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Juan M. González
Cesáreo Saiz-Jiménez*

Natural Resources and Agrobiology Institute, CSIC, Seville, Spain

Application of molecular nucleic acid-based techniques for the study of microbial communities in monuments and artworks

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*Corresponding author:
J.M. González
Instituto de Recursos Naturales y Agrobiología-CSIC
Apartado 1052
41080 Sevilla, Spain
Tel. +34-954624711. Fax +34-954624002
E-mail: jmgrau@irnase.csic.es

Summary. Microorganisms play critical roles in every kind of habitat on Earth, including those constructed by humans. Thus, our cultural heritage is affected by microbial colonization. While classical microbiological methods based on culturing procedures have provided important, but limited information on the microbial diversity of natural samples, novel molecular techniques have been extremely valuable in unraveling the diversity of microbiota involved in the biodeterioration of our monuments and artworks. The knowledge gained from these approaches has allowed the design of strategies for conserving and protecting monuments for the benefit of future generations. This review describes the state-of-the-art of the application of molecular methods to the analysis of cultural assets, and provides near-future perspectives on the subject. [**Int Microbiol** 2005; 8(3):189-194]

Key words: molecular techniques · microbial communities · cultural heritage

Introduction

Monuments and works of art are exposed to the effects of physical, chemical, and biological factors. The latter includes damage caused by microorganisms in a process referred to as biodeterioration. Identifying the microorganisms involved in biodeterioration is the first and a very necessary step for understanding the effects of microorganisms on cultural assets. The second step is elucidating the functional properties of these microorganisms and their role in biodeterioration. The third and final step is to use the accumulated information to devise strategies to conserve and protect monuments and works of art from microbial colonization and its consequent effects.

Until recently, the only information available on microorganisms inhabiting cultural assets came from the use of classical microbiology methods. However, those methods, based on the cultivation of microorganisms, detect only a minor fraction (less than 1%) of the total number of microbial communities [9,14,29]. There are multiple reasons for this bias: The growth requirements of many species of microorganisms are unknown. In addition, a large fraction of microorganisms in natural communities are at inactive stages of their life cycle, and thus carry out very limited metabolic activity in their environment at a specific time. Several studies have proposed solutions involving culture-independent techniques to solve the problem of detecting these types of microorganisms in natural samples [5,14,29].

Molecular biology techniques have been successfully used for the detection of microorganisms in their environments. Such techniques are based on the detection of nucleic acids, as every microorganism holds unique sequences, which allows the differentiation of microorganisms within complex microbial communities. The results of thid kind of molecular surveys have revealed that the microbial diversity on Earth is much greater than previously believed based on culturing methods [14,16,29]. As a consequence, current knowledge of microbial diversity thriving on monuments has moved away from the classical vision that only a few microbial species can grow on cultural assets. On the contrary, an enormous microbial diversity threatens our cultural heritage and we need to understand its significance in order to design appropriate strategies for controlling microbial colonization of cultural assets.

In this review, we will discuss current molecular techniques that are used in microbiological studies of our cultural assets. Novel methodologies and standard molecular protocols that facilitate the detection of microorganisms on cultural sites are described, including specific examples of the application of these techniques to protect our cultural heritage.

Molecular detection of microorganisms

Generally, the first step in the molecular detection of microorganisms consists of extracting nucleic acids from collected samples. In studies of monuments and artworks, the samples are very small (often less than 1 mg), which makes analyses difficult. Most molecular strategies used in the field have followed the basic protocol outlined in Fig. 1 and previously described [26]. The detection of microorganisms is mainly based on the sequences of the small subunit (16S for prokaryotes and 18S for eukaryotes) ribosomal RNA (rRNA) genes. This is a universal gene present in every living organism. The existence of complete DNA databases for rRNA genes guarantees optimal identification of the microorganisms detected through their sequences and the possibility of carrying out phylogenetic analysis with their closest relatives. rRNA genes are highly conserved and contain a level of divergence that allows microorganisms to be unambiguously differentiated.

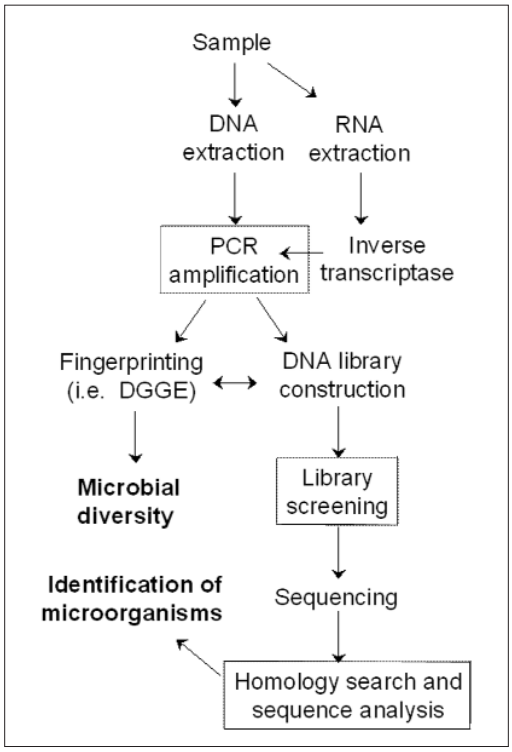

Fig. 1. Outline of a standard protocol for the molecular survey of microbial communities residing on cultural assets. Recent improvements commented in the text are boxed.

Amplification of target genes. Samples collected from cultural assets are highly limited in size, which makes many forms of analyses difficult, if not impossible to carry out. In the basic molecular protocol, specific target genes (rRNA genes) are PCR-amplified in order to obtain a large number of copies of these DNA fragments [30]. The PCR technique requires two gene-specific primers and is carried out through 25–35 thermal cycles consisting of a denaturation step, annealing of the primers, and extension of the newly synthesized DNA fragment. Currently available primers are able to target every class of microorganism within a microbial community, such as *Bacteria*, *Archaea*, or *Eukarya*, or the range of microorganisms to be detected can be restricted to group-specific amplifications. Common primer pairs used for the amplification of 16S rRNA from the Domain *Bacteria* are 27F (5′-AGAGTTTGATYMTGGCTC AG) and 1522R (5′-AAGGA GGTGATCCAGCCGCA) [21]. The 16S rRNA genes from the Domain *Archaea* can be satisfactorily amplified using the primer pair 20bF (5′-YTCCSGTTGATCCYGCSRGA) and 1492bR (5′-GGYTACCTTG TKWCGACTT) [21]. The 18S rRNA gene from the Domain *Eukarya* could be specifically detected using the primer pair EukA (5′-AACCTGGTTGATCCTGCCAGT) and EukB (5′-TGATCCTTCTGCAGGTTCACCTAC) [6]. The use of these primer pairs permits the detection and distinction of the three domains of life. Similarly, the sequences of microbial group-specific primers, e.g. for sulfate-reducing bacteria [4], nitrate-reducing bacteria [22], and methanotrophs [13], are available in the literature, and these primers can be applied in monitoring our cultural assets.

Community fingerprinting. PCR amplification products can be processed to construct a rRNA gene library or to obtain a microbial community fingerprint. In the latter, the amplified rRNA genes from different microorganisms result in different electrophoretic patterns of migration. As a consequence, the microbial community of a sample can be characterized by its electrophoretic profile, which produces a so-called microbial community fingerprint. This allows the microbial diversity in each analyzed sample to be easily visualized and compared to the fingerprint from other samples, sites, or temporal series [9].

Currently, there are several methods to obtain microbial community fingerprints from natural samples. For studies of monuments and artworks, the technique most often used is denaturing gradient gel electrophoresis (DGGE) [9]. Other techniques, such as analysis of terminal restriction fragment length polymorphisms (t-RFLP), have been frequently used in molecular surveys of microbial communities in ecological studies. DGGE analysis has been previously described in detail [20]. Briefly, it requires previous amplification of a specific

portion of the 16S (or 18S for eukaryotes) rRNA genes. These DNA fragments are then separated in a chemical denaturing gradient (formed by urea and formamide) and then amplified with a set of one of the following primers: 341F-GC (5′-tail-CCTACGGGAGGCAGCAG) and 518R (5′-ATTACCGCG GCTGCTGG) [20] for Bacteria; 344F-GC (5′-tail-ACGGGG CGCAGCAGGCGCGA) and 518R for Archaea [24]; and Euk1209F-GC (5′-tail-GCAGGTCTGTGATGCCC) and Uni1392R (5′-ACGGGCGGTGTGTRC) for Eukarya [6]. Primers 341F-GC, 344F-GC and Euk1209F-GC have a 40-bp GC-rich tail (5′-CGCCCGCCGCGCGCGGCGGGCGGGG CGGGGGCACGGGGGG), which is required to stabilize the migration of the DNA fragments during DGGE [9,20].

Experimental strategies. The need to identify microorganisms or to visualize and compare the complexity of microbial communities will determine the most appropriate experimental strategy. Microorganisms forming a given microbial community in a sample are usually identified through cloning and sequencing of the amplified PCR products obtained from the studied samples [7]. The products are cloned into adequate vectors, screened, and then sequenced. A homology search of the sequence against DNA databases provides information on the taxonomic and phylogenetic lineage of the microorganism corresponding to that sequence. The most commonly used homology search algorithm is Blast [1], which is available online at the US National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov/BLAST/].

Sequencing and visual analysis of microbial diversity provides key information on the composition of microbial communities thriving on cultural assets. Once some of the members of a given microbial community have been detected, in situ identification procedures, such as fluorescent in situ hybridization (FISH), can be used to detect specific microbial groups [2]. FISH has been recently applied in biodeterioration studies [17]. Recent advances introduced by our group to improve the above-described experimental approaches are presented below.

Recent strategies for molecular survey of microorganisms involved in the biodeterioration of cultural assets

As briefly noted above, in the conservation of artworks and monuments, the size of the sample that can be studied is extremely small, and the use of non-destructive techniques is encouraged whenever possible [27,28]. These restrictions have led to the development of novel, highly sensitive methods for the amplification of DNA fragments. While standard PCR can be carried out with a minimum amount of template DNA, natural samples often contain inhibitory substances (e.g. humic acids) that negatively affect the reaction. Recently, a two-step procedure for amplifying DNA fragments from natural samples was tested [10] (Fig. 2). In the first step, a multiple-displacement amplification (MDA) reaction is carried out. This novel pre-PCR amplification strategy consists of a non-specific amplification using random hexamers and ϕ29 DNA polymerase. The reaction is carried out at a constant temperature of 30ºC and generates non-specific genomic amplification products. In the second step, DNA generated in the first step serves as template for a PCR reaction using 16S-rRNA-specific primers, a thermostable DNA polymerase, and standard thermal conditions (see above). This procedure results in the amplification of 16S rRNA gene fragments from natural samples at concentrations 10-fold lower than used in standard PCR amplifications [11]. In addi-

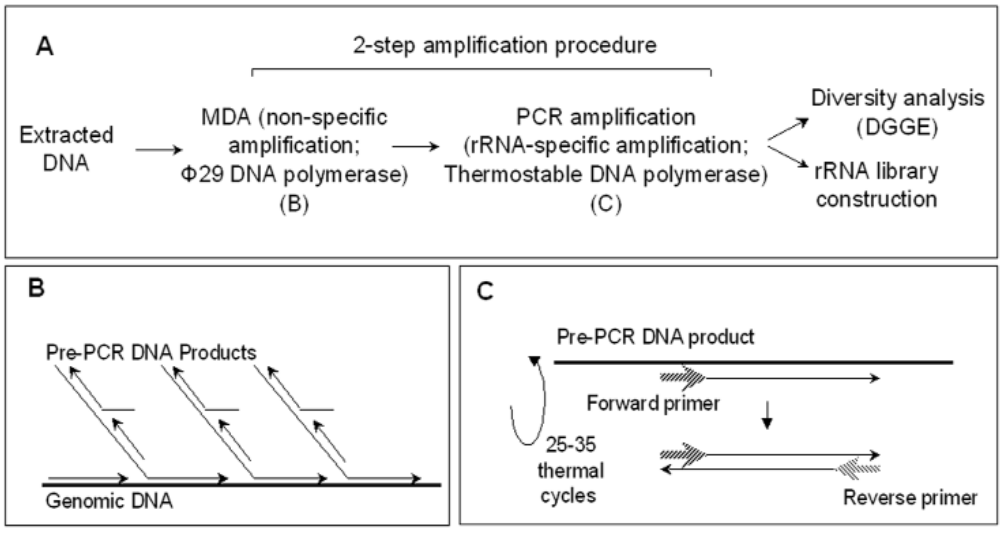

Fig. 2. Development of a two-step amplification procedure and insertion into the basic protocol for the molecular analysis of microbial communities from cultural heritage samples. (**A**) Modification of the standard protocol to accommodate a two-step amplification procedure. (**B**) Multiple-displacement amplification (MDA) using random hexamers, a constant amplification temperature, and ϕ29 DNA polymerase. (**C**) PCR amplification using rRNA -specific primers, thermal cycling conditions (denaturation, annealing, and extension), and a thermostable DNA polymerase. The pre-PCR DNA product obtained during the first step (MDA reaction) is the template used during the second step (PCR amplification).

Int. Microbiol.

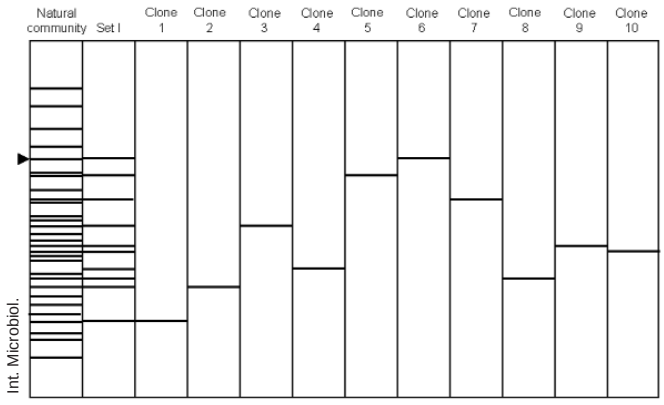

Fig. 3. An outline of the screening strategy proposed by Gonzalez et al. [8] for the selection of single clones from complex rRNA libraries. The example compares a theoretical microbial community fingerprint obtained from a natural sample with a set of ten clones in which the clone of interest (arrow) has been identified. The individual bands correspond to the analysis of the individual clones (clones number 1-10) included in the selected set. In this example, the desired clone is clone 6, which would be selected for sequencing and further analysis.

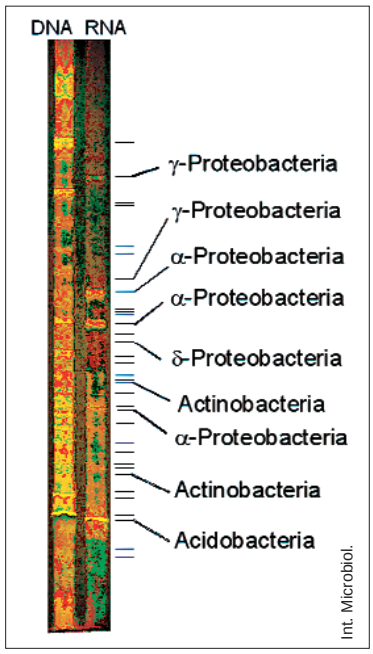

Fig. 4. Comparative DGGE analysis of microbial community fingerprints based on DNA and RNA from the same sample point. RNA-based analysis focuses on specific, active microorganisms, while DNA-based analysis shows a biodiversity too complex for reasonable in-depth study. Microorganisms identified from the RNA-based analysis are reported, while the bars on the right of the fingerprints indicate microorganisms identified from DNA-based analysis.

tion, amplification can be carried out at concentrations of PCR inhibitory substances that are at least 10-fold higher than those completely inhibiting standard PCR amplifications. Thus, the two-step procedure greatly improves the molecular detection of microorganisms using minute samples, even if they contain a high proportion of PCR-inhibiting compounds.

After successfully obtaining the desired PCR products, microorganisms forming the studied microbial community must be identified. This is done by constructing a 16S rDNA library and then following a generally time-consuming screening process to select different clones while avoiding replicated copies. Scientists are not usually interested in identifying all of the microorganisms in complex microbial communities, but rather in a few that are most representative of a specific process. This can readily be done by comparative DGGE analysis of several samples [9]. Furthermore, compared to the standard procedure, which calls for the analysis of individual clones, savings in labor and costs of up to 90% can be gained by processing the clones in groups [8]. The clones are initially organized in sets (generally 10 clones per set), which are then PCR-amplified and analyzed by DGGE (Fig. 3). Once a set that includes the clone of interest is detected, clones forming this set are analyzed individually. Finally, the selected clone is processed for sequencing and analyzed as described above.

Using DGGE, the microbial fingerprints of different communities can be easily compared. In addition, by the end of the screening procedure [8], the DNA bands from a community fingerprint can be assigned to their corresponding microorganisms. Recent improvements in molecular studies have shown the advantages of RNA-based molecular analyses [7], RNA-based analyses require the synthesis of a DNA (cDNA) complementary to the RNA extracted from the studied samples. The cDNA is obtained by a reaction using an inverse transcriptase and either a single 16S rRNA-specific primer or a random-hexamers priming alternative [3]. In an RNA-based approach, not only the presence of a species of microorganism but also its metabolic activity [19] can be determined, since the levels of RNA in a cell are proportional to the need of that cell for synthesizing proteins required for metabolism. This type of microbial community survey thus provides information on the fraction of the microbial community actually involved in the metabolic activity of a given sample. Consequently, microorganisms comprising that community are the ones directly responsible for any biodeterioration processes occurring on the artwork under study. Figure 4 shows a comparative DGGE analysis of community fingerprints based on DNA and RNA samples obtained from microorganisms in Altamira Cave. RNA-based fingerprints simplify the study, since the number of active microorganisms is generally much lower than the total number of microorganisms present in a sample. The drawback to RNA-based molecular detection of microorganisms is that, since RNA is very labile due to the omnipresence of RNases, the procedure is complicated and requires the use of extremely clean facilities and extra care in sample handling and amplification.

Among the sequences obtained in molecular microbial surveys, the generation of chimeric sequences has been frequently reported [15]. Chimeras, or sequences with a chimeric origin, are those originating from two different DNA templates, thus representing non-existing microorganisms. Chimeras can lead to false results and so controls for their detection should be included in studies that evaluate biodeterioration of cultural

assets. Recently, two novel procedures and computer programs for this purpose have become available [11,15].

Homology searches and phylogenetic analyses permit the identified microorganisms to be grouped according to similarities in their sequences (mainly using their rRNA genes). Since phylogenetically similar microorganisms tend to have relatively comparable metabolisms, molecular detection is generally used to infer the potential roles of microorganisms in biodeterioration. This is often a valid alternative in the absence of other sources of information; however, this inference implies certain risks since related microorganisms do not always show similar metabolism.

Future strategies and perspectives

Strategies aimed at conserving and protecting artworks and monuments are rapidly adapting molecular techniques for the detection of microorganisms. While these techniques have developed very quickly over the last few years, many problems nonetheless remain. For example, linking function to phylogeny is a point of interest in molecular microbial surveys [12], and novel approaches are being proposed [18]; however, they have not yet been applied to microorganisms involved in the biodeterioration of cultural assets. Moreover, while it is a common practice to infer phylogeny from function, and is an appropriate application when comparing highly related microorganisms, it may be risky in microorganisms with increasing divergence.

Microbial surveys carried out using molecular techniques have revealed the enormous microbial diversity of our planet. The long-held perspective classifying the living world into five Kingdoms has evolved into a far more complicated perspective [31]. At present, 52 bacterial divisions (excluding the Archaea) [23] have been discovered, 26 of which consist of microorganisms detected only by molecular methods, with no cultured representatives. Of those 52 divisions, 13 comprise less than five cultivated species. An example is the Acidobacteria; although nearly a thousand sequences are deposited in DNA databases, only three species have been cultured and taxonomically described [14]. Considering that cultivation is still required for studying the physiology and metabolism of bacterial species, it is easy to understand the need for complementary approaches. The combination of molecular techniques and culturing methods facilitates the study of natural microbial communities, including those involved in the biodeterioration of artwork and monuments.

Developments mainly borrowed from biomedical sciences, such as the use of DNA microarrays, are being incorporated into the analysis of microbial communities. For example, 16S rDNA microarrays [25] containing nucleic acid probes targeting most of the microbial species expected in a sample allow the rapid detection of known microbial species. Obviously, since sequence information from unknown microorganisms cannot be incorporated into such microarrays; their usefulness is restricted [9].

Finally, in the last few years, studies aimed at assessing biodeterioration of monuments and artworks have not only been improved by incorporating techniques developed in other scientific fields, they have also exported molecular methods, such as those mentioned herein, which have applications in microbial ecology surveys and clinical analyses. These novel approaches are improving our understanding of microbial communities thriving on cultural assets which is essential for the development of strategies for conserving our cultural heritage.

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References

1. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403-410
2. Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143-169
3. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1992) Short protocols in molecular biology. Wiley, New York
4. Daly K, Sharp RJ, McCarthy AJ (2000) Development of oligonucleotide probes and PCR primers for detecting phylogenetic subgroups of sulfate-reducing bacteria. Microbiology 146:1693-1705
5. DeLong EF (2001) Microbial seascapes revisited. Curr Opin Microbiol 4:290-295
6. Diez B, Pedros-Alio C, Marsh TL, Massana R (2001) Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. Appl Environ Microbiol 67:2942-2951
7. Gonzalez JM (2003) Overview on existing molecular techniques with potential interest in cultural heritage. In: Saiz-Jimenez C (ed) Mol Biol Cultural Heritage, Balkema, Lisse, pp 3-13
8. Gonzalez JM, Ortiz-Martinez A, Gonzalez-delValle MA, Laiz L, Saiz-Jimenez C (2003) An efficient strategy for screening large cloned libraries of amplified 16S rDNA sequences from complex environmental communities. J Microbiol Methods 55:459-463
9. Gonzalez JM, Saiz-Jimenez C (2004) Microbial activity in biodeteriorated monuments as studied by denaturing gradient gel electrophoresis. J Separ Sci 27:174-180
10. Gonzalez JM, Portillo MC, Saiz-Jimenez C (2005) Multiple displacement amplification as a pre-PCR reaction to process difficult to amplify samples and low copy number sequences from natural environments. Environ Microbiol 7:1024-1028
11. Gonzalez JM, Zimmermann J, Saiz-Jimenez C (2005) Evaluating putative chimeric sequences from PCR amplified products and other crossover events. Bioinformatics 21:333-337
12. Gray ND, Head IM (2001) Linking genetic identity and function in communities of uncultured bacteria. Environ Microbiol 3:481–492
13. Horz HP, Yimga MT, Liesack W (2001) Detection of methanotroph diversity on roots of submerged rice plants by molecular retrieval of

pmoA, mmoX, mxaF, and 16S rRNA and ribosomal DNA, including pmoA-based terminal restriction fragment length polymorphism profiling. Appl Environ Microbiol 67:4177-4185
14. Hugenholtz P, Goebel BM, Pace NR (1998) Impact of Culture-independent studies on the emerging phylogenetic view of bacterial diversity. J Bacteriol 180:4765-4774
15. Hugenholtz P, Huber T (2003) Chimeric 16S rDNA sequences of diverse origin are accumulating in the public databases. Intl J Syst Evol Microbiol 53:289-293
16. Laiz L, Gonzalez JM, Saiz-Jimenez C (2003) Microbial communities in caves: Ecology, physiology, and effects on paleolythic paintings. In: Koestler RJ, Koestler VR, Charola AE, Nieto-Fernandez FE (eds) Art, biology, and conservation: Biodeterioration of works of art. The Metropolitan Museum of Art, New York pp 210-225
17. La Cono V, Urzì C (2003). Fluorescent in situ hybridization (FISH) applied on samples taken with adhesive tape strips. J Microbiol Methods 55:65-71
18. Manefield M, Whiteley AS, Griffiths RI, Bailey MJ (2002) RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. Appl Environ Microbiol 68:5367-5373
19. Molin S, Givskov M (1999) Application of molecular tools for *in situ* monitoring of bacterial growth activity. Environ Microbiol 1:383-391
20. Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695-700
21. Orphan V J, Hinrichs K-U, Ussler III W, Paul CK, Taylor LT, Sylva SP, Hayes JM, DeLong EF (2001) Comparative analysis of methane-oxidizing Archaea and sulfate-reducing bacteria in anoxic marine sediments. Appl Environ Microbiol 67:1922-1934
22. Petri R, Imhoff JF (2000) The relationship of nitrate reducing bacteria on the basis of *narH* gene sequences and comparison of *narH* and 16S rDNA based phylogeny. Syst Appl Microbiol 23:47-57
23. Rappé MS, Giovannoni SJ (2003) The uncultured microbial majority. Ann Rev Microbiol 57:369-394
24. Raskin L, Stromley JM, Rittmann BE, Stahl DA (1994) Group-specific 16S rRNA hybridisation probes to describe natural communities of methanogens. Appl Environ Microbiol 60:1232-1240
25. Rudi K, Flateland SL, Hanssen JF, Bengtsson G, Nissen H (2002) Development and evaluation of a 16S ribosomal DNA array-based approach for describing complex microbial communities in ready-to-eat vegetable salads packed in a modified atmosphere. Appl Environ Microbiol 68:1146-1156
26. Schabereiter-Gurtner C, Pinar G, Lubitz W, Rolleke S (2001) An advanced molecular strategy to identify bacterial communities on art objects. J Microbiol Methods 45:77-87
27. Urzì C, De Leo F (2001) Sampling with adhesive tape strips: an easy and rapid method to monitor microbial colonization on monument surfaces. J Microbiol Methods 44:1-11
28. Urzì C, De Leo F, Donato P, La Cono V (2003) Study of microbial communities colonizing hypogean monument surfaces using destructive and non-destructive sampling methods. In: Koestler R, Koestler VH, Charola AE, Nieto-Fernandez FE (eds) Art, biology, and conservation: biodeterioration of works of art. The Metropolitan Museum of Art New York, pp 316-325
29. Ward DM, Weller R, Bateson MM (1990) 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature 345:63-65
30. White BA (ed) (1993) PCR protocols, current methods and applications. Humana Press, Totowa, NJ
31. Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms. Proposal for the domains Archaea, Bacteria and Eukarya. Proc Natl Acad Sci USA 87:4576-4579

Aplicación de técnicas moleculares basadas en ácidos nucleicos al estudio de comunidades microbianas en monumentos y obras de arte

Resumen. Los microorganismos desempeñan papeles críticos en todo tipo de hábitat de la Tierra, incluso en los que han sido construidos por los humanos. Así, el patrimonio cultural también se ve afectado por la colonización de microorganismos. Mientras los métodos clásicos de microbiología basados en procedimientos de cultivo han proporcionado información importante, pero limitada, de las muestras naturales, las nuevas técnicas moleculares son muy valiosas para descubrir la diversidad de la microbiota causante del biodeterioro de monumentos y obras de arte. El conocimiento alcanzado con estos métodos ha permitido diseñar estrategias adecuadas para la conservación y protección de monumentos, en beneficio de generaciones futuras. Esta revisión describe la situación actual de la aplicación de métodos moleculares al análisis de bienes culturales y aporta perspectivas para un futuro próximo. [**Int Microbiol** 2005; 8(3):189-194]

Palabras clave: técnicas moleculares · comunidades microbianas · patrimonio cultural

Aplicação de técnicas moleculares basadas em ácidos nucleicos ao estudo de comunidades microbianas em monumentos e obras de arte

Resumo. Os microorganismos desempenham papéis críticos em todo tipo de hábitat da Terra, inclusive aquelos que foram construídos pelos humanos. Assim, o patrimônio cultural também se vê afetado pela colonização de microorganismos. Enquanto os métodos clássicos de microbiologia baseados em procedimentos de cultivo proporcionaram informação importante, mas limitada, das amostras naturais, as novas técnicas moleculares são muito valiosas para descobrir a diversidade da microbiota causadora do biodeterioro de monumentos e obras de arte. O conhecimento alcançado com estes métodos permitiu desenhar estratégias adequadas para a conservação e proteção de monumentos, em benefício de gerações futuras. Esta revisão descreve a situação atual da aplicação de métodos moleculares à análise de bens culturais e contribui perspectivas para um futuro próximo. [**Int Microbiol** 2005; 8(3):189-194]

Palavras chave: técnicas moleculares · comunidades microbianas · patrimonio cultural