Elbaz-Poulichet · J.-C. Personné · R. Duran

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Abstract The acid waters (pH = 2.73–3.4) that originate from the Carnoulès mine tailings (France) are known for their very high concentrations of As (up to 10,000 mg l^{-1}) and Fe (up to 20,000 mg l^{-1}). To analyze the composition of the archaeal community, (their temporal variation inside the tailing and spatial variations all along the Reigous Creek, which drains the site), seven 16S rRNA gene libraries were constructed. Clone analysis revealed that all the sequences were affiliated to the phylum Euryarchaeota, while Crenarchaeota were not represented. The study showed that the structure of the archaeal community of the aquifer of the tailing stock is different to that of the Reigous Creek. Irrespective of the time of sampling, the most abundant sequences found inside the tailing stock were related to *Ferroplasma acidiphilum*, an acidophilic and ferrous-iron oxidizing Archaea well known for its role in bioleaching. Inversely, in Reigous Creek, a sequence affiliated to the uncultured Thermoplasmatales archaeon, clone YAC1, was largely dominant. This study provides a better understanding of the microbial community associated with an acid mine drainage rich in arsenic.

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O. Bruneel (✉) · N. Pascault · M. Egal · C. Bancon-Montigny · F. Elbaz-Poulichet · J.-C.Personné
Laboratoire Hydrosciences Montpellier, UMR 5569, IRD, CNRS, Universités Montpellier 1 et 2, Université Montpellier 2, Place E. Bataillon, Case MSE, 34095 Montpellier Cedex 05, France
e-mail: bruneel@msem.univ-montp2.fr

M. S. Goñi-Urriza · R. Duran
Equipe Environnement et Microbiologie UMR CNRS 5254, IPREM, EEM, Université de Pau et des Pays de l'Adour, Avenue de l'Université, IBEAS, BP 1155, 64013 Pau Cedex, France

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Introduction

The processing of sulfide-rich ores in the recovery of base metals such as copper, lead, zinc, and gold, has produced large quantities of pyrite wastes (Langmuir 1997). When exposed to rain, this material generates acid mine drainage (AMD) which contains large quantities of sulfate, iron, arsenic and heavy metals. Despite their toxicity, these waters are colonized by iron- and sulfur-oxidizing prokaryotes and form stable microbial communities with obligate acidophilic eukaryotes (fungi, yeasts, algae and protozoa; Johnson 1998; Zettler et al. 2002). The metabolic activities of such communities lead to solubilization (leaching) of the heavy metals from the sulfidic ores and pollution of surface and subsurface waters fed by the run-off.

For several decades, bacteria-like *Acidithiobacillus* or *Leptospirillum* have been considered to be the principal acidophilic sulfur- and iron-oxidizing microorganisms in AMD. They were believed to be responsible for pyrite oxidation and for the release of associated metals. However, during the last 10 years, several studies have evidenced the presence of archaeal communities in acidic waters (Edwards et al. 2000; Dopson et al. 2004). Previously, Archaea were renowned for their ability to inhabit extreme environments and specialized niches but their widespread presence in non-extreme environments, such as marine and terrestrial soils, was also recently revealed (Chaban et al. 2006).

Archaeal communities are often better adapted to low pH, high concentrations of total and ferrous iron and other

metals, and moderately elevated temperatures than classical bioleaching mesophilic bacteria (*Acidithiobacillus* spp. and *Leptospirillum* spp.). Archaea were seen as numerically significant members in these environments (Bond et al. 2000; Edwards et al. 2000; Johnson and Hallberg 2003). Furthermore, it has been suggested that Archaea could play a major role in the generation of AMD (Baker and Banfield 2003) with oxidation of iron. Some members of the Archaea that respire As(V) like *Pyrobaculum aerophilum* and *Pyrobaculum arsenaticum* have been discovered (Huber et al. 2000; Oremland and Stolz 2003). Furthermore, *Pyrobaculum arsenaticum*, forms realgar (As_2S_2) as a precipitate under organotrophic conditions in the presence of thiosulfate and arsenate. These findings suggest that Archaea may play a significant role in the biogeochemical cycling of arsenic (Huber et al. 2000; Chaban et al. 2006).

Highly acidic environments are relatively scarce worldwide and are generally associated with mining activities. The oxidation by meteoric water of the pyrite-rich wastes from the abandoned Pb–Zn Carnoulès mine generates low pH (2.7–3.4) water containing high concentrations of As and Fe, up to 10,000 and up to 20,000 mg l^{-1}, respectively (Casiot et al. 2003a). We previously characterized the bacterial communities and showed that populations related to sulfate-reducing bacteria and *Gallionella ferruginea* seem to play a key role in AMD functioning (Bruneel et al. 2005, 2006). To know how a system is structured and how it functions, we first have to address the diversity of the whole community. We used a molecular phylogenetic approach to characterize the microbial structure and infer a corresponding ecosystem function where appropriate. The aim of the present study was to investigate the archaeal community in water samples from an AMD very rich in As, to improve our understanding of the implication of these microorganisms in AMD functioning. This is the first molecular analysis of the archaeal community present in the Carnoulès mine system.

Materials and methods

Description of the study site

The lead and zinc mine of Carnoulès, which has been abandoned since 1963, produced 1.2 MT of spoil material containing sand, sulfide minerals, heavy metals (Pb, Zn, Tl) and metalloids (As, Sb). The material is deposited in the middle of and across the upstream part of a creek (the Reigous) at the site of its natural spring. The Reigous collects downstream seepage waters from the surroundings before joining, at 1.5 km, the relatively pristine Amous river.

The source of the Reigous Creek, now located at the foot of the dike retaining the mining spoil, is acid (pH 2.7–3.4) and very rich in dissolved arsenic and iron (80–350 and 750–2,700 mg l^{-1} respectively, Leblanc et al. 2002) predominantly in their reduced forms: As(III) and Fe(II). The water discharge is comprised between 0.8 and 1.7 l s^{-1}.

In the Reigous Creek, As(III) is the dominant As species whereas Fe occurs as Fe(II). Along the first 30 m of the creek (about 1 h residence time), the microbial mediated oxidation of Fe(II) leads to the coprecipitation of 20–60% of the dissolved As. As-rich (up to 20%) yellow sediments cover the bottom of the creek. The precipitate is mainly composed of amorphous Fe(III)–As(III) associated with tooeleite, a rare nanocrystal mineral of Fe(III)–As(III) during the winter period and with amorphous Fe(III)–As(V) the rest of the year (Casiot et al. 2003b; Morin et al. 2003). Bacteria play an essential role in the oxidation of Fe and As (Casiot et al 2003b). Bacterial diversity is lower than in unpolluted water. Sequences related to *G. ferruginea*, a neutrophilic Fe-oxidizing bacterium, are dominant (Bruneel et al. 2006).

The biogeochemical processes that occur in the Carnoulès spoil heaps are more complex than those in the creek. The general hydrochemistry and aquifer hydrodynamics have already been broadly characterized (Koffi et al. 2003; Casiot et al. 2003a). The spoil heaps are covered by an impermeable layer of clay which prevents rainwater percolation from the surface towards the unsaturated zone. The aquifer originates from former natural springs that were buried under the tailings (Koffi et al. 2003). Therefore, the primary region of oxidation is located at the base of the tailing, where the oxygen rich rainwater can penetrate directly. The dominant organisms (27–65%) are related to *Desulfosarcina variabilis* a sulfate-reducing bacterium. *Acidithiobacillus ferrooxidans* represent the second most important group (8–14%).

Cultivable bacterial strains of *A. ferrooxidans* and *Thiomonas* (shown to be very active in the oxidation of As) were identified both in the tailing stock and in the Reigous Creek (Bruneel et al. 2003).

Sampling and analysis

Three surveys were carried out in November 2004, April 2005, and September 2005 in the tailing stock. Groundwaters were collected in a borehole (S5, between 10 and 12 m deep) located in the center of the tailings. Samples were also taken along the Reigous Creek, (collecting downstream seepage waters from the surroundings) in November 2005, at the spring (S1), 30 m downstream from the spring (station COWG), 150 m downstream (COWS), and 1,500 m (CONF) upstream from the confluence between the Reigous and the Amous river. Water samples

(300 ml) were filtered through sterile 0.22 μm Nuclepore filters that were then transferred to cryotubes, frozen in liquid nitrogen, and stored at −80°C until further analysis.

The main physicochemical parameters [pH, T°C, dissolved oxygen (DO), etc.] were measured at the sampling points. Measurements of pH and water temperature were made in the field with an Ultrameter Model 6P (Myron L 125 Company, Camlab, Cambridge). Water samples were immediately filtered through 0.22 μm Millipore membranes fitted on Sartorius polycarbonate filter holders. Samples for total Fe and As determination were acidified to pH = 1 with HNO_3 (14.5 M), and stored at 4°C in polyethylene bottles until analysis. The samples for Fe and As speciation and sulfate determination were stored in the dark and analyzed within 24 h.

DNA isolation

Genomic DNA was extracted from filtered water using the UltraClean Soil DNA Isolation Kit according to the recommendations of the manufacturer (MoBio Laboratories Inc., USA). All the extracted genomic DNA samples were stored at −20°C until further processing.

PCR amplification

Amplification of archaeal 16S rRNA genes was obtained using primers Arch21F (5′-TTCCGGTTGATCCYGCCG GA-3′) and Arch958R (5′-YCCGGCGTTGAMTCCAA TT-3′) (Delong 1992). The PCR amplifications were performed as previously described (Bruneel et al. 2006). The amount of PCR product was determined by comparison to known concentrations after migration on agarose gel.

Archaeal 16S rRNA gene library analysis

Archaeal 16S rRNA gene libraries were constructed to characterize the archaeal populations. Archaeal 16S rRNA genes were amplified with Arch21F and Arch958R primers. These PCR products were cloned in *E. coli* TOP 10 using the pCR2.1 Topo TA cloning kit (Invitrogen, Inc.), according to the manufacturer's instructions. Cloned 16S rRNA gene fragments were reamplified using the primers TOP1 (5′-GTGTGCTGGAATTCGCCCTT-3′) and TOP2 (5′-TATCTGCAGAATTCGCCCTT-3′) located on the vector and surrounding the inserted PCR fragment, and then digested with the enzymes *Hae*III or *Hin*fI. Restriction profiles were analyzed using 2.5% agarose gel electrophoresis (small-fragment resolution agarose; QA agarose, QBiogène, Inc.). Around 60–70 clones from each library were analyzed and grouped according to their RFLP patterns (*Hae*III and *Hin*fI digestion). The sequences of clones from dominant groups were determined.

16S rRNA gene sequencing

Partial sequences of the 16S rRNA gene were determined by the dideoxy nucleotide chain-termination method using the BigDye 3.1 kit (Applied Biosystems) on an ABI PRISM 3730XL Genetic analyzer (Applied Biosystems). Sequences were checked for chimeras using the CHIMERA CHECK function of the Ribosomal Database Project II (Maidak et al. 2001). DNA sequence analyses were performed using the BLAST, ALIGNN, and CLUSTALW programs (Altschul et al. 1990; Felsenstein 1993; Thompson et al. 1994). A phylogenetic tree was constructed using the PHYLIP computer package (Felsenstein 1993). The confidence level of the phylogenetic tree topology was evaluated by performing 100 bootstrap replications with the SEQBOOK program. All the sequences obtained were submitted to the EMBL databases under accession numbers AM765808 to AM765809 and AM778965 to AM778977.

Chemical analysis

The determination of total dissolved As was performed by hydride generation atomic fluorescence spectrometry (HG-AFS). Analyses of As species were carried out using coupled anion-exchange chromatography–HG-AFS. This method, described by Bohari et al. (2001), has a detection limit of 2.3 nM for As(III) and 6.1 nM for As(V). The precision is better than 5%. Total dissolved Fe was determined by flame atomic absorption spectrometry. Fe(II) was determined using colorimetry at 510 nm after complexation with 1,10-phenanthrolinium chloride solution in buffered samples (pH 4.5) (Rodier et al. 1996). The detection limit is 0.2 μM and the precision better than 5%. The sulfate concentration was determined after precipitation of $BaSO_4$ with $BaCl_2$ and spectrophotometric measurement at 650 nm.

Rarefaction analysis, diversity index, and coverage values

PAST (PAleontological STatistics v 1.19) software from the website http://folk.uio.no/ohammer/past/ was used for different diversity indices (Rarefaction analysis, Taxa, Total clones, Singletons, Dominance, Coverage, Shannon, Equitability, and Simpson) for each clone library. To perform rarefaction analysis, the total number of clones obtained compared with the number of clones representing each unique phylotype was used to produce the rarefaction curves. Coverage values were calculated to determine how efficiently the libraries described the complexity of a theoretical community like an original archaeal community. The coverage (Good 1953) value is given as

$C = 1 - (n1/N)$ where $n1$ is the number of clones that occurred only once in the library.

Results

Aqueous chemistry

The physicochemical composition of the water is presented in Table 1. The pH inside the piezometer was between 3.73 and 5.78. The temperature varied from 15.5 to 20.6°C and was relatively stable throughout the year (Koffi et al. 2003). The DO was quite low particularly in April 2005 (between 0.1 and 0.2 mg l^{-1}). The concentration of As inside the tailing stock varied greatly. As(III) was predominant, comprised between 78 and 277 mg l^{-1}, and As(V) varied between 42 and 66 mg l^{-1}. The concentration of Fe(II) (Fe(III) not detected, data not shown) varied greatly, i.e. between 778 and 1,299, and sulfate between 3,264 and 4,195 mg l^{-1}. The concentrations of As(III), Fe and SO_4^{2-} were highest in November 2004.

In the Reigous creek, the 2.5 pH at the spring increased along the creek to reach 3.43 at COWS and 3.25 just before the confluence with the Amous (CONF), 1.5 km away. The DO content was 1 mg l^{-1} in the spring but it increased along the creek to reach 5–6 at COWS and 3–4 mg l^{-1} at CONF. Dissolved As and Fe concentrations decreased at varying degrees along the course of the creek, (30 mg l^{-1} for As(III), 39 mg l^{-1} for As(V), 879 mg l^{-1} for Fe(II) and 4,388 mg l^{-1} for sulfate at the spring station (S1) but only 0.53 for As(III and V), 25 mg l^{-1} for Fe(II) and 749 for sulfate at the CONF station. These elements are removed by coprecipitation with Fe(III). This process results from bacterially mediated As- and Fe-oxidation (Casiot et al. 2003b). Furthermore, the increase in pH as a result of dilution by unpolluted tributaries after COWG also contributes to an increase in As and Fe precipitation. During this sampling period, the concentrations of As, Fe and SO_4^{2-} were not particularly high in comparison to the concentrations usually found in these waters (up to 10,000 and 20,000 mg l^{-1} for As and Fe, respectively) in the tailing stock (Casiot et al. 2003a) and from 80 to 350 mg l^{-1} for As, 750 to 2,700 mg l^{-1} for Fe, and 2,000 to 7,500 mg l^{-1} for sulfate in the head waters of the Reigous creek (Leblanc et al. 2002).

Composition of archaeal communities

16S rRNA gene library analyses were performed to identify the dominant groups of archaeal populations. The most representative sequences of the dominant clones are summarized in Table 2 and the phylogenetic filiations of the sequences obtained are presented in Fig. 1. DNA could be extracted from all sampling sites except the S5 borehole in November 2005. In the Carnoulès mine drainage, numerous sequences in the libraries are related to sequences previously found in AMD, showing that the clone libraries were not contaminated.

Clones analysis revealed that all the sequences were affiliated to the phylum Euryarchaeota, while Crenarchaeota were not represented. The most abundant sequence types present in the water of the tailing (S5) displayed from 99 to 100% homology with *Ferroplasma acidiphilum* strain DR1, that was detected in microbial consortia from AMD and in industrial bioleaching environments (Dopson et al. 2004, AY22042). They were recovered in the groundwater in November 2004 and April 2005, representing a large majority of the clones (65–72%). The second most abundant group (9% in November 2004 but 65% in September 2005) was similar (99–100%) to the uncultured archaeon clone ant h4 (Table 2, Fig. 1) found in two anaerobic sludges (DQ462728, unpublished). The sequences representing the second most abundant type in April 2005 (15%) were similar (91%) to clones of the uncultured archaeon clone YAC1 (Table 2, Fig. 1) found in communities of different hot springs (DQ237924, unpublished). In September 2005, the second most important group (20%), (Table 2), was related to the uncultured archaeon clone ASL1 found in AMD (Baker and Banfield 2003; AF544224).

Table 1 Physico-chemical characteristics of the water (mg l^{-1}) during the sampling in S5, S1, COWG, COWS and CONF

Sampling station		Sampling period	pH (±SD)	T (°C)	DO (±SD)	As(III) (±SD)	As(V) (±SD)	Fe (II) (±SD)	SO_4^{2-} (±SD)
Tailing stock	S5	November 2004	5.78 (±0.05)	15.5	2	277 (±14)	42 (±2)	1299 (±104)	4195 (±420)
		April 2005	4.05 (±0.05)	17.3	0.1–0.2	128 (±6)	66 (±3)	784 (±62)	3264 (±326)
		September 2005	3.73 (±0.05)	20.6	4–5	78 (±4)	53 (±3)	778 (±62)	3629 (±363)
Reigous Creek	S1	November 2005	2.5 (±0.05)	14.6	1	30.0 (±0.8)	39 (±2)	879 (±70)	4388 (±441)
	COWG		2.74 (±0.05)	10.6	5–6	22.0 (±0.8)	22.0 (±0.8)	501 (±40)	1785 (±182)
	COWS		3.43 (±0.05)	7.2	5–6	4.5 (±0.2)	1.50 (±0.08)	95 (±8)	902 (±90)
	CONF		3.25 (±0.05)	6.7	3–4	0.53 (±0.02)	0.53 (±0.02)	25 (±2)	749 (±75)

SD Standard deviation

Table 2 Archaeal clones found in Carnoulès mine drainage with closest match organism or clone name, percent similarity, phylogenetic group, closest relative and percent number of each group compared to the total number of clones

Sampling station		Sampling period	Clones	Phylum	Closest relative (accession number)	Number of bp identical and % similarity	Relative abundance of clones (%)[a]
Tailing stock	S5	November 2004	S5Nov04 73	Euryarchaeota	*F. acidiphilum* strain DR1 (AY222042)	100	72
			S5Nov04 82	Euryarchaeota	Uncultured archaeon clone ant h4 (DQ303256)	100	9
		April 2005	S5Apr05 12	Euryarchaeota	*F. acidiphilum* strain DR1 (AY222042)	99	65
			S5Apr05 47			99	
			S5Apr05 45	Euryarchaeota	Uncultured archaeon clone YAC1 (DQ237924)	91	15
		September 2005	S5Sep05 53	Euryarchaeota	Uncultured archaeon clone ant h4 (DQ303256)	99	65
			S5Sep05 56	Euryarchaeota	Uncultured archaeon clone ASL1 (AF544224)	97	20
Reigous Creek	S1	November 2005	S1Nov05 90	Euryarchaeota	*F. acidiphilum* strain DR1 (AY222042)	99	54
			S1Nov05 58	Euryarchaeota	Uncultured archaeon clone YAC1 (DQ237924)	93	21
	COWG		CGNov05 19	Euryarchaeota	Uncultured archaeon clone YAC1 (DQ237924)	93	59
			CGNov05 94			93	
			CGNov05 32	Euryarchaeota	*F. acidiphilum* strain DR1 (AY222042)	100	4
	COWS		CSNov05 10	Euryarchaeota	Uncultured archaeon clone YAC1 (DQ237924)	92	93
			CSNov05 20	Euryarchaeota	Uncultured archaeon clone ant g10 (DQ303253)	99	6
	CONF		CFNov05 6	Euryarchaeota	Uncultured archaeon clone YAC1 (DQ237924)	94	74

[a] The abundance of clones was calculated for each library

In the Reigous creek during the sampling campaign in November 2005, the most abundant group (21% at the spring S1, 59% at COWG, 93% at COWS and 74% at CONF) was related (92–94%) to the uncultured archaeon clone YAC1. These clones were found in low abundance (15%) in the groundwater and only in April 2005. The second most abundant group in the creek was similar (99–100%) to *F. acidiphilum*, also numerically significant members in Carnoulès tailing stock. The abundance of this group decreased along the creek, representing 54% of the clones at the spring S1, but only 4% at COWG and was undetected at COWS and CONF. The least abundant sequences (6%) found only at the COWS station was related (99% similarity) to the uncultured archaeon clone ant g10 isolated in macroscopic filaments from an extremely acidic environment, Tinto River (DQ303253, unpublished). Phylogenetic analyses (Fig. 1) did not enable affiliation of the clone sequences with any representative of the subdivision. The closest relative (91%) was *Thermoplasma* sp. SO2 (AB262009, unpublished).

Rarefaction analysis, diversity index and coverage values of the clone libraries analyzed

Table 3 shows Dominance, Shannon, Equitability, Simpson index and Coverage values calculated for each library.

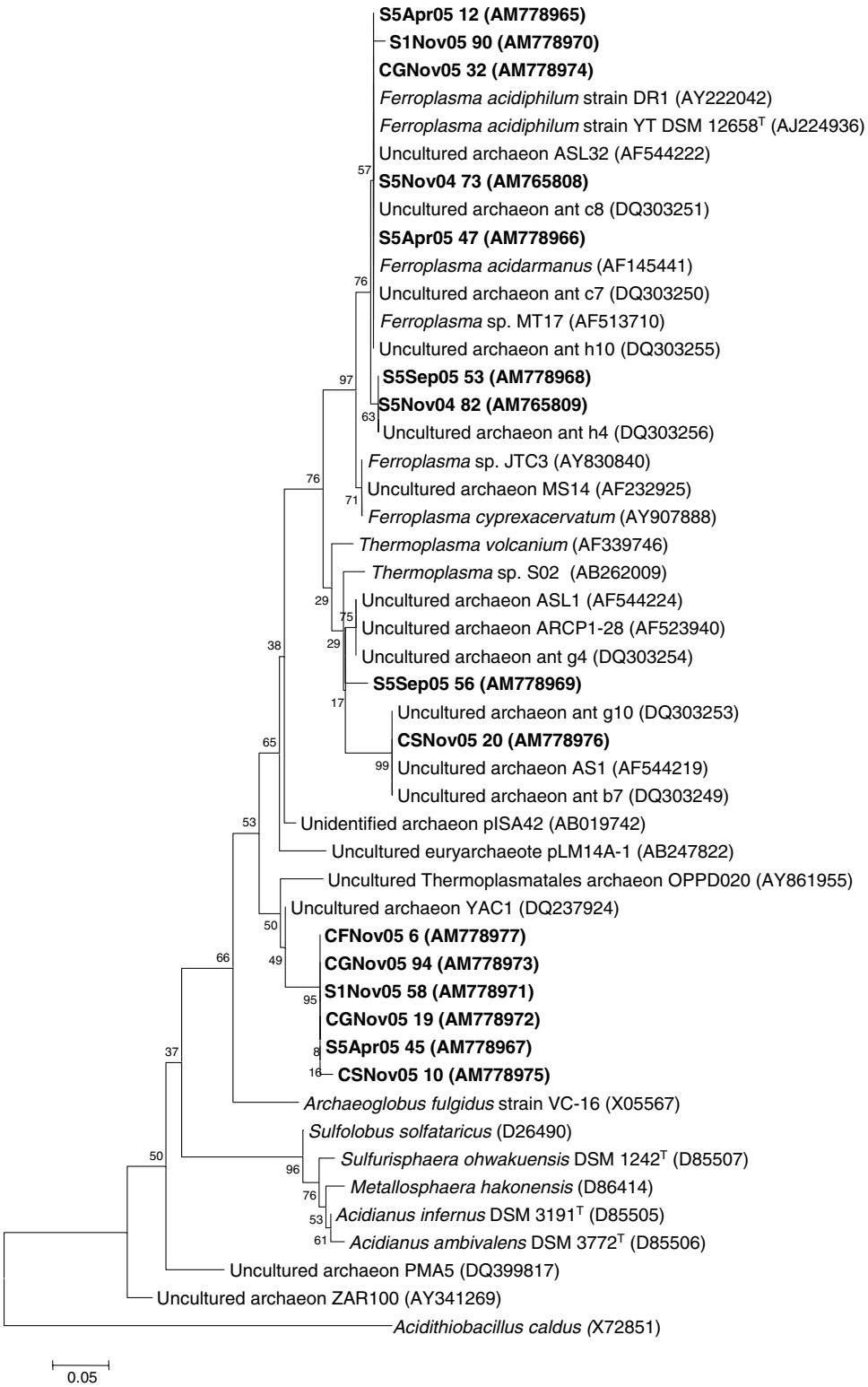

Fig.1 Phylogenetic analysis of 16S rRNA gene sequences affiliated with Archaea members from the AMD of Carnoulès (France). Clone names in *bold* correspond to sequences found in the Carnoulès mine drainage

To estimate diversity coverage and to determine whether a sufficient number of clones from each library had been sequenced, rarefaction analysis was performed. The generated curves were near saturation (data not shown), consistent with the high coverage values (between 0.82 and 0.93). In November 2005, the COWS library showed lower diversity indices (Shannon: 0.5704; Simpson: 0.2397) than the other libraries (Shannon ranging from 1.151 to 1.604; Simpson from 0.4488 to 0.6545). Inversely, in November 2005, the COWS library presented a higher Dominance

Table 3 Diversity indices calculated for the seven clone libraries from different stations in Carnoulès mine drainage

Clone library	Taxa	Total clones	Singletons	Dominance	Coverage(C)	Shannon (H)	Equitability	Simpson (1-D)
S5 November 2004	9	66	5	0.4913	92	1.151	0.5241	0.5087
S5 April 2005	11	66	6	0.4564	90	1.277	0.5323	0.5436
S5 September 2005	10	61	6	0.4512	90	1.234	0.5361	0.5488
S1 November 2005	14	57	10	0.3456	82	1.604	0.6077	0.6545
COWG November 2005	13	69	9	0.3989	86	1.424	0.5552	0.6011
COWS November 2005	6	61	4	0.7603	93	0.5704	0.3183	0.2397
CONF November 05	12	61	6	0.5512	90	1.189	0.4785	0.4488

index (0.7603) than the other libraries (from 0.3456 to 0.5512).

Discussion

In the AMD site of Carnoulès, more than 65% of the archaeal sequences could not be closely related to cultured organisms, suggesting that they may constitute new taxa. Only sequences close to *F. acidiphilum* were related to cultured organisms. Rarefaction data and percent coverage calculations suggested that the archaeal 16S rRNA gene libraries reach saturation.

Whatever the sampling period, the water of S5 inside the tailing stock, where intensive pyrite oxidation takes place, was numerically dominated by sequences clearly related to *F. acidiphilum*, or to the uncultured clone ant h4 which showed more than 98% similarity with *F. acidiphilum*. This isolate was an acidophilic, mesophilic, ferrous-iron oxidizing, cell-wall lacking microbe that became the basis of a new archaeal lineage: the new genus *Ferroplasma* within the new family *Ferroplasmaceae*, in the order *Thermoplasmatales*, which includes the families *Thermoplasmaceae* and *Picrophilaceae* (Golyshina and Timmis 2005). These two populations represented 81% of clones in November 2004, 65% in April 2005, and 65% in September 2005. Previous analysis of the bacterial community in the Carnoulès tailing showed that the dominant population was related to the sulfate-reducing bacteria *Desulfosarcina variabilis* (Bruneel et al. 2005). This population could not clearly explain the leaching of the Carnoulès tailing as it is well known that it was mostly acidophilic ferrous iron-oxidizing microorganisms that were found to be involved in the production of acid mine drainage (Baker and Bandfield 2003). Iron oxidizing bacteria like *A. ferrooxidans* and *Sulfobacillus* spp. were also present in the Carnoulès mine tailing but represented a minor population (Bruneel et al. 2005). Thus, *F. acidiphilum* could explain the intensive leaching observed in the Carnoulès tailing and the high concentration of As, up to 10,000 mg l^{-1}, one of the highest concentrations reported in the world. Furthermore, some strains of this genus like *Ferroplasma acidarmanus* Fer1 was shown to be an arsenic-hypertolerant acidophilic archaeon (Gihring et al. 2003; Baker-Austin et al. 2007). This strain, isolated from the Iron Mountain mine, California, was able to grow with up to 10 g arsenate per litre but his growth was reduced with 5 and 10 g of arsenite per litre. This population, which is more acid-resistant than iron- and sulfur-oxidizing bacteria, is in fact known to mobilize metals from sulfide ores, e.g. pyrite, arsenopyrite and copper-containing sulfides. According to Golyshina and Timmis (2005) *Ferroplasma* spp. are probably the major players in the biogeochemical cycling of sulfur and sulfide metals in highly acidic environments, and may have considerable potential for biotechnological applications such as biomining and biocatalysis under extreme conditions. These results are consistent with those of Edwards et al. (2000) at the Iron Mountain acid-generating site (United State), where the microbial community is dominated (85%) by an archaeon of the genus *Ferroplasma*. For these authors, the presence of this population and other closely related Thermoplasmatales suggests that these acidophiles are important contributors to acid mine drainage and may substantially impact iron and sulfur cycles. The growth of *F. acidiphilum* occurs between 20 and 45°C with an optimum at 35°C and at pH 1.3–2.2 with an optimum at pH 1.7 (Golyshina et al. 2000). Surprisingly, we detected this population in a less acidic environment (3.73–5.7). Isolation and characterization of members of this population are needed to determine their physiological capabilities especially at the pH range found in Carnoulès waters.

The clone sequences from the Reigous Creek were related to the same groups detected in the tailing S5 but the abundance of each varied. The dominant population in the Reigous Creek (21% of total clones at the spring S1, 59% at COWG, 93% at COWS and around 74% CONF) was related to the uncultured archaeon clone YAC1 found in communities in different hot springs. Phylogenetic analyses (Fig. 1) did not enable affiliation of the clone sequences with any cultured representative of the subdivision and this clone could thus represent a new species. The closest

relative (91%) was the uncultured Thermoplasmatales archaeon found in the Yellowstone geothermal ecosystem (Spear et al. 2005). The order Thermoplasmatales includes the families Ferroplasmaceae, Thermoplasmaceae and Picrophilaceae (Golyshina and Timmis 2005). The known members of the Thermoplasmales are all acidophilic. Some groups, like the family Ferroplasmacea within this order, are capable of iron oxidation (Edwards et al. 2000; Golyshina and Timmis 2005). A previous study of bacterial populations in the Carnoulès creek showed that the dominant bacterial population was related to *G. ferruginea*, a neutrophilic bacterium that oxidizes Fe (Bruneel et al. 2006). Consistent with previous observations demonstrating that *G. ferruginea* efficiently remove As (III and V) in water by coprecipitation with Fe (Katsoyiannis and Zouboulis 2004), this population may play a key role in the remediation process observed in the Reigous creek (Casiot et al. 2003b). If the uncultured archaeon clone YAC1 oxidizes Fe, this population could play a role in the natural remediation processes occurring in the Reigous Creek in association with *G. ferruginea*, but until the archaeal strains are isolated, their physiological role in the creek ecology will remain uncertain. Environmental genome data like those obtain with analysis of assembled random shotgun sequence data can also provide detailed insight into the metabolic potential of uncultivated organisms (Tyson et al. 2005).

Our study demonstrated the existence of a complex prokaryotic community in the Carnoulès AMD where bacterial and archaeal populations are present. Both phylotype communities were significantly altered in terms of size and structure with microhabitats varying inside the AMD particularly in underground water from the tailing and in the Reigous and the small creek draining the site. The occurrence of different dominant communities is likely associated with the formation of environmental gradients of temperature, pH, oxidation–reduction potential, etc. Other methods such as fluorescence in situ hybridization (FISH) will help to clearly assess the relative proportion of population. However, this method has not been widely applied to samples of thermophilic archaea and may be limited by cross-hybridization. Furthermore, methods such as metagenomic research (study of the entire genetic composition of communities of an environment) could help to study the total diversity, physiology, ecology and phylogeny of microbial population but all of the approaches that are available today have advantages and limitations (Pontes et al. 2007). Only, the isolation of archaeal strains at the Carnoulès mine will extend our understanding of the ubiquity of archaea in such environments, and help elucidate the microbial component driving the biogeochemical processes present in this and other extreme AMD sites.

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