Saving A Fragile Legacy: Big History And Biotechnology

Priya Sundarrajan

Big History is a fast-emerging study that provides a holistic approach for inquiry into the Earth and all its constituent parts, from the big bang to the present-day. Its method is multidisciplinary and combines evidences from humanities, science, commerce and other fields, so as to understand the development of Earth and facets of its evolution – physical, chemical, biological. Its scope of study also includes human history.[1]

Advances in science and technology have been crucial to a variety of fields in the last fifty years. These gains have been especially noticeable in areas like physics and chemistry, which have seen huge breakthroughs in electronics and drug therapy, or in biological sciences, as with recent developments in bioengineering. Big history is among these milestones in thinking.

In the area of social studies, the civilizations that have existed on Earth, from the Mycenaean Greeks and New Kingdom Egyptians to the Indus Valley Civilization, have all left behind traces of their existence, in the form of early paintings, tools, and monuments. This allows us to study the evolution of their societies and help us date back human advances, such as early Neanderthal art, along with their fossil record.

1 David Christian 1991; David Christian and William McNeill 2008; David Christian et al. 2013.

The surviving heritage is invaluable but fragile, so its conservation is of great importance as a legacy for our future.[2]

As a result, preservation is an important job for anthropologists, historians and other scholars of humanity. Furthermore, knowing what we have learned about the adverse impacts of chemicals and other products on life, it is imperative to find a sustainable way to do this conservation. The techniques and materials must not have negative consequences or impede future treatments. In other words, the methods need to be sustainable and environmentally friendly. As a biologist and biotechnologist, I ask: Can Big History make an impact in the field of heritage conservation?

The Problem: Artefact Degradation

Degradation of artefacts, as well as natural calamities, lead to their loss, and indeed many items are in a state of decomposition today. A major cause is due to the nature of the artefacts themselves, since they are often made of fragile organics like paper, cloth or wood. In addition, polishing and repairs have frequently been done in earlier times with animal or plant-based materials, such as oils, waxes or glues. These overlays act as ideal ecological niches in which microorganisms can grow, and their metabolic products, like acids, inorganic salts and sulphates, result in decay. Increasing levels of pollution in the environment, as well as climate change, cause more decline from sulphates, hydrocarbons, nitrates and other contaminants.

Even stone artefacts undergo natural aging and progressive deterioration, which alters their originality and aesthetic value. Over the years, increasing levels of tourism have resulted in more rapid deterioration.[3] Organisms that grow on stone can be of two types – autotrophs (bacteria, algae, lichens or mosses) that live on inorganic nutrients in the stone and heterotrophs (bacteria and fungi) that feed on organic substances and use the stone as a support on which to grow. Many of these microbes form biofilms over the artefacts and excrete toxins and substances that lead to deterioration of the heritage materials.

2 There are several big historians who work in the area of human studies, see, for example, the following. Barry Rodrigue 2014; Craig Benjamin 2015; Shweta Sinha Deshpande 2016; Sada Mire 2016; John Mears 2016; Kathy Schick and Nicholas Toth 2017.

3 European Molecular Biology Organization (EMBO) reports, 2006

The application of physical, chemical and material science techniques in the restoration and conservation of heritage materials has been practised for a long time. However, these methods can have serious side-effects, since the chemicals that have been used can be toxic to the conservation technicians, causing adverse effects to their health and on the environment. Moreover, the chemicals may damage and corrode the artefacts in different ways.[4]

Since the beginning of twentieth century, biotechnology-based methods have been used to diagnose deterioration of heritage materials and develop strategies for sustainable conservation.[5] Microbes are omnipresent in the world, but they can only be observed under a microscope. Molecular biology and biotechnology have been used to identify new and uncultured microorganisms growing on artefacts. Genomic DNA analysis of these microbes have proven to be very useful in identifying microbial particles in the bio-aerosols of indoor environments that can pose a potential threat to heritage workers and tourists.[6]

Let us look at some of the bio-based strategies used in conservation and restoration:

> Bio-Cleaning: Microorganisms are often responsible for the deterioration and destruction of artefacts, but they can also be used for preservation.[7] As the name suggests, bio-cleaning employs microbes or the products synthesized by them on the surface of artefacts to remove stains, rust and sulphur deposits, as well as calcite/calcium-carbonate deposits in clefts. This treatment dates back to 1970, when enzymes in buffer solutions were applied to erode animal glues from pages of books. Later, lipases were employed to remove acrylic coatings from paintings, which had been applied in earlier times as protective layers. The bacteria, *Desulfovibriae vulgaris*, was successfully applied to the surface of Carrara marble in the Florence Cathedral (Italy) to clean black-sulphur crust. The method proved to be

4 Barbabietola, et.al, 2012, European Molecular Biology Organization (EMBO) reports, 2006.

5 Palla 2012.

6 Palla et al. 2003, 2007, 2013.

7 Eleonora Balliana 2016.

efficient and convenient, as the activity of the bacteria could be controlled, preventing further damage to the marble.[8] Thus, the destructive activity of microorganisms has been well exploited to restore artefacts.[9]

Consolidation: This is a process by which an artefact is strengthened by filling in gaps or crevices that weaken it. Traditional methods involved the use of products that were not only harmful to the restorer but also caused damage to the artefact, such as yellowing, production of hazardous substances, or uneven distribution over a surface. Synthetic products, such as acrylic or vinyl polymers and silicon-based compounds, take a high toll on the environment. These items also require the use of volatile substances like white spirit methyl-ethyl ketones, which are potential carcinogens.[10] In order to keep in line with green regulations, alternate methods such as bio-consolidation were developed.

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8 E. Gioventù, 2013. Another example is the use bacteria entrapped in laponite (clay) to solubilize patinas (a greenish brown film that develops on walls, furniture, marble, and brass due to long-term oxidation) on the loggia (gallery) of Casina Farnese in Rome. Three strains of bacteria were trapped within the laponite (*Pseudomonas koreensis, Cellulosmicrobium cellulans and Stenotrophomonas maltophilia*), which was then placed over the patinas. Each bacteria had their own function: *Pseudomonas* solubilised phosphates, *Cellulosmicrobium* solubilised carbonates and sulphates, and *Stenotrophomonas* digested proteins. The laponite was used to restrict the bacterial effect to only the surface of the gallery. The outcome was efficient, highly specific, gradual and non-invasive; it removed the patinas while ensuring the safety of the workers and the environment. Matteo Mazzoni 2014. *Desulfovibrio desulficans* on a carbogel support allowed the removal of black crusts, reducing sulphates to hydrogen sulphide, while *Pseudomonas stutzeri* (A29 strain) was used to resolve animal glues from paintings on the wall of the cemetery in Pisa.. The metabolic activity of *Pseudomonas* removed the glue with 80–90 per cent efficiency without damaging the original pigments. The remainder was dissolved using proteases. G. Ranalli 2005.

9 Cappitelli et al., 2006, 2007, Sorlini, 2010. Wendelbo used trypsin in a phosphate buffer solution (pH 8.0, @ 40oC for 10 mins) to dissolve the animal glue that had sealed book pages together. Wendelbo O., 1970. Segel and Cooper, used a dual enzyme method consisting of amylase and protease to remove glues that were comprised of starch and proteins. The amylase was in a phosphate buffer pH7.0, after the application of amylase the book was incubated at 38oC for 60 mins (1 hour), while the protease also in a phosphate buffer of a pH 7.5, was applied to the book and then incubated at 38oC. Segel J., 1977. Vokic D., 2005

10 Eleonora Balliana 2016

Bio-Consolidation: This is a new approach that makes use of microorganisms or their products that are similar to the makeup of the artefact itself. The application strengthens the artefact, either by removing or neutralizing weakening agents on the artefact and by filling cavities as binding agents and barriers. This method mostly uses organisms capable of attaching to or growing on stone and that can synthesize binding agents like calcium-carbonate precipitation. The first bio-consolidation took place in 2005 using Organic Matrix Macromolecules (OMM) from the shells of molluscs.[11]

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11 Nicoletta Barbabietola 2012. Two experiments were carried out under lab conditions to determine the feasibility of this method. The first experiment used a bacterium known as *Myxococcus xanthus*. The culture was inoculated in the stone from a liquid-culture medium. The results observed were that calcite and vaterite crystals were precipitated in the crevices of the limestone and cemented the limestone granules together while at the same time not affecting the porosity of the limestone, allowing for vapour permeability. The problem though was that the precipitate did not penetrate more than 1 mm into the stone. C. Rodriguez-Navarro, 2003. Another experiment used the indigenous organisms of the stone to precipitate calcite. They concluded that the rate of calcite precipitation by the indigenous microflora was much lower than that of *M. xanthus*. There are several issues with this method – all the results were observed under lab conditions but not in the field, the precipitate could change the aesthetics of the artefact, there could be behavioural change of the organisms over time and when subjected to chemical cleaning, since the strains of organisms chosen must be non- pathogenic and non-sporulating. C. Jimenez-Lopez 2007. This method is sustainable, efficient and safe to the environment and the restorer. In 2010, three strains of bacteria were used to cause bio-mineralization. Organisms were isolated from marine sediments and checked for their ability to precipitate calcium carbonate. The bacteria observed belonged to the genera *Sporosarcina sp, Bacillus sp, and Brevundimonas sp. Sporosarcina soli,* dominated in terms of growth as compared to other bacteria, but *Bacillus lentus* (CP 28) showed the highest urease activity and hence the highest calcium-carbonate collection and precipitation rate. It was observed that the urease activity would increase the pH of the environment by releasing ammonia, thus making it more feasible for calcium to be collected and then precipitated in the form of a extracellular polymeric substance i.e. calcium carbonate or other polymorphs. Shiping Wei, 2015 The strain TNSD 13 belonging to genus *Rhinococcus* was isolated from the Etruscan tomb of *Mercareccia*. This bacterium has shown the best results, since it is able to form biofilms resulting in an increased calcium-carbonate precipitation and reduction of water absorption due to capillary action. Another advantage was the lack of any change in colour due to the bacterium and that it does not form spores and is non-pathogenic. Nicoletta Barbabietola 2012.

> Biotechnology-Based Methods / Enzymes: Biotechnology-based methods have recently come into use for conservation and restoration of artefacts. These methods have greater advantage than traditional chemical and physical restoration methods by their selectivity for weathered material, safety of the artefacts, and economical costs. The advantages of using these enzymes (or living bacteria) are that they generally induce more highly selective processes and are therefore non-invasive and noncorrosive. Typically, they act only on target compounds without attacking molecules other than those for which they are intended. They are also safe for the heritage workers and have low environmental impact with respect to being hazardous and causing pollution. Further research is in progress to identify ideal sources for the enzymes, the optimum conditions in which they are effective, and the types of artefacts to which they can be applied.[12]

Biotechnology is a powerful field of science that aids in medicine and industrial production and environmental management. It is the approach that we have come to favour for heritage conservation at our laboratory.

Our Research and Effort

I am an associate professor of life science and biochemistry at St. Xavier's College in Mumbai, where I teach genetics, biotechnology and environmental science to undergraduate and postgraduate students. As director of the Caius Research Laboratory, with a background in enzyme biotechnology and an interest in multidisciplinary approaches, I engaged in this question of heritage conservation with the Heras Institute of Indian History and Culture at our college, and received a TATA – Heras Post-Doctoral Fellowship to carry out this work.

The Heras Institute was founded in 1926 by Fr. Henry Heras SJ. Its objective is to strengthen Indian identity by fostering research into Indian history, archaeology, religion, and culture. There is a museum associated with the institute, which has a rich collection of antiquities and rare books, so we adopted a biotechnology approach to restore and conserve its artefacts.

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12 Webster & May 2006; Palla, 2013.

If microorganisms are a problem, they can also become a solution, since they produce enzymes (proteases) like cellulases, proteases, lipases and amylases that break down specific substrates. The organisms that produce these enzymes can be isolated on media from sources like soil, dairy sludge, etc., and then the enzymes can be manipulated as bio-cleaning agents. This is an environmentally friendly and efficient way of restoring and conserving the artefacts.

This can be achieved by first analysing the microorganisms on the artefacts, which allows us to evaluate the microbial load, along with the type and extent of damage. So we began to study the microbial diversity on the artefacts in the Heras Museum. Artefacts of various material from different locations of India were selected, such as statues of sandstone, basalt, marble and granite, as well as wooden artefacts:

- Devotee – Bhramhachari, black basalt, 9th to 10 century CE, South India;
- Ganapathi, white marble, 20th century CE, Maharashtra;
- Parsvanatha, green quartzite, 13th to 14th century CE, Baindur, North Kanara;
- Nandi, basalt, 13th century CE, Goa;
- Pillar, wood, 20th century CE, Gujarat;
- Sapta Martika, basalt, 11th to 12th century CE, Karnataka;
- Surasundari, sandstone, 12th century CE, Central India;
- Standing Buddha, red sandstone, 7th century CE, Bihar.

The artefacts were wiped with sterile swabs, which we then spread onto nutrient agar, a media gel that enables the growth of microorganisms. The agar plates were placed overnight in a 37°C incubator, and, if microbes were present, they grew in colonies. Each type of colony represents a different microbe that grows in a unique way.

Plate 1: A sample of the artefacts conserved: Jina, black basalt, 10th century CE (left), Tabernacle, wood, 20th century CE (right). Courtesy of the Heras Institute of Indian History and Culture, St. Xavier's College – Autonmous, Mumbai, Maharashtra, India. Photographs by Nythan D'Cunha and Merlyn Cherusserikkaran.

Plate 2: Microbial Diversity: Colony characteristics of different cultures isolated from the various artefacts. Courtesy of the Caius Research Laboratory, St. Xavier's College – Autonmous, Mumbai, Maharashtra, India. Original work and photographs taken, using the Alpha Digi Doc – Gel documentaion system, by Nythan D'Cunha and Merlyn Cherusserikkaran.

The microbes were also distinguished by various other techniques, such as Gram-staining, a method that differentiates microorganisms into two main groups based on their cell-wall composition – as Gram positive (purple) and Gram negative (pink). Once the microbes from the artefacts have been identified, the question arises of how to restore the artefacts in a sustainable way.

Proteases and lipases are enzymes that degrade proteins and lipids respectively. Many microbes produce these enzymes, and some do so in especially large amounts. The first step was therefore to isolate the enzyme-producing microorganisms (from sources like soil, oil and sludge) on special media.[13] We then harvested the enzymes being produced by the microbes by centrifugation – spinning the overnight grown culture at high speed. The collected protease and lipases were then brushed onto the artefact surfaces, leading to their restoration and conservation.

Conclusion

Biotechnological restoration of heritage structures and artefacts has proven to be economical and environmentally beneficial, in addition to not having adverse effects on the health of workers or the aesthetic beauty and functionality of the artefacts. The value of the artefact is not damaged significantly in comparison to traditional chemical methods, most of which have been banned. The result is a highly effective and specific removal of black crusts, glues and overlaid paints from walls, bronze, paper and other materials, without any loss of pigmentation or degradation of the artefact. The use of enzyme based technology is a more natural method of restoration of artefacts.

In terms of consolidation, the calcium-carbonate precipitation induced by indigenous microorganisms result in an increase in the strength of the structure, reduced absorption of water, with no net decrease in vapour permeability, and the formation of a bio-film that

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13 For protease producers, the specific media used was SMA (skim milk agar media) that has protein-rich milk powder. A sterile suspension of the source, for example soil, is spread on the media and, if protease producers are present, they secrete the enzyme into the surroundings and degrade the protein-rich media, clearing them in the process. These isolates, which are probable protease producers, are picked up for further characterization. Similarly, lipase producers were isolated on lipid-rich media, namely TBA (Tributyrin Agar). Suspensions from the source was spread on the media, and those which showed clearing due to their degradation of the lipid in the media are lipase producers. These isolates are then picked up for further characterization.

protects the artefact from future damage, giving the artefact resistance to sulphate-crust formations. These organisms are also capable of retarding the growth of microorganisms that would cause damage to the artefact as well as inhibiting pathogenic and spore forming organisms.

The drawbacks of this method are the variability of penetration of the calcium-carbonate precipitate with respect to bio-consolidation. It requires knowledge of microbiology and provisions of special conditions to ensure viability of the bacterium and the enzymes. Also, the behaviour of organisms in response to chemical cleaning is not easily predictable of if there is a potential to become pathogenic or form spores. The organisms chosen must be non-pathogenic, non-spore forming and must be easily removable once their function in restoration has ended. For this method, many inert support-gels are being devised so as to entrap the organisms, restricting their spread and action to the surface.

The protese- and lipase-producing strains have been identified and isolated. The work is now currently focusing on carrying out restoration work using the enzymes produced by these strains on the heritage artefacts. Since, these as strong producers of lipases and proteases, we expect to get promising results.[14]

The question that comes to my mind about the art / science interface is whether science can be useful and have applications in the field of art. From this work, it is evident that biotechnology comes under the umbrella of Big History – a unique combination of science having an immense application in the field of art. The main intent of Big History is to connect and integrate various field of arts, science and commerce. The above study is a good example of this connection of multidisciplinary interaction.

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Priya Sundarrajan, PhD is an Associate Professor, Department of Life Science and Biochemistry, St. Xavier's College – Autnomous, Mumbai, India. She has taught in the college since January 2001. She is also Director of the Caius Research Laboratory and member secretary of its Institutoinal Biosafety Committee. As a geneticist and biotechnologist, her main research focus is on bioethanol and biofuel production, especially using yeasts, enzyme biotechnology and human genetics. Priya teaches cell biology, biotechnology, genetics, molecular biology, and environmental science to undergraduate and postgraduate students. She has a particular interest in integrative and multidisciplinary approach to teaching and learning. She may be contacted at <priya.s@ xaviers.edu>.

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