Saving a fragile legacy

Biotechnology and microbiology are increasingly used to preserve and restore the world's cultural heritage

Science and technology interact with art and culture in many ways. Both involve knowledge and investigation—some philosophers would maintain—and nature and its representation lie at their core (Frazzetto, 2004). But their interactions extend further: the arts draw both inspiration and new materials from science, while the scientific examination of art and artefacts has provided us with important insights into the progress of human civilizations. Now, science—in particular the biological sciences—might have an even more important role: to protect and conserve mankind's often fragile, cultural heritage for future generations. This task is particularly difficult given the magnitude and diversity of objects involved, and the enormous variety of different materials used—for example, stone, metal, ceramics, synthetic substances, and organic matter derived from plants and animals.

A classic example of art under threat is found in the cave of Lascaux in southwest France. Discovered accidentally in 1940, the cave contains some of the finest paleolithic drawings and polychrome rock paintings in the world, dating back some 17,000 years. Herds of giant aurochs and other wild mammals—realistically depicted in vivid colours—still seem to roam the prairies while seeking salvation from hunting men (Fig 1). These strikingly beautiful images testify to the birth of humans using the abstract to represent reality, and the location was listed as a UNESCO World Heritage site in 1979. But an invading army of fungi, bacteria, algae and moss—their lives made easier by disputing conservators—threatens the artwork.

The paintings remained intact and fresh until the cave was exposed to tourists after the Second World War, when the rise in temperature and humidity caused a burst of microbial growth, which was first noticed in 1955. The French authorities—worried about the future of the paintings—closed the cave to the public in 1963; a duplicate cave with facsimile frescoes was opened in 1983. Without the negative effects of breathing and sweating visitors, the situation in the original cave stabilized until 2000, when a new air-conditioning system was installed. Within months, a devastating infection of *Fusarium* fungus and other molds covered the floor and banks of the main decorated chamber.

Fig 1 | White aurochs, Hall of the Bulls, Lascaux (Montignac, France). Image courtesy of Wikimedia Commons.

> Science and technology interact with art and culture in many ways

Controversy spread as rapidly as the fungi, and was further fed by national and international media coverage. Who was at fault this time, if tourists were not to blame? More importantly, what should be done to remove this new infestation? The ill-conceived air-conditioning system soon came under scrutiny as the direct cause of the cave's climate change. The Laboratoire de Recherche des Monuments Historiques in Champs-sur-Marne, France, was charged with developing a research programme to disclose the precise identity of the microbial invaders of the cave. Restorers applied fungicides, antibiotics, ammonium disinfectants and quicklime, but the results of these treatments have not been fully disclosed and the current status of the cave paintings remains unclear.

Last June, Lascaux curator Jean-Michel Geneste told the *Wall Street Journal* that there was no danger to the paintings and that the microbial growth had disappeared naturally (Rosenbaum, 2006). But Laurence Léauté Beasley, founder and chair of the US-based International Committee for the Preservation of Lascaux (ICPL), has a very different opinion. "The fungus is still present in the cave. Art restorers continue to manually pluck the roots [mycelia] of the fungus from the affected paintings. However, as the fungus is removed, dark and grey spots are left," she said. Léauté Beasley added that new black spots have appeared in large numbers near the entrance of the cave. "To date, they have not been reported by authorities for scientific analysis," she said. "Calcite is growing on

Table 1 | Projects involving novel microbiological tools for the conservation of cultural heritage funded under the European Union's Fifth Framework Programme

Project	Description	Cost (€)	Time span
BACPOLES	Preserving cultural heritage by preventing bacterial decay of wood in foundation poles and shipwrecks	1.75 million	2002–2005
BIOBRUSH	Novel approaches to conserve our European heritage: bioremediation for building restoration of the urban stone heritage in European states	1.74 million	2002–2005
BIODAM	Inhibitors of biofilm damage on mineral materials	1.44 million	2002–2005
BIOREINFORCE	Biomediated calcite precipitation for monumental stone reinforcement	5.48 million	2001–2004
CATS	Cyanobacteria attack rocks: control and preventive strategies to avoid damage caused by cyanobacteria and associated microorganisms in Roman subterranean monuments	1.68 million	2001–2003
COALITION	Concerted action on molecular microbiology as an innovative conservation strategy for indoor and outdoor cultural assets	2.52 million	2000–2003

Source: European Commission's Research DG, http://ec.europa.eu/research

some of the unpainted walls; some colour tones in the paintings are fading and ladders of workmen lean against unpainted walls, breaking off ledges of calcite."

Léauté Beasley criticized the French authorities for not using every means possible—including modern science—to deal with the fungi and preserve the original paintings. "The serious science needed to preserve the cave has been hampered by French bureaucracy," she remarked. "One must ask why French authorities are employing art restorers, not scientists and microbiologists, to remove fungus from the paintings. There is a real and present danger to the survival of world heritage when governments disregard and stand in the way of competent science and technology."

Unfortunately, Lascaux is not the only case in which a cultural treasure is suffering from microbial invaders. Other paintings share the same fate, such as those in the Altamira cave in Santillana del Mar, Spain, and the earliest known Christian paintings that adorn Roman catacomb walls. To rescue these and other cultural testimonies, curators might team up with new kinds of scientists. "In the last decade, chemistry, physics and material science have been important for many aspects of cultural heritage conservation, like the use and optimization of analytical and examination techniques," said Francesca Cappitelli, a microbiologist working on art conservation at the University of Milan, Italy. "Recently, however, biotechnology has surely dominated the scene." It is used not only to study the organisms that gnaw away at cultural heritages, but also to clean, recover and consolidate works of art (González & Saiz-Jiménez, 2005; Cappitelli *et al*, 2006a). "In the near future, the expanding frontiers of biotechnology in conservation will include, as a few examples, the control of quorum sensing to avoid the formation of biofilms on cultural heritage objects and the use of single-cell biosensors for the museum environment monitoring and control," she said.

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Placing trust in biotechnology and microbiology to help conserve its cultural heritage—which has a significant impact on the economy of many nations—the European Union is supporting a number of innovative projects. These range from the development of new methods to preserve waterlogged archaeological wood (see sidebar), to finding new inhibitors of microbial growth and preventive strategies to avoid the formation of biofilms, which damage stones in historical buildings and subterranean monuments (Table 1).

But it is not only in Europe where historic and archaeological artefacts need protection. The Mayan archaeological sites in southern Mexico and Guatemala, for example, are the remnants of a highly developed civilization that flourished for many centuries. Reaching its peak around 700 AD, Mayan society thrived with intellectual and artistic prowess, proclaimed by a sophisticated writing system and by great cities such as Tikal, Chichén Itza and Palenque, which are dominated by magnificent pyramids and palaces adorned with carved stones (Figs 2,3).

Much of the material that the Mayans used to construct their buildings is soft limestone. Its mechanical weakness, combined with the tropical forest's high humidity and temperature, which favours microbial growth, poses great challenges for the conservation of these structures. A research group led by Ralph Mitchell at Harvard University (Cambridge, MA, USA) is investigating the role of microflora in the degradation of limestone at several Mayan sites. Various metabolites produced by microbial biofilms—such as organic and inorganic acids, substrate-adhering exopolymers and other exudates—might cause the direct dissolution of different stone mineral structures, and the chelation and leaching of calcium and other cations, or affect the monument's aesthetic value through discoloration or pigmentation. Moreover,

Fig 2 | The South Tower of Structure I at the Mayan archaeological site at Xpujil in Yucatán, Mexico. Photograph by Holger Breithaupt.

increasing air pollution further stimulates biodeterioration, although the details of this interaction are not fully understood.

In a recent study conducted at the Ek' Balam site in Yucatán, Mexico, researchers extracted total flora community DNA from stone samples and constructed a clone library to obtain a detailed picture of the microbial diversity that lives on and in Mayan ruins (McNamara *et al*, 2006). Their analysis indicated substantial differences between the epilithic and endolithic communities: Proteobacteria dominated the surface, along with a large number of Actinobacteria and photosynthetic microorganisms, whereas Actinobacteria, Acidobacteria and Firmicutes permeated the ruins. The previous studies on the biodeterioration of Mayan monuments were carried out using microbial culture methods and microscopy, whereas the molecular techniques applied at Ek' Balam provided a more accurate description of the microflora involved, especially the phyla that penetrate deep into the limestone. "The presence of differing epilithic and endolithic bacterial communities may be a significant factor for conservation of stone cultural heritage materials and quantitative prediction of carbonate weathering," the authors wrote (McNamara *et al*, 2006).

Fig 3 | Entrance to Structure I at the Mayan archaeological site at Xpujil in Yucatán, Mexico. Photograph by Holger Breithaupt.

> Although microbes are the problem, they can also be the solution

Studies such as those conducted on Mayan buildings have the ultimate aim of developing strategies to arrest and possibly reverse the biodeterioration of stone. That is not an easy task, particularly when the objects are outdoors, but several promising approaches have emerged. To some extent, regular cleaning can prevent the formation of biofilm and subsequent degradation of lithic materials. In the long term, the application of biocides and consolidating agents might be an effective treatment to counteract the biodeterioration of historic buildings and monuments. However, although it has been common practice for quite some time, the use of biocides can cause serious environmental problems. Moreover, these chemicals generally lack specificity for selected microorganisms; biofilm bacteria tend to have a lower susceptibility to these agents, and a surge of microbial resistance is possible, which could render the application ineffective. Consolidation of archaeological stone—preserving the physical integrity and mechanical performance to resist biological, chemical and physical weathering—can be achieved with a coat of slaked lime or by using ethoxysilanes and acrylic resins, but most polymers that are commercially available are still susceptible to biodegradation. A viable solution might be to tailor appropriate combinations of biocides and consolidating agents to monuments with a specific and well-defined chemical, physical and microbiological environment.

Although microbes are the problem, they can also be the solution. Several research groups have shown that the anaerobic sulphate-reducing bacteria *Desulfovibrio desulfuricans* and *D. vulgaris* can efficiently remove the black sulphate crusts that often tarnish buildings (Webster & May, 2006). Other studies reported that the bioformation of oxalic acid could generate a protective calcium oxalate patina on stone surfaces (Garcia-Valles *et al*, 1997).

Biomineralization is another emerging interdisciplinary research field with high applicative potential in the consolidation and restoration of deteriorated ornamental stone. Several bacterial strains have been shown to precipitate calcium carbonate—a process known as carbonatogenesis—mainly in the form of calcite, which can form a protective layer on the surface of weathered stones and even penetrate the stone matrix to act as a bioconsolidating cement (Rodriguez-Navarro *et al*, 2003; Webster & May, 2006). Other strains, such as the microbe *Shewanella oneidensis* MR-1, inhibit the rate of calcite dissolution under laboratory conditions (Lüttge & Conrad, 2004).

Given the widespread occurrence of carbonate stone—such as limestone, dolostone and marble—in architectural and sculptural artworks, the potential use of biomineralization and bioremediation as supplemental conservation technologies is of great importance (Table 1). "Although the technology is still in its infancy and, therefore, not readily available, the results so far indicate that it promises to offer a viable alternative to those working to preserve our cultural heritage," noted microbiologists Alison Webster and Eric May from the University of Portsmouth, UK (Webster & May, 2006).

Art has long since broken the boundaries of natural materials—such as wood, plant and mineral pigments, and stone—to explore new forms of expression by moulding man-made materials. Many contemporary art objects now include, or are made entirely from, plastic and synthetic fibres. Despite their

ANCIENT SHIPS IN TROUBLED WATERS

An acid ghost haunts old shipwrecks even after they have been saved from their marine graves. 'Time capsules', such as the Swedish warship *Vasa* and the Tudor flagship *Mary Rose*, are being corroded by tonnes of sulphuric acid that accumulated in their timber during their rest on the seafloor. This creates a huge challenge for conservationists to prevent these magnificent ships from being destroyed.

The *Vasa*, as it appears today after conservation treatment in the Vasa Museum, Stockholm, Sweden. By March 2005, more than 2,000 areas of acidified wood and visible precipitates of salts had been identified on the warship. Photograph by Hans Hammarskiöld/Vasa Museum.

Commissioned by King Gustav II Adolf in 1625, the *Vasa* was one of the mightiest warships of its age, bursting with an impressive battery of 64 guns on its 61-m, 1,210-tonne hull. But the ship lacked stability and never made it to battle—it sank in Stockholm harbour on its maiden voyage on 10 August 1628. The *Vasa* was eventually rediscovered in 1956 at a depth of 32 m and raised five years later. After the first phase of drying and stabilizing the old oak timbers, it soon appeared that the general state of conservation was excellent, thanks to the anoxic environment that prevented any biodegradation of the wood. Swedish experts began to preserve this fascinating example of seventeenth century naval carpentry (left). Salvage measures included inserting some 8,500 iron bolts to replace the originals that had rusted away, and spraying the wood with aqueous solutions of polyethylene glycol (PEG). This water-soluble, slowly evaporating inert polymer penetrates waterlogged wood, replacing the water to prevent shrinkage and the formation of cracks while the wood is drying. PEG treatment is now routine for the treatment of ancient shipwrecks, but when it was first used on the *Vasa* no one knew whether the method could be successfully applied on such a large scale.

Nothing apart from ordinary conservation work occurred for about a decade after the ship was put on display in 1990 in the Vasa Museum in Stockholm. However, in 2000, areas of acidified wood and deposits of sulphates became apparent, giving rise to a general alarm and an in-depth inspection of the ship's conditions. X-ray analyses, performed by a team led by Magnus Sandström at the University of Stockholm, has identified gypsum and other crystalline hydrated sulphates on the wooden surfaces, and revealed that about two tonnes of sulphur, in various reduced forms, is slowly oxidizing to sulphuric acid (Sandström *et al*, 2002), which could sink the *Vasa* forever.

Apparently, bacteria in the anoxic water and on or below the sea floor reduced sulphate to hydrogen sulphide, which penetrated the wood and was oxidized—either chemically or bacterially—to elemental sulphur. Once the *Vasa* was raised from the sea and exposed to the air, the sulphur slowly began to oxidize to sulphuric acid, in a process probably catalysed by the iron released from both the original and the newly inserted bolts. Paradoxically, what preserved the *Vasa* in the cold brackish waters of Stockholm harbour is now the cause of its deterioration.

In 2003, the international 'Preserve the *Vasa*' project was launched to remove or stabilize the sulphur and iron compounds, neutralize the acid, and prevent more from forming. One of the key questions is whether the breakdown process is influenced by the bacteria present in the wood microenvironment. "By using molecular techniques and traditional culturing techniques we were able to demonstrate the presence of a diverse and active bacterial community within the *Vasa*'s timbers," said Sarah Hotchkiss from the University of Portsmouth, UK. Her research team identified the presence of RNA signature molecules for heterotrophic bacteria, potential cellulolytic and PEG-degrading bacteria, sulphur oxidizers and even enteric bacteria. "What we have not demonstrated as yet is whether the activity of these organisms is of direct concern to the future conservation of the *Vasa*," said Hotchkiss.

At the time of sampling, she explained, potentially dangerous bacteria—that is, acid-producing and cellulolytic strains—were present in relatively low abundance. But bacteria have the capacity to increase rapidly in number when conditions are favourable, so attention must be continuous. "One thing that is so difficult with this kind of research is that we are very limited in the samples that can be taken," said Hotchkiss. "Obviously, the last thing we want is to start drilling holes all over something as precious as the *Vasa* or *Mary Rose* and so we must be aware of this when interpreting results from a limited number of samples."

King Henry VIII's warship *Mary Rose*, foundered in 1545 outside Portsmouth, and was partially recovered in 1982 (right). It experienced post-conservation problems similar to those of the *Vasa*, and research is now being conducted in parallel on these and other shipwrecks (Fors & Sandström, 2006). "The work will probably be a constant struggle against the ravages of time and acid, but the *Vasa* is well worth all efforts," wrote Sandström and colleagues in an online account of their work on the *Vasa* (www.fos.su.se/~magnuss/). "The *Vasa* may be under acid attack, but she will prevail in her first real battle!"

The hull of the *Mary Rose*, now at the Portsmouth Historic Dockyard (UK), being sprayed with polyethylene glycol solution. Photograph by Farideh Jalilehvand, University of Calgary, Canada.

Fig 4 | *Futuro*, designed in 1965 by Matti Suuronen, showing evidence of microbial growth. Images courtesy of Tim Bechthold (Museum Die Neue Sammlung, Munich, Germany) and Francesca Cappitelli (Cappitelli *et al*, 2006b). Reprinted with permission from Elsevier.

design, these modern materials are not indestructible; what is surprising is that they are prone not only to physical and chemical damage but also to microbial attacks. This 'appetite for culture' sometimes has serious consequences. "The microbial contamination of modern materials in contemporary collections is still an underestimated concern," wrote Cappitelli and colleagues in a recent overview of this aspect of cultural heritage conservation (Cappitelli *et al*, 2006b). She added, "while it is possible that Renaissance masterpieces will remain for many other centuries, a conspicuous part of contemporary art made of modern materials will be lost in the next few decades."

> Whether new or old, inside or out, two-dimensional or three-dimensional, human art and artefacts are part of our common heritage and must be preserved for future generations

Cappitelli and her group focus on the microbial communities growing on synthetic polymers, the genes encoding enzymes involved in the degradation of these polymers and the mechanisms of their deterioration. Recently, they studied *Futuro*, a 'space age' mobile house designed in 1965 by the Finnish architect Matti Suuronen. Made from fibreglass-reinforced polyester plastic with Perspex windows, *Futuro* gained considerable notoriety owing to a mix of innovative features including serial production. One example of the structure, hosted in a private collection but looked after by the art conservators of the Museum Die Neue Sammlung in Munich, Germany, now shows extensive damage caused by microbes. Coupling *in situ* hybridization of fluorescent nucleic acid probes against specific microbial ribosomal RNAs and epifluorescence microscopy, Cappitelli and colleagues were able to identify Cyanobacteria and Archaea as the microbes to blame (Fig 4; Cappitelli *et al*, 2006b). "The aim of these studies is to provide effective tools to avoid microbial colonization of synthetic polymeric surfaces," Cappitelli said. "For example, in fighting biofilm formation, we are now testing several products of natural origin to be used as antifouling agents."

Whether new or old, inside or out, two-dimensional or three-dimensional, human art and artefacts are part of our common heritage and must be preserved for future generations. To stop them from rotting away or falling apart, scientific efforts from all quarters are needed, unhindered by national pride. "Lascaux does not belong solely to France or Europe, although it rests in their soil. The past, Lascaux, belongs to all mankind," wrote the ICPL (2005). To state that cultural heritage is common property is of course a noble approach, but it also requires a common sense of responsibility, which calls for unrestricted, interdisciplinary and international efforts to protect this legacy.

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