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Short communication

Feasibility study involving the search for natural strains of microorganisms capable of degrading graffiti from heritage materials

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ABSTRACT

Microorganisms found on graffiti and associated environments are potential candidates for biological removal of undesirable graffiti on cultural heritage structures and materials. A feasibility study involving the isolation of natural strains of microorganisms that are capable of degrading graffiti as possible candidates for use in biocleaning treatments for heritage monuments was carried out. A total of 54 different strains were obtained from various sources, recent and old graffiti, the bodywork of a car in a scrapyard and the soil beneath it, an acrylic wall painting and the interior of spray paint cans. The strains were isolated under aerobic conditions and subjected to preliminary laboratory tests to determine their potential as bioremediation agents; i.e., their ability to remove and degrade samples of paint on glass microscope slides. Only those showing such potential were further characterized by sequencing of 16S rDNA and ITS regions. Sequence results identified the isolated strains as bacteria belonging to the genera *Arthrobacter*, *Bacillus*, *Gordonia*, *Microbacterium*, *Pantoea* and *Pseudomonas* and fungi belonging to the genus *Alternaria*. These findings suggest that existing graffiti surfaces are a good source for putative biodegradative microbial populations, which may aid in the remediation of damaged surfaces.

1. Introduction and research aims

Although microorganisms are commonly associated with negative effects on the integrity of buildings, materials and structures, there is growing evidence that they can be used for the purpose of bioremediation (viz. the use of living organisms to remove environmental pollutants or undesirable materials through biodegradation), in a procedure that is safe for the artwork, the restorer's health and the environment (for details, see e.g. Ranalli et al., 1997, 2000; Cappitelli et al., 2007; Bosch-Roig and Ranalli, 2014).

The first studies demonstrating the positive role of microorganisms in cleaning cultural heritage structures date from the late 1980s/early 1990s. Most of those studies used anaerobic microorganisms, mainly sulphate-reducing bacteria (SRB). Although interest in the topic declined for some time shortly afterwards, biocleaning and biorestoration of cultural heritage structures have received renewed attention with the development of new microbial and biotechnological approaches. For example, Alfano et al. (2011) presented a case study addressing the removal of nitrate and sulphate salts from the sandstone (tuff stone) surfaces of the Matera Cathedral (Italy), which dates from the 1100s. *Pseudomonas pseudoalcaligenes* KF707 (cultivated in aerobic conditions) and *Desulfovibrio vulgaris* subsp. *vulgaris* ATCC 29579 cells (cultivated in anaerobic conditions), which are nitrate- and sulphate-reducing bacteria respectively, were applied. After 24 h, the microorganisms had removed 55% of the nitrate and 85% of the sulphate deposits, respectively. Lustrato et al. (2012) managed to remove organic matter from past restorations (e.g. thin layers of animal glue and casein) carried out on the surface of the "Stories of the Holy Fathers" fresco, painted by Buffalmacco Buonamico in the 1300s and located at the Monumental Cemetery in Pisa (Italy). Viable bacterial cells of *Pseudomonas stutzeri* (strain A29) were applied after a preliminary traditional cleaning. The bioremediation was highly successful, rapid and no bacterial cells were found in the fresco after the treatment. Similarly, Bosch-Roig et al. (2013) used *P. stutzeri* strain (DSMZ 5190) and agar (as application support) to clean nitrate salt efflorescences (mainly potassium nitrate) from wall paintings dating from the 1800s and located in the Santos Juanes church in Valencia (Spain). Removal of insoluble salt

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efflorescences was highly efficient, with a reduction of 92% in the amount of salt after 90 min, and did not damage the paint. Troiano et al. (2013) demonstrated the synergic effect of chemical and biological treatments in cleaning stone artwork. Application of a soft non-ionic detergent (Tween 20) followed by *Desulfovibrio vulgaris* subsp. *vulgaris* ATCC 29579 was tested on a stone column affected by black crusts and subsequently on a one-century-old artistic marble statue weathered by sulphate-based crusts and grey deposits. The results indicated that a single application of Tween 20 softened the black crust, enhancing the biocleaning process by reducing the cleaning time and the number of treatment applications required. Finally, Mazzoni et al. (2014) carried out a case study in which deposits that were classified hard-to-remove by curators were removed from mural paintings, dating from the late 1500s, in the loggias (exterior galleries) of the Casina Farnese on the Palatine Hill (Rome, Italy). The deposits were composed of gypsum, calcium oxalate dihydrate (weddellite), calcium carbonate, apatite and a protein compound (probably aged casein). *Cellulosimicrobium cellulans* TBF11E, *Stenotrophomonas maltophilia* UI3E and *Pseudomonas koreensis* UT30E bacteria were applied, individually (which produced the best results), in combination, and in succession. Strain TBF11E removed the inorganic (gypsum and carbonates) darker layer, UI3E dissolved the protein brownish layer and UT30E removed the mixed deposits (phosphates and proteins).

These studies clearly demonstrate that the use of microorganisms has important advantages over traditional physical and chemical cleaning treatments, especially when the substances to be removed are complex and incrusted (Bosch-Roig et al., 2013). This is the case of graffiti paintings, which represent an escalating deterioration factor in urban fabrics. Until now graffiti cleaning has always represented a balance between removal of unwanted material and substrate damage, as none of the traditional cleaning methods available at present are capable of removing the graffiti from substrates without also affecting the underlying material itself in some way (Sanmartín et al., 2014, 2015).

Graffiti spray paint contains a large variety of biodegradable organic and inorganic components (including additives such as emulsifiers and thickeners), which can be used by many different microbial species for growth (Ciferri, 1999; Cappitelli and Sorlini, 2008). In addition, the high carbon (C) content of graffiti paints (above 50%, see e.g. Sanmartín et al., 2014) may represent a potential source of C for bacteria and fungi. In this sense, microorganisms found in aged and waste graffiti paint will presumably be good candidates for graffiti removal. However, to date, no studies of the microbial ecology of graffiti wall paintings and associated environments have been published. Cases have been reported wherein some microorganisms, such as fungi and particularly bacteria, can modify spray paint in-can (Bentley and Turner, 1998; Horie, 2010). Microorganisms can contaminate paint via infected intermediates and raw materials (including water) or non-sterile equipment. For example, the presence of bacteria has been found to lead to reduced viscosity, gassing and color drift in latex paint (Bentley and Turner, 1998). In this respect, the components of spray paint affect microorganisms, either by inhibiting or by stimulating their development. For example, cellulose derivatives can act as nutrients for fungal cells (Winters and Guidetti, 1976; Allsopp et al., 2004), whereas organic solvents and heavy metals in pigments can adversely affect the cells (Gaylarde et al., 2011). Higher proportions of resin in gloss paint may also yield greater bioresistance (Gaylarde et al., 2011).

The aim of the present study was to isolate and screen (under aerobic conditions) bacterial and fungal strains that are able to thrive on graffiti wall paintings and associated environments; and then, to determine their potential as bioremediation agents; i.e., their ability to remove and degrade samples of graffiti spray paint on glass microscope slides. The final goal of the wider project is to establish a biological method of using aerobic and mesophilic culturable bacteria and/or fungi to remove spray-painted graffiti. These microorganisms must demonstrate the ability to remove the substances contained in the graffiti paint, playing a destructive role (causing deterioration of painting) in a natural process. Indeed, without resorting to the use Genetically Modified Organism (GMO), which could lead to unforeseen risks to safety (Bosch-Roig and Ranalli, 2014).

2. Materials and methods

2.1. Sample collection and isolation of microorganisms

Samples were collected from different sources (some of them as shown in Fig. 1) by using swabs moistened with sterile buffer solution (5 mL). In the case of soil samples, they were collected with a shovel forced into the soil to a depth of approximately 5 cm. Five different locations were selected for sampling in two areas in the Northeastern United States. New graffiti artwork was located in an alley off Central Square, Cambridge, MA (latitude: 42°21′53.5″N, longitude: 71°06′08.4″W). Old graffiti artwork, a car bodywork in a scrapyard and the soil beneath it, and an acrylic wall painting were located on Columbia Street, Cambridge, MA (approximately 1.6 km from the other graffiti) (latitude: 42°22′28.9″N, longitude: 71°05′38.0″W). The samples were serially diluted and the different dilutions were spread on culture plates containing – Tryptic Soy Agar (TSA), Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) (Becton Dickinson Company, USA) at 1/10th strength.

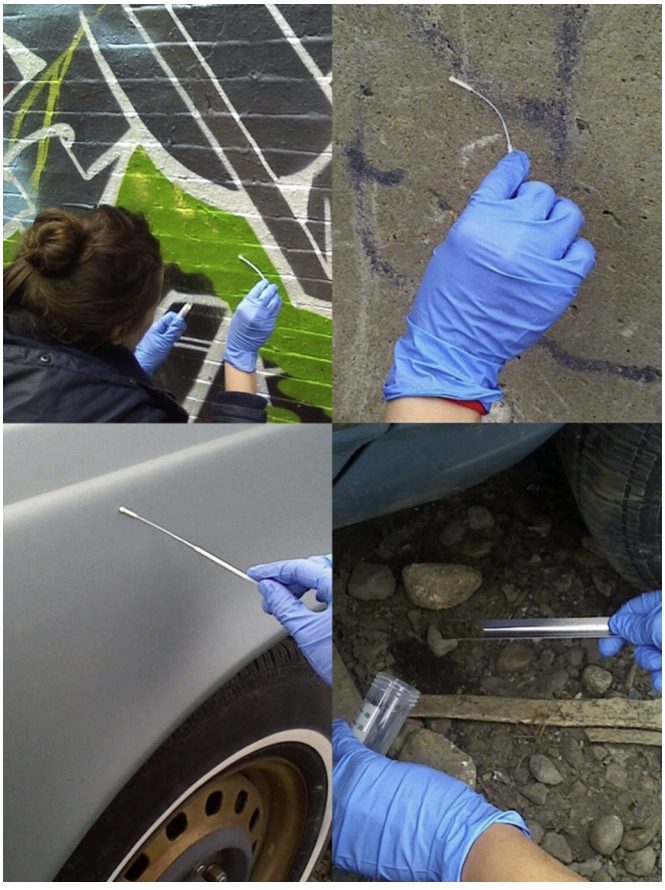

Fig. 1. Some of the sampling places. From left to right and top to bottom: recent graffiti, old graffiti, the bodywork of a car in a scrapyard and the soil beneath it.