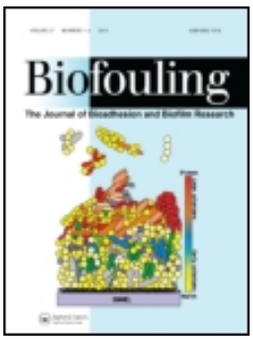

Taylor & Francis
Taylor & Francis Group

Biofouling
The Journal of Bioadhesion and Biofilm Research

ISSN: 0892-7014 (Print) 1029-2454 (Online) Journal homepage: http://www.tandfonline.com/loi/gbif20

Biofouling on the Walls of a Spent Nuclear Fuel Pool with Radioactive Ultrapure Water

Eduardo Chicote , Diego A Moreno , Ana M Garcia , M Isabel Sarro , Petra I Lorenzo & Felipe Montero

To cite this article: Eduardo Chicote , Diego A Moreno , Ana M Garcia , M Isabel Sarro , Petra I Lorenzo & Felipe Montero (2004) Biofouling on the Walls of a Spent Nuclear Fuel Pool with Radioactive Ultrapure Water, Biofouling, 20:1, 35-42, DOI: 10.1080/08927010410001662670

To link to this article: http://dx.doi.org/10.1080/08927010410001662670

Published online: 25 Jan 2007.

Biofouling, February 2004 Vol 20 (1), pp 35–42

Taylor & Francis
Taylor & Francis Group

Biofouling on the Walls of a Spent Nuclear Fuel Pool with Radioactive Ultrapure Water

EDUARDO CHICOTE[a], DIEGO A MORENO[a,*], ANA M GARCIA[a], M ISABEL SARRO[a], PETRA I LORENZO[a] and FELIPE MONTERO[b]

[a]*Universidad Politécnica de Madrid, Departamento de Ingeniería y Ciencia de los Materiales, Escuela Técnica Superior de Ingenieros Industriales, José Gutiérrez Abascal 2, E-28006 Madrid, Spain;* [b]*Iberdrola Generación, SA, Tecnología de Materiales, Paseo de la Virgen del Puerto 53, E-28005 Madrid, Spain*

(Received 18 June 2003; in final form 21 November 2003)

Microbial activity in spent nuclear fuel pools which contain ultrapure and radioactive water has been previously observed. The aim of the present research was to isolate and identify the microorganisms attached to the nuclear pool wall of a Spanish nuclear power plant. Amplification of 16S rDNA fragments from the culturable microorganisms by PCR using universal primers for the domain 'Bacteria', followed by Denaturing Gradient Gel Electrophoresis analysis revealed the presence of six different bacteria. The complete gene for 16S rDNA of each one was sequenced and identified as belonging to three different phylogenetic groups, *viz*. *β*-Proteobacteria, Actinomycetales and the *Bacillus/Staphylococcus* group. A fungus was also found and identified as *Aspergillus fumigatus* by sequencing the D2 region of the large subunit rDNA gene. The isolation of these microorganisms in oligotrophic and radioactive conditions is of great interest due to the possibility of their use in bioremediation processes of radionuclide-contaminated environments.

Keywords: biofouling; DGGE; nuclear pool; nuclear power plant (NPP); PCR; radioactive water; ultrapure water

INTRODUCTION

Microorganisms have been shown to survive and grow in extreme habitats (Rothschild & Mancinelli, 2001; Cavicchioli, 2002), such as ultrapure water (Kvasnikov *et al*., 1978; McFeters *et al*., 1993; Kulakov *et al*., 2002) or highly radioactive conditions (Booth, 1987). In natural oligotrophic environments, biofilm formation is a survival strategy for bacterial communities (Characklis & Marshall, 1990). Many bacteria can excrete extracellular polymeric substances (EPS) allowing both adherence to surfaces (George *et al*., 2003) and potential resistance to adverse conditions (Wingender *et al*., 1999). The EPS matrix acts as a diffusion barrier to nutrients and cellular products (Wolfaardt *et al*., 1999). The chemical conditions in this matrix can be completely different from the bulk medium, creating microhabitats that protect microorganisms (Costerton *et al*., 1995).

The sensitivity of microorganisms to high-energy radiation varies greatly among genus, species and strains (Farkas *et al*., 2002) and can be modified by temperature, oxygen and another environmental factors (Gazsó, 1997). Microorganisms can attach to surfaces, grow and form biofilms leading to different processes of biofouling and/or biocorrosion (*e.g*. metallurgical deterioration, production of explosive gases, fouling and plugging of orifices) (Wolfram & Dirk, 1997; Diósi *et al*., 2003; Hamilton, 2003). In different environments, microbiologically influenced corrosion (MIC) appears frequently under the biofilms. The microorganisms modify the environment, creating crevices, differential aeration zones or a more aggressive environment with the presence of metabolites (Moreno *et al*., 2000). Biofilm formation can accelerate rates of partial reactions in corrosion processes (Geesey, 1991; Little & Wagner, 1997). It seems to be a universal requirement for biocorrosion, although it is not necessarily a sufficient condition.

There is increasing evidence of biofouling in different nuclear power facilities such as nuclear

*Corresponding author; fax: 34 913 363 007; e-mail: moreno@materials.upm.es

ISSN 0892-7014 print/ISSN 1029-2454 online
DOI: 10.1080/08927010410001662670

reactor pools, radioactive waste repositories and primary cooling systems of nuclear reactors (Maltsev *et al*., 1996; Zavilgelsky *et al*., 1998; Pitonzo *et al*., 1999). Thus, the identification of microorganisms able to grow at highly radioactive sites is of great interest in nuclear power facilities. Even more interesting is the potential application of such microbes to bioremediation of waters containing radionuclides (Macaskie, 1991; Nealson *et al*., 2002). Microorganisms can control the transfer of trace elements of radionuclides in four main ways: (i) biodegradation; (ii) bioaccumulation; (iii) biosorption and (iv) bioprecipitation (Hambuckers-Berhin & Remacle, 1997). The knowledge of the variety of microorganisms able to grow in different radioactive environments would be of great help in order to select the most appropriate microorganism to be used in each bioremediation situation. Members of the family Deinococcaceae are one of the most radio-resistant microorganisms (Makarova *et al*., 2001). These Gram-positive cocci are being widely studied in order to understand the mechanisms allowing them to survive in such extreme conditions (Bauche & Laval, 1999; Venkateswaran *et al*., 2000). Nevertheless, little is known about other families of microorganisms able to grow in highly radioactive environments (Binks, 1996; Asgarani *et al*., 2000).

The main goal of the present study was to isolate and identify sessile microorganisms able to attach to and grow on the nuclear pool wall in such an extreme environment (oligotrophic and radioactive), in order to consider their application in a radionuclide-bioremediation process.

MATERIALS AND METHODS

Sampling Site

This study was carried out in the upper transference pool in the Cofrentes Nuclear Power Plant (NPP), in Valencia, Spain. The NPP is a Boiling Water Reactor (BWR) type plant. The transference pool is made of austenitic stainless steel, and the ultrapure water is treated in a closed-loop system including filtration and demineralisation processes.

Water Analysis

The water quality is analysed once a week, as a routine test in the NPP. The concentrations of nitrates, sulphates and chlorides were measured by gradient ion chromatography (Dionex IC, Model DX500) with a microbore configuration, conductivity detection and Peaknet 5.1 software. Conductivity, pH, temperature and TOC were analysed according to AWWA-APHA-WEF (1998).

Following the recommendations of Debertin and Helmer (1988), radiochemical analysis was carried out by a Canberra gamma-spectrometer system consisting of a high purity p-type coaxial germanium detector, 40.6% efficiency, with Canberra associated electronics and a Canberra Genie2k gamma spectrometry software package.

Microbial Isolation and Identification

Samples from the microorganisms attached to the nuclear pool wall were collected at a depth of 25 cm, taking advantage of a descent in the water level of the pool, in two different ways, *viz*. using NA (nutrient agar) contact plates (Microkit SL) and scraping with cotton swabs. Contact plates were incubated at 30°C for 72 h and the isolated colonies were then plated on BHI (Brain Heart Infusion, Oxoid, supplemented with agar (UPS), purissimum, Panreac) solid medium and incubated at 30°C for further analysis. The swabs were spread onto different solid media, *viz.* BHI, NA (nutrient agar, Oxoid CM3) and STC (starch from potato soluble for analysis, Panreac, 10 g l^{-1}; casein hydrolysate (acid), Oxoid, 1 g l^{-1}; potassium phosphate monobasic, KH_2PO_4, Panreac, 0.5 g l^{-1}; agar (UPS); purissimum, Panreac, 20 g l^{-1}; pH = 7.2) and in BHI liquid medium and incubated for 72 h at 30°C. After that, the liquid cultures were plated onto BHI and STC solid media and incubated at 30°C to isolate the colonies observed.

Genomic DNA was extracted from all colonies cultured in solid media using the commercial product PrepMan (PE Applied Biosystems) according to the protocol given by the manufacturer. PCR amplification of 16S rDNA fragments corresponding to nucleotides 5 to 531 in the *Escherichia coli* sequence was performed using the bacterial universal primers 5F (5′-TGGAGAGTTTGATCCTGGCTCAG-3′) containing a 40-base GC-clamp at its 5′end (CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG) and 531R (5′-TACCGCGGCTGCTGGCAC-3′). To increase the specificity of the amplification and to reduce the formation of spurious by-products a 'touchdown' PCR was performed. The annealing temperature was lowered from 50°C to 40°C over 20 cycles (0.5°C every cycle) and then another 20 cycles at 43°C. The mixture was preincubated for 5 min at 94°C. Each cycle consisted of an initial denaturing step of 45 s at 94°C, an annealing step of 30 s at the appropriate temperature and a final 45 s step of elongation at 72°C. Following the final cycle, the reaction was extended for 7 min at 72°C. In all cases the PCR mixture, containing 5 μl of DNA; 12.5 μl of PCR Master kit 1 × (1.5 mM $MgCl_2$; 50 mM KCl; 10 mM Tris-HCl; 1.25 U *Taq* DNA polymerase; 0.2 mM dNTPs; Roche Diagnostics); 25 pmol of each primer and 0.4 mM $MgCl_2$, was made up to 25 μl

with sterile water (Roche Diagnostics). The PCR was carried out in a GeneAmp PCR System 2400 (Perkin Elmer). Before being subjected to further analysis all PCR products were checked by electrophoresis in 1% (wt/vol) agarose gels in 1 × TBE buffer with ethidium bromide (0.5 μg ml^{-1}).

Amplified fragments from bacteria were analysed by Denaturing Gradient Gel Electrophoresis (DGGE) in 6% polyacrylamide gels (37.5:1, acrylamide:bisacrylamide) in 1 × TAE buffer containing a linear chemical gradient ranging from 30% to 50% denaturant (100% denaturant contains 7 M urea and 40% [vol/vol] formamide). Electrophoresis was performed at 60°C for 6 h at 110 V on a Dcode™ Universal Mutation Detection System (Bio-Rad). Following the electrophoresis, the gel was incubated for 10 min in an ethidium bromide solution (0.5 μg ml^{-1}) and visualised in an image analyser (Kodak Image Station 440CF) with a shortcut to KDS1D 3.0.2 software.

DGGE was used as a pre-screening technique to select microorganisms to be sequenced. Identification of bacteria presenting different migration patterns by DGGE was accomplished by sequencing 1500 bp with the MicroSeq™ Full Gene 16S rDNA Bacterial Sequencing Kit (PE Applied Biosystems) in an ABI PRISM™ 310 Genetic Analyser following the manufacturer's instructions. The sequences obtained were compared to those deposited in the European Molecular Biology Laboratory (EMBL) and the National Center of Biotechnology Information (NCBI) Databases using the Fasta3 and Blastn programs, respectively.

The identification of the fungus was performed by using the MicroSeq™ D2 LSU rDNA Fungal Identification System (PE Applied Biosystems) in the ABI PRISM™ 310 Genetic Analyser, according to the instructions given by the manufacturer. This system was designed to amplify and sequence the D2 region of the large subunit (LSU) rDNA gene from fungal genomic DNA. The sequence obtained was compared to those deposited in the NCBI Database using the Blastn program.

Alignments of the sequences were performed by using the ClustalX software v 1.81 (Thompson *et al.*, 1997). Phylogenetic and molecular evolutionary analysis were conducted using Molecular Evolutionary Genetics Analysis (MEGA) v 2.1 (Kumar *et al.*, 2001). Phylogenetic trees were constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPBMA) method with the Jukes-Cantor model (Jukes & Cantor, 1969). A total of 1000 bootstrapped replicate resampling data sets were generated. The *Escherichia coli* 16S rDNA sequence was used as an outgroup.

All samples were collected and manipulated following the recommendations of the Nuclear Security Council to avoid a possible radiological contamination (Council Directive 96/29/Euratom, 1996) and the materials used in this research were managed as radioactive waste.

RESULTS

The results of the water quality analysis are shown in Table I. The low concentration of nitrates, sulphates and chlorides and the TOC values indicate the oligotrophic character of the water.

The radiochemical analysis of the water by gamma spectrometry showed radiation levels between 2.5 and 5.0 Bq g^{-1}. The most common radionuclides found in the water were ^{60}Co, ^{137}Cs, ^{134}Cs, ^{54}Mn and ^{65}Zn, which usually appear in the NPP operating with BWR.

Following the isolation of the microorganisms in solid media, the first approach was observation of the morphology of the colonies and the visualization of the microorganisms by microscopy. Once morphologically grouped, all the isolated bacteria were analysed by PCR-DGGE, which allows the differentiation of the microorganisms based on their nucleotides sequences. Different migration patterns were observed (Figure 1), indicating the presence of 6 distinct bacteria attached to the nuclear pool wall. From the contact plates, 4 morphologically different colonies were observed among the 24 that initially grew but only 3 showed different migration patterns by DGGE, which were named C1, C3 and C4. From the swabs that were spread onto different solid media, growth was observed only on the BHI medium. In this medium only a single bacterial colony (C5) and a fungus (C6) were able to grow. After the incubation of the swabs in liquid media, a total of 17 colonies were isolated: 9 from STC medium (C7 to C15) and 8 from BHI medium (C16 to C23). Two different microorganisms were detected by comparing their migration patterns. They were named C16 and C18.

In order to identify the isolated bacteria and the fungus, the 16S rDNA full gene (1500 bp) of each bacterium and the D2 region of the LSU rDNA gene of the fungus were sequenced and compared with those consigned in the NCBI and EMBL Databases. In Table II, the closest relative species to each one, with their accession numbers, and the homology between

TABLE I Quality analysis of the nuclear pool water

Parameter	Measurement
Conductivity (μS.cm^{-1})	0.8 – 1.5
pH	5.0 – 6.0
Temperature (°C)	27 – 34
TOC (ppm)	0.02 – 0.10
Chlorides (ppb)	0.05 – 2.5
Nitrates (ppb)	0.04 – 2.0
Sulphates (ppb)	0.14 – 0.50

them are presented. Among the isolated bacteria Gram-positive and Gram-negative types were found. They belonged to 3 different phylogenetic groups, *viz*. *β*-Proteobacteria (C1), Actinomycetales (C3 and C5) and the *Bacillus/Staphylococcus* group (C4, C16 and C18). A phylogenetic tree was constructed from the evolutionary distances by the UPGMA method based on the whole sequence of the 16S rDNA gene (Figure 2).

The sequences of the DNA from the isolated bacteria have been submitted to GenBank under the accession numbers AF397059, AF397060, AF397061, AF397062, AF397063, AY479983 and AY479984.

DISCUSSION

As can be deduced from this work, microorganisms belonging to different phylogenetic groups are viable in an oligotrophic and radioactive environment such as the wall of the nuclear pool. Gram-positive (C3, C4, C5, C16 and C18) and Gram-negative (C1) bacteria have been found among the isolated microorganisms. The Gram-positive bacteria were the most abundant, not only in terms of number of microorganisms isolated, but also in diversity. These results are in agreement with other studies that have demonstrated that Gram-positive microorganisms are more radioresistant than Gram-negative bacteria (Gazsó, 1997). Although the radioresistance of the microorganisms isolated in this research has not been tested, other bacteria isolated in the same pool water belonging to similar genera presented D10 values of about 1400 Gy (data not published). Some of these microorganisms have been found in other radioactive environments. For example, *Bacillus* sp. has been isolated from radioactive soil samples (Bondar *et al*., 1997; Romanovskaia *et al*., 2002) and nuclear fuel waste disposal containers (Stroes-Gascoyne *et al*., 1997). Members of this genus have exhibited resistance to gamma and UV radiation, H_2O_2 and desiccation irrespective of whether they were isolated from a radioactive environment or outside it (Romanovskaia *et al*., 2002; Venkateswaran *et al*., 2003). *Nocardia* sp. also has been isolated from radioactive environments (Filicheva *et al*., 1978; Stroes-Gascoyne *et al*., 1997; Romanovskaia *et al*., 2002) as well as other nocardioform actinomycetes, but in some cases they have showed gamma radiation sensitivity. *Ralstonia* sp. is another genus found in radioactive environments (Nikitin *et al*., 1993) showing resistance to gamma radiation and other extreme conditions such as oligotrophy. With respect to the *Staphylococcus* group, some species belonging to this genus have been studied and have showed gamma radiation resistance like *S. caprae, S. epidermidis* and *S. haemolyticus* (Kumar *et al*., 2000). No other findings on the genus *Gordona* in radioactive environments have been reported.

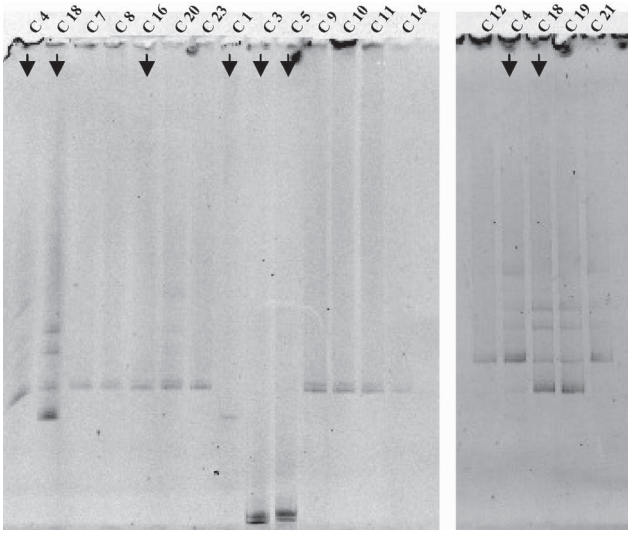

FIGURE 1 DGGE patterns of PCR-amplified 16S rDNA segments from the isolated bacteria.

The growth of these microorganisms attached to the nuclear pool wall is of great interest. The pool is made of austenitic stainless steel, because of its greater resistance to corrosion in different aggressive environments. However, this material is known to be affected by various processes and types of corrosion (Medilanski *et al*., 2002). Some of these, mainly pitting and occasionally stress corrosion cracking, are activated and developed in the presence of microorganisms (Ibars *et al*., 1992a; 1992b). In these circumstances a local break of the passive layer is produced, which impedes the repassivation process

TABLE II Phylogenetic identification of microorganisms isolated from the nuclear pool wall

	Closest GeneBank Relative (NCBI)			
Clone	Phylogenetic group	Strains or species	Accession number	% Similarity to closest relative
C 1	*β*-Proteobacteria	*Ralstonia* sp. APF11	AB045276	100%
C 3	Actinomycetales	*Nocardia seriolae*	Z36925	99.98%
C 4	*Bacillus/Staphylococcus* group	*Staphylococcus saccharolyticus*	L37602	99.99%
C 5	Actinomycetales	*Gordona terrae*	AF154833	100%
C 16	*Bacillus/Staphylococcus* group	*Bacillus licheniformis* (strain M1-1)	AB039328	99.99%
C 18	*Bacillus/Staphylococcus* group	*Bacillus licheniformis*	AB039328	100%
C 6	Fungi-Trichocomaceae	*Aspergillus fumigatus*	AF397059	100%

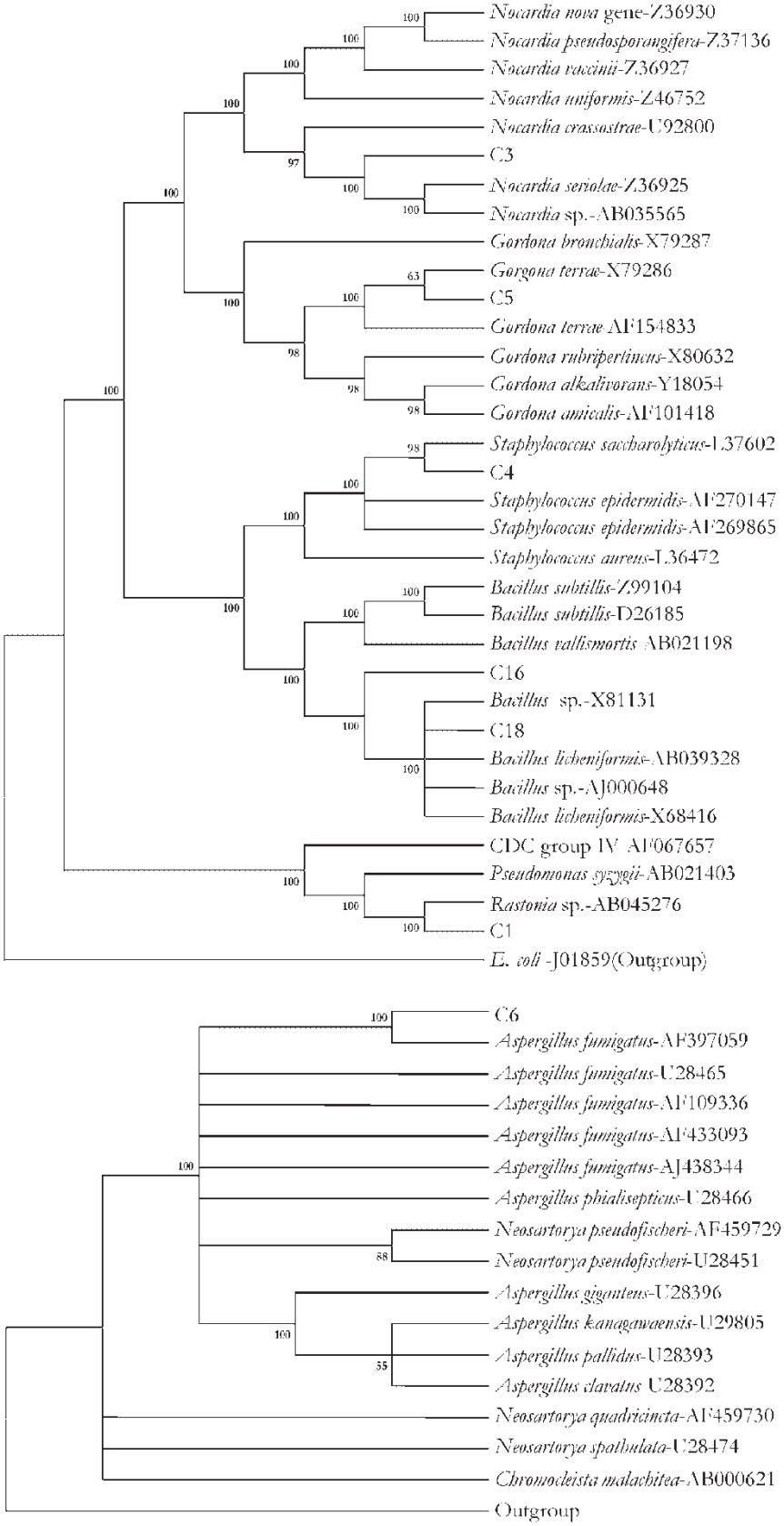

FIGURE 2 Phylogenetic analysis of the 16S rDNA (positions 5 to 1500) from the strains isolated from the nuclear pool wall and the D2 region of the LSU rDNA gene of the fungus. The trees were constructed by the UPGMA approach using the MEGA program. Bootstrap values (in percentages) are given at the nodes. The 16S rDNA sequence from *E. coli* was used as the outgroup.

(Moreno *et al.*, 2000). Jain (1995) observed the biofouling of this material in a deionized water system, and the authors also have seen microbial colonization and biofilm formation on the surface of stainless steel coupons in a previous study (Sarró *et al.*, 2003). In this oligotrophic situation the biofilm could affect the interaction between the metal surface and the environment, contributing to microbiologically influenced corrosion. No signs of corrosion were observed on the wall of the nuclear pool under study, nor on the stainless steel coupons previously tested (Sarró *et al.*, 2003). Nevertheless, the need to apply microbiological control in these kinds of systems should be considered.

On the other hand, the isolation of microorganisms different from those belonging to the family Deinococcaceae in such environments could be useful for the improvement of bioremediation processes to eliminate or reduce the concentration of radionuclides from contaminated sites. It has previously been noticed that biofilms developed on the surfaces of stainless steel coupons are able to accumulate radionuclides, reaching radioactivity values of about 5500 Bq cm^{-2} (Sarró *et al.*, 2003). In these processes, the EPS of the biofilm seemed to have a very important role in the accumulation of radionuclides through the formation of phosphate biominerals (Lloyd & Lovley, 2001). These are initiated by nucleation at the phosphate groups of the EPS with crystal growth driven by enzymatically generated phosphate (Macaskie *et al.*, 2000).

The microorganisms found attached to the nuclear pool wall are similar to those forming the biofilm on the coupons previously tested (Sarró *et al.*, 2003), so they should be able to accumulate radionuclides. Moreover, they belong to groups frequently used in bioremediation of heavy metals and organic compounds (Lange *et al.*, 1998; Brim *et al.*, 2000; Daly, 2000). In this way, *Bacillus* sp. has been used in the biosorption of some radionuclides, such as uranium (Lloyd & Macaskie, 2000), cesium (Hughes *et al.*, 1993) and copper (White *et al.*, 1995) from radionuclide-containing wastewaters. *Ralstonia* sp. is able to form hydroxides and carbonates of radionuclide metallic compounds (Lloyd & Macaskie, 2000), so their isolation in a radioactive environment is very encouraging due to the possibility of their being used in the bioremediation of radionuclides.

The finding of *Aspergillus fumigatus* is also interesting, because of the potential use of fungi in the biosorption of radionuclides (White & Gadd, 1990; Liu *et al.*, 2002). The chitin on the cell wall of filamentous fungi is a very good ligand for radionuclides (Glombitza *et al.*, 1997), and some studies also have shown that oxalate production by fungi can result in the precipitation of radionuclides as insoluble metal oxalates (Tait *et al.*, 1999). The biosorption of uranium, americium and strontium has been observed in different fungi, like *Aspergillus niger, Rhizopus arrhizus* and *Penicillium italicum* (White & Gadd, 1990; Macaskie, 1991; Lloyd & Macaskie, 2000). The application of *A. niger* and *R. arrhizus* in the bioremediation of americium has been studied in different types of bioreactors (White & Gadd, 1990). *A. niger* has been use to eliminate uranium in bioreactors showing higher efficiency than ionic exchange resins (White & Gadd, 1990).

Although these processes of bioremediation have not yet been developed on an industrial scale, the isolation of these types of microorganisms is interesting, as they could be used in the elimination of radionuclides from polluted waters.

SUMMARY

Due to the difficulties involved in investigating nuclear fuel storage facilities, there is a lack of information in this field. Thus, it is important to identify opportunities to achieve this purpose. Some of the studies conducted on nuclear power facilities referred to the presence of microbial activity (Santo Domingo *et al.*, 1998), although the microorganisms have not been completely identified.

In this study, seven different microorganisms have been isolated from the wall of a nuclear pool, and they have been identified as belonging to several phylogenetic groups. These culturable microorganisms represent only a small part of the total consortium of microorganisms. It has been demonstrated that bacteria are capable of developing a survival strategy, *i.e.* the viable but non-culturable state (VBNC), when under adverse environmental conditions (Oliver, 1993; del Mar Lleo *et al.*, 1999). Molecular microbiology techniques allow the study of the microbial communities without cultivation and the detection of VBNC microorganisms (Ward, 1990). However, it was not possible to use these kinds of techniques in this research due to the high radiation at the sampling site and the limitations imposed by the Nuclear Security Council with respect to taking out samples contaminated with radionuclides.

The results of this study suggest that the oligotrophic and radiological environment of the nuclear pool and the deionization treatments commonly applied to prevent electrochemical corrosion in NPP facilities do not prevent microbial growth. The microorganisms identified were capable of forming biofilms, which can accumulate radionuclides (Sarró *et al.*, 2003), thus increasing the radiation levels in the pool walls. Therefore, it is important to apply microbiological control in NPP facilities to avoid problems related to biofouling or biocorrosion of the materials used in the cooling water system and to

prevent the increase in the radioactivity in the pool water.

Acknowledgements

This project has been supported by grants from Ministerio de Ciencia y Tecnología, Spain (CICYT-FEDER, 2FD97-0530-MAT), and Iberdrola Generación, SA (6276-99). We thank Iwona B Beech (University of Portsmouth, UK) for her comments.

References

Asgarani E, Terato H, Asagoshi K, Shahmohammadi H R, Ohyama Y, Saito T, Yamamoto O, Ide H (2000) Purification and characterization of a novel DNA repair enzyme from the extremely radioresistant bacterium *Rubrobacter radiotolerans*. *J Radiat Res* **41**: 19–34

AWWA-APHA-WEF (1998) *Standard Methods for the Examination of Water and Wastewater*, 20th Edition. American Public Health Association, Washington DC

Bauche C, Laval J (1999) Repair of oxidized bases in the extremely radiation-resistant bacterium *Deinococcus radiodurans*. *J Bacteriol* **181**: 262–269

Binks P R (1996) Radioresistant bacteria: have they got industrial uses? *J Chem Tech Biotechnol* **67**: 319–322

Bondar Y I, Galeeva A G, Gerasimova L V, Tchernetsova I B (1997) Influence of the microbiological processes in soil on vertical migration of radionuclides. In: Wolfram J H, Rogers R D, Gazsó L G (eds) *Microbial Degradation Processes in Radioactive Waste Repository and in Nuclear Fuel Storage Areas*. Kluwer Academic Publishers Group, NATO ASI Series 1, Disarmament Technologies, Volume 11, pp 71–80

Booth W (1987) Postmortem on Three Mile Island. *Science* **238**: 1342–1345

Brim H, McFarlan S C, Fredrickson J K, Minton K W, Zhai M, Wackett L P, Daly M J (2000) Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. *Nat Biotechnol* **18**: 85–90

Cavicchioli R (2002) Extremophiles and the search for extraterrestrial life. *Astrobiology* **2**: 281–292

Characklis W G, Marshall K C (eds) (1990) *Biofilms*. Willey & Sons, NY

Costerton J W, Lewandowski Z, Caldwell D E, Korber D R, Lappin-Scott H M (1995) Microbial biofilms. *Annu Rev Microbiol* **49**: 711–745

Council Directive 96/29/Euratom (1996) Laying down basic safety standards for the protection of the health of workers and the general public against the dangers arising from ionizing radiation. Official Journal L 159, 29/06/1996, pp 0001–0114

Daly M J (2000) Engineering radiation-resistant bacteria for environmental biotechnology. *Curr Opin Biotechnol* **11**: 280–285

Debertin K, Helmer R G (1988) *Gamma- and X-ray Spectrometry with Semiconductor Detectors*. Elsevier Science BV

del Mar Lleo M, Tafi M C, Signoretto C, Dal Cero C, Canepari P (1999) Competitive polymerase chain reaction for quantification of nonculturable *Enterococcus faecalis* cells in lake water. *FEMS Microbiol Ecol* **30**: 345–353

Diósi G, Telegdi J, Farkas G, Gazsó L G, Bokori E (2003) Corrosion influenced by biofilms during wet nuclear waste storage. *Int Biodeterior Biodegr* **51**: 151–156

Farkas G, Gazsó L G, Diósi G (2002) Radiosensitivity of subterranean bacteria in the Hungarian Upper Permian Silstone Formation. *J Environ Radioact* **61**: 233–239

Filicheva V A, Lapchinskaia O A, Saburova T P, Siniagina O P, Ponomarenko V I (1978) Selection of active strains of *Actinomadura carminata*, the producer of carminomycin. *Antibiotiki* **23**: 18–22

Gazsó L G (1997) Basic radiation microbiology. In: Wolfram J H, Rogers R D, Gazsó L G (eds) *Microbial Degradation Processes in Radioactive Waste Repository and in Nuclear Fuel Storage Areas*. Kluwer Academic Publishers Group, NATO ASI Series 1, Disarmament Technologies, Volume 11, pp 93–103

Geesey G (1991) What is biocorrosion? In: Flemming H-C, Geesey G G (eds) *Biofouling and Biocorrosion in Industrial Water Systems*. Springer-Verlag, Berlin, Heidelberg, pp 155–164

George R P, Muraleedharan P, Sreekumari K R, Khatak H S (2003) Influence of surface characteristics and microstructure on adhesion of bacterial cells onto a type 304 stainless steel. *Biofouling* **19**: 1–8

Glombitza F, Eckhart L, Hummel A (1997) Fundamentals of the application of biosorption to the separation of uranium from mining drainage waters. *Res Microbiol* **148**: 517–518

Hambuckers-Berhin F, Remacle J (1997) Activities of microorganisms and transfers of trace elements of radionuclides in microhabitats. In: Wolfram J H, Rogers R D, Gazsó L G (eds) *Microbial Degradation Processes in Radioactive Waste Repository and in Nuclear Fuel Storage Areas*. Kluwer Academic Publishers Group, NATO ASI Series 1, Disarmament Technologies, Volume 11, pp 17–22

Hamilton W A (2003) Microbially influenced corrosion as a model system for the study of metal microbe interactions: a unifying electron transfer hypothesis. *Biofouling* **19**: 65–76

Hughes M N, Poole R K, Sami C H K (1993) Accumulation of cesium by microorganisms and clays. In: Torma A E, Wey J E, Lakshmanan V L (eds) *Biohydrometallurgical Technologies*. TMS The Minerals, Metals & Materials Society, Volume II, pp 95–102

Ibars J R, Moreno D A, Ranninger C (1992a) Microbial corrosion of stainless steels. *Microbiologia* (Madr) **8**: 63–75

Ibars J R, Moreno D A, Ranninger C (1992b) MIC of stainless steels: a technical review on the influence of microstructure. *Int Biodeterior Biodegr* **29**: 343–355

Jain D K (1995) Microbial colonization of the surface of stainless steel coupons in a deionised water system. *Water Res* **29**: 1869–1876

Jukes T H, Cantor C R (1969) Evolution of protein molecules. In: Munro H N (ed) *Mammalian Protein Metabolism*. Academic Press, pp 21–132

Kulakov L A, McAlister M B, Odgen K L, Larkin M J, ÓHanlon J F (2002) Analysis of bacteria contaminating ultrapure water in industrial systems. *Appl Environ Microbiol* **68**: 1548–1555

Kumar J K, Sharma A K, Kulkarni P R (2000) Effect of gamma irradiation on staphylococcal thermonuclease (Tnase). *Int J Food Sci Nutr* **51**: 55–58

Kumar S, Tamura K, Jakobsen I B, Nei M (2001) MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**: 1244–1245

Kvasnikov E I, Kliushnikova T M, Mitropolskaya N Yu (1978) [Microflora of the Truskavetz waters with a low content of mineral substances]. *Mikrobiologiia* **47**: 505–509

Lange C C, Wackett L P, Minton K W, Daly M J (1998) Engineering a recombinant *Deinococcus radiodurans* for organopollutant degradation in radioactive mixed waste environments. *Nat Biotechnol* **16**: 929–933

Little B, Wagner P (1997) Microbiologically influenced corrosion in nuclear waste storage environments. In: Wolfram J H, Rogers R D, Gazsó L G (eds) *Microbial Degradation Processes in Radioactive Waste Repository and in Nuclear Fuel Storage Areas*. Kluwer Academic Publishers Group, NATO ASI Series 1, Disarmament Technologies, Volume 11, pp 55–69

Liu N, Yang Y, Luo S, Zhang T, Jin J, Liao J, Hua X (2002) Biosorption of 241Am by *Rhizopus arrihizus*: preliminary investigation and evaluation. *Appl Radiat Isot* **57**: 139–143

Lloyd J R, Macaskie L E (2000) Biorremediation of radionuclide-containing wastewater. In: Lovley D R (ed) *Environmental Microbe-Metal Interactions*. ASM Press, Washington DC, pp 277–327

Lloyd J R, Lovley D (2001) Microbial detoxification of metal and radionuclides. *Curr Opin Biotechnol* **12**: 248–253

Macaskie L E (1991) The application of biotechnology to the treatment of wastes produced from the nuclear fuel cycle: biodegradation and bioaccumulation as a means of treating radionuclide-containing streams. *Crit Rev Biotechnol* **11**: 41–112

Macaskie L E, Bonthrone K M, Yong P, Goddard D T (2000) Enzymically mediated bioprecipitation of uranium by a *Citrobacter* sp.: a concerted role for exocellular lipopolysaccharide and associated phosphatase in biomineral formation. *Microbiology* **146**: 1855–1867

Makarova K S, Aravind L, Wolf Y I, Tatusov R L, Minton K W, Koonin E V, Daly M J (2001) Genome of the extremely radiation-resistant bacterium *Deinococcus radiodurans* viewed from the perspective of comparative genomics. *Microbiol Mol Biol Rev* **65**: 44–79

Maltsev V N, Saadawi A, Ayad A, Elgawi O, Shlip M (1996) Microecology of nuclear reactor pool water. *Radiats Biol Radioecol* **36**: 52–57

Medilanski E, Kaufmann K, Wick L Y, Wanner O, Harms H (2002) Influence of the surface topography of stainless steel on bacterial adhesion. *Biofouling* **18**: 193–203

McFeters G A, Broadawaty S C, Pyle B, Egozy Y (1993) Distribution of bacteria within operating laboratory water purification systems. *Appl Environ Microbiol* **59**: 1410–1415

Moreno D A, Ibars J R, Ranninger C (2000) Influence of microstructure on the microbial corrosion behaviour of stainless steels. *Rev Metal* **36**: 266–278

Nealson K H, Belz A, McKee B (2002) Breathing metals as a way of life: geobiology in action. *Antonie Leeuwenhoek* **81**: 215–222

Nikitin D I, Tashtemirova M A, Pitriuk I A, Sorokin V V, Oranskaia M S, Nikitin L E (1993) High resistance of some oligotrophic bacteria to ionizing radiation. *Mikrobiologiia* **62**: 1064–1071

Oliver J D (1993) Formation of viable but non culturable cells. In: Kjelleberg S (ed) *Starvation in Bacteria*. Plenum Press, New York, pp 239–272

Pitonzo B J, Amy P S, Rudin M (1999) Effect of gamma radiation on native endolithic microorganisms from a radioactive waste deposit site. *Radiat Res* **152**: 64–70

Romanovskaia V A, Rokikto P V, Mikheev A N, Gushcha N I, Malashenko Iu R, Chernaia N A (2002) The effect of gamma-radiation and desiccation on the viability of the soil bacteria isolated from the alienated zone around the Chernobyl Nuclear Power Plant. *Mikrobiologiia* **71**: 705–712

Rothschild L J, Mancinelli R (2001) Life in extreme environments. *Nature* (*Lond*) **409**: 1092–1101

Santo Domingo J W, Berry C J, Summer M, Fliermans C B (1998) Microbiology of spent nuclear fuel storage basins. *Curr Microbiol* **37**: 387–394

Sarró M I, Moreno D A, Chicote E, Lorenzo P I, García A M, Montero F (2003) Biofouling on austenitic stainless steels in spent nuclear fuel pools. *Mater Corros* **54**: 535–540

Stroes-Gascoyne S, Pedersen K, Haveman S A, Dekeyser K, Arlinger J, Daumas S, Ekendahl S, Hallbeck L, Hamon C J, Jahromi N, Delayne T L (1997) Occurrence and identification of microorganisms in compacted clay-based buffer material designed for use in a nuclear fuel waste disposal vault. *Can J Microbiol* **43**: 1133–1146

Tait K, Sayer J A, Gaharieb M M, Gadd G M (1999) Fungal production of calcium oxalate in leaf litter microcosms. *Soil Biol Biochem* **31**: 1189–1192

Thompson J D, Gibson T J, Plewniak F, Jeanmougin F, Higgins D G (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **24**: 4876–4882

Venkateswaran K, Kempf M, Chen F, Satomi M, Nicholson W, Kern R (2003) *Bacillus nealsonii* sp. nov., isolated from a spacecraft-assembly facility, whose spores are gamma-radiation resistant. *Int J Syst Evol Microbiol* **53**: 165–172

Venkateswaran A, McFarlan S C, Ghosal D, Minton K W, Vasilenko A, Makarova K, Wackett L P, Daly M J (2000) Physiologic determinants of radiation resistance in *Deinococcus radiodurans*. *Appl Environ Microbiol* **66**: 2620–2626

Ward D M, Weller R, Bateson M M (1990) 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature* (*Lond*) **345**: 63–65

White C, Gadd G M (1990) Biosorption of radionuclides by fungal biomass. *J Chem Technol Biot* **49**: 331–343

White C, Wilkinson S C, Gadd G M (1995) The role of microorganisms in biosorption of toxic metals and radionuclides. *Int Biodeterior Biodegr* **35**: 17–40

Wingender J, Neu T R, Flemming H-C (1999) What are bacterial extracellular polymeric substances? In: Wingender J, Neu T R, Flemming H-C (eds) *Microbial Extracellular Polymeric Substances. Characterization, Structure and Function*. Springer-Verlag, Berlin, Heidelberg, pp 1–19

Wolfaardt G M, Lawrence J R, Korber D R (1999) Functions of EPS. In: Wingender J, Neu T R, Flemming H-C (eds) *Microbial Extracellular Polymeric Substances. Characterization, Structure and Function*. Springer-Verlag, Berlin, Heidelberg, pp 171–200

Wolfram J H, Dirk W J (1997) Biofilm development and the survival of microorganisms in water systems of nuclear reactors and spent fuel pools. In: Wolfram J H, Rogers R D, Gazsó L G (eds) *Microbial Degradation Processes in Radioactive Waste Repository and in Nuclear Fuel Storage Areas*. Kluwer Academic Publishers Group, NATO ASI Series 1, Disarmament Technologies, Volume11, pp 139–147

Zavilgelsky G B, Abilev S K, Sukhodolets V V, Ahmad S I (1998) Isolation and analysis of UV and radio-resistant bacteria from Chernobyl. *J Photochem Photobiol* B: *Biol* **43**: 152–157