Biodegradation of pollutants in urban environments

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ABSTRACT: Microbial degradation of phenanthrene is a common process in weathered stones from urban polluted environments. On the surfaces of building stones from the cathedral of Sevilla, Spain, there is an active community of bacteria able to grow using this polycyclic aromatic hydrocarbon. The rapid bacterial growth in cultures with phenanthrene as unique carbon source suggests that microbial transformation reactions might occur in situ. The particular conditions prevailing in the stone niches promote the selection of microorganisms able to transform anthropogenic compounds that have been so far considered as recalcitrant.

RESUMEN: Sobre la superficie de la piedra de la catedral de Sevilla, España, existe una microflora capaz de degradar hidrocarburos policíclicos aromáticos como demuestra el rápido crecimiento de bacterias que utilizan fenantreno como única fuente de carbono en medios de cultivos. Es posible que este tipo de degradación ocurra in situ.

1 INTRODUCTION

The study of microbial communities is usually accomplished using standard culture methods. Microorganisms traditionally are characterized by phenotype (morphology, biochemical tests, lipid composition, etc.). However, it is believed that fewer than 20% of the extant microorganisms have been discovered, and that culture methods are inadequate for studying microbial community composition. There are many reasons for the routine failure of the usual cultivation strategies, the most common is that selective enrichment cultures fail to reproduce the conditions that particular microorganisms require for proliferation in their natural habitat. However, when bacteria with the ability to degrade a specific compound are searched, enrichment cultures is the method of choice.

Although biodeterioration processes were recently discussed (Saiz-Jimenez 1999), biodegradation of organic compounds in monuments has barely been investigated. Bacteria on stone can be extremely versatile and could maintain their activity during nutrient perturbations, operating at low nutrient levels and utilizing what the environment has to offer. As a consequence, bacterial populations may be able to maintain their involvement in the biodeterioration process during periods of nutrient flux. Recently, biodegradation of polycyclic aromatic hydrocarbons by heterotrophic bacteria was reported (Ortega-Calvo & Saiz-Jimenez 1997) and new insights on the microbial ecology of black crusts were presented.

Phenanthrene concentrations at contaminated sites and urban environments are among the highest of individual polycyclic aromatic hydrocarbons (PAHs); therefore the selection of phenanthrene to estimate the activity of PAH-degrading bacteria and subsequent PAH degradation in building stones seems an appropriate choice.

PAH-degrading bacteria have been used previously as indicators of the microbial activity in polluted environments. For instance, Bogardt & Hemmingsen (1992) detected and enumerated the phenanthrene-degrading bacteria in petroleum-contaminated sites; and phenanthrene-utilizing and phenanthrene-cometabolizing microorganisms have been evidenced in estuarine sediments (Cerniglia 1993). For this reason, it was considered that these bacteria could be present on weathered stones in urban environments subjected to heavy air pollution, and a sampling was perfomed in the cathedral of Sevilla, Spain.

2 MATERIALS AND METHODS

Black crust samples were taken during the restoration works of the south façade of the cathedral on 30 January 1998 (Fig. 1) at two different heights (1C and 2C), as well as the same samples of stone without black crusts (1S and 2S).

The black crusts from this façade (Figs 2 and 3) are well characterized (Saiz-Jimenez 1993). The abundant crust formation in the south front is related to the fact

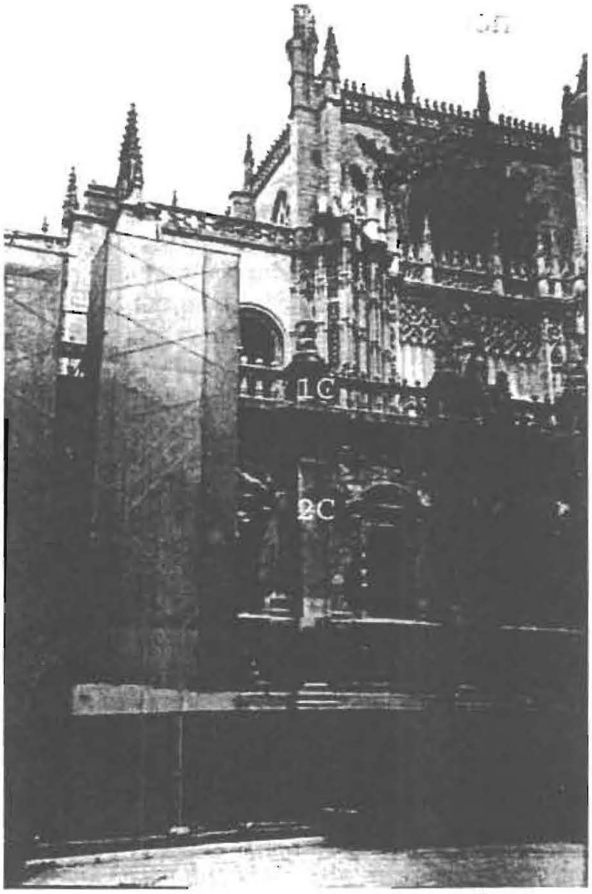

Figure 1. Cathedral of Sevilla, south façade.

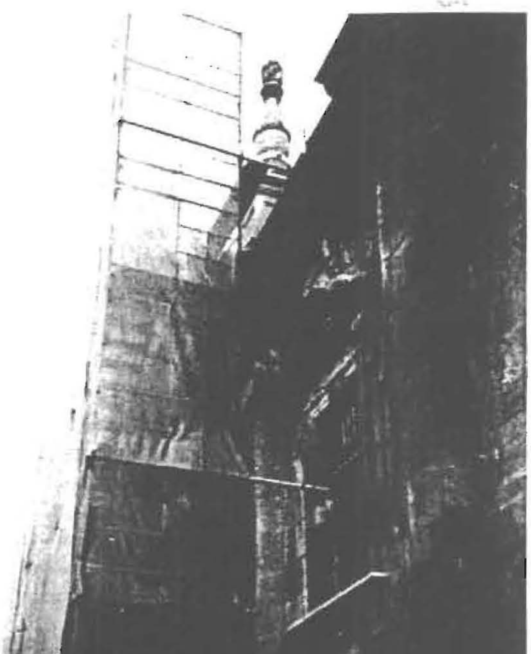

Figure 2. Blackening of the façade.

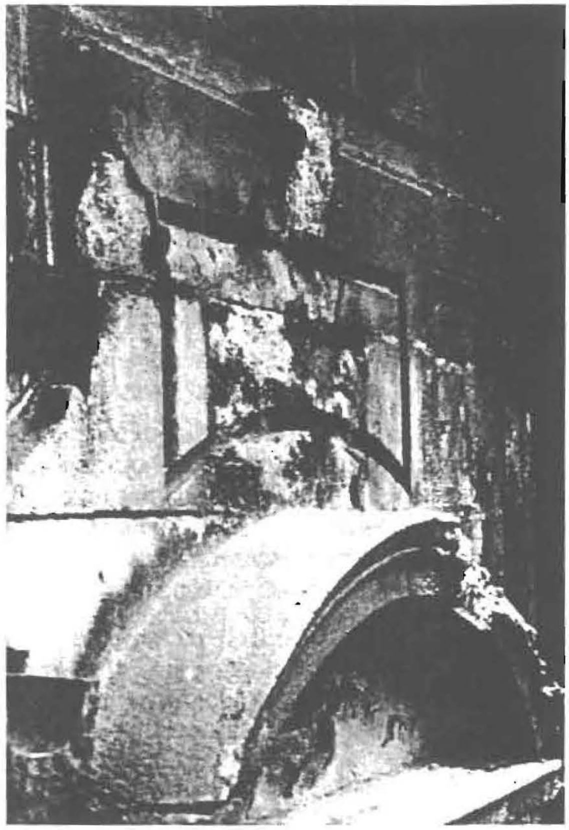

Figure 3. Detail of the façade.

that one of the main municipal bus terminals, operating for more than 20 years since the 1960s was just in front and less than 10 m away from this wall. Exhaust gases and particulate matter heavily affected the stone.

Samples from the two different heights were inoculated in different culture media as reported by Laiz et al. (1999), such as TSA, starch–casein agar, malt–yeast extract agar, TSA + 5% NaCl and starch–casein agar + 5% NaCl. The plates were cultured at 28°C and the colonies isolated.

In parallel, 3 g of each black crust sample was added to a 50 ml Erlenmeyer containing a culture medium composed of inorganic salts and 2.5 mg of phenanthrene as unique carbon source. The Erlenmeyer was incubated at 30°C on a rotary shaker operating at 100 rpm and the bacteria were isolated after 1 month.

Individual colonies were isolated randomly and purified by streak plating onto TSA and a medium composed of inorganic salts and 2.5 mg of phenanthrene until pure culture plates were obtained. Isolates were characterized by morphological and physiological properties, the latter using standard microbiological methods, API and Biolog. Total cellular fatty acid methyl esters (FAMEs) were analysed using the Microbial Identification System. Delaware, USA (MIDI) in accordance with protocols for cultures grown on solid medium and recommended instrument specifications. This permitted automatic identification of bacteria by comparison with the MIDI Sherlock Standard Aerobe and Actin1 databases.

The bacteria were maintained for 3 years either in solid or liquid media containing phenanthrene as unique carbon source.

3 RESULTS

A total of 51 bacteria were isolated from the different culture media. They belong to the genera *Arthrobacter*, *Bacillus*, *Cellulomonas*, *Kocuria*, *Micrococcus*, *Paenibacillus* and *Staphylococcus*. In addition, 16 sporoactinomycetes were isolated, most of them tentatively identified as *Streptomyces* sp. (Table 1).

From the Erlenmeyers with phenanthrene only 12 bacteria were isolated, from which nine were identified as species of the genera *Arthrobacter*, *Brevibacillus*, *Brevibacterium*, *Micrococcus* and *Pseudomonas* (Table 2). *Brevibacterium* and *Pseudomonas* species were isolated only in the medium enriched with phenanthrene. No phenanthrene-degrading bacterium was isolated from the samples without black crusts.

Table 1. Isolates from the south façade, cathedral of Sevilla.

Taxa	1C*	1S	2C	2S
Actinomycetes	1 + 0		13 + 0	2 + 0
Arthrobacter	3 + 3		1 + 0	
Bacillus	2 + 0	1 + 0	2 + 0	2 + 0
Brevibacillus			0 + 1	
Brevibacterium	0 + 1			
Cellulomonas		1 + 0		
Kocuria		2 + 0	1 + 0	1 + 0
Micrococcus	1 + 1	2 + 0	1 + 1	1 + 0
Paenibacillus			2 + 0	
Pseudomonas			0 + 2	
Staphylococcus		2 + 0		
Not identified	7 + 1	3 + 0	3 + 2	1 + 0

* First figure corresponds to isolates in different media, second one corresponds to isolates from phenanthrene enrichment.

Table 2. Bacteria identified in phenanthrene enriched medium.

Strain	Identification	SI*
1CA4	*Arthrobacter ramosus*	0.795
1CA10	*Arthrobacter oxydans*	0.606
1CA13	*Arthrobacter protophormiae*	0.755
1CB16	*Brevibacterium mcbrellneri*	0.653
1CB19	*Micrococcus* sp.	0.440
2CA4	*Pseudomonas putida*	0.413
2CB1	*Pseudomonas putida*	0.685
2CB5	*Micrococcus luteus*	0.752
2CB12	*Brevibacillus laterosporus*	0.632
1CB1	NI**	
2CB15	NI	
2CB17	NI	

* SI: Similarity index according to Microbial Identification System MIDI; ** NI: not identified.

4 DISCUSSION

Anthropogenic activities, particularly the burning of fossil fuels, are significant sources of PAH released into the environment via atmospheric deposition. As a result, their concentration on the façades of historic monuments has tended to increase, particularly in urban areas.

In previous studies, (Saiz-Jimenez 1993, Fobe et al. 1995) reported that black crusts coating the surfaces of buildings in urban environments contained a diversity of organic compounds: aliphatic and isoprenoid hydrocarbons, fatty acids, diterpenoids, tricyclic terpanes, steranes, triterpanes, PAH, etc.

The presence in the black crusts of geologically mature compounds such as the tricyclic terpanes, triterpanes, steranes, and the isoprenoid hydrocarbons pristane and phytane, all characteristic molecular markers of petroleum, indicates a direct input of traffic-derived combustion products on the cathedral façade. Polycyclic aromatic hydrocarbons have usually a pyrolytic origin, such as the combustion of fossil fuel. These compounds have been identified in aerosols (Simoneit 1986), and diesel engine soot (Yu & Hites 1981, Bayona et al. 1988).

From literature data it appears that non-volatile PAH are degraded by a variety of bacteria, yeasts and fungi which generally belong to the autochthonous flora (Cerniglia 1993). The PAH-utilizing bacteria most frequently reported include several species of *Pseudomonas*, and a few of *Arthrobacter*, *Acinetobacter*, *Alcaligenes*, and *Streptomyces*. The bacteria isolated from black crusts using phenanthrene-enriched medium are mainly *Arthrobacter*, *Pseudomonas* and *Micrococcus* species, which is in agreement with literature data.

This and previous studies have shown that weathered building stones contain molecular markers that are characteristic of petroleum derivatives and bacteria are able to utilize some of the most recalcitrant petroleum derivatives, the PAH. This kind of microflora is not only restricted to fuel-contaminated soils or sediments, PAH-contaminated wastes, etc. where they are usually isolated, but can also be found in urban environments, where the bacteria have adapted to specific site conditions such as a strong air pollution due to the combustion of petroleum derivatives, which include high PAH concentrations in particulate matter for long periods.

The data suggest that on the surfaces of building stones there is an active microflora of PAH degraders and that microbial degradation of phenanthrene is common in weathered stones from urban polluted environments. Although the experiments performed in this work required the removal of the samples from the cathedral walls, the rapid bacterial growth in cultures with phenanthrene as unique carbon source indicates a significant phenanthrene utilization, suggesting that microbial transformation reactions occur in situ. It is also possible that the particular conditions prevailing in the stone niches promote the selection of microorganisms able to transform other anthropogenic compounds that have been so far considered as recalcitrant.

Due to its lipophilic nature, phenanthrene is associated to lipids deposited on the stone surface or remains adsorbed to airborne carbonaceous particles. Microorganisms may be able to transform phenanthrene either as such or after spontaneous or microbiologically-induced desorption in the water present in the porous stone. However, it can be expected that phenanthrene entrapment in the weathering black crust during gypsum crystal formation may cause some decrease in bioavailability, and this therefore contributes to its persistence in the stone.

5 CONCLUSIONS

The data herein reported indicate that microorganisms are able to growth and eventually remove some of the most abundant components of black crusts, such as PAH.

Although the use of microorganisms is not foreseen as a method for cleaning façades due to some obvious limitations (dimension of monuments, time needed, wetting of weathered surfaces, economy, etc.), it must be emphasized that nature develops strategies for biodegradation of pollutants in urban environments. In fact, the continuous input of inorganic and organic compounds modifies the chemical composition of building stones, resulting in the selection of microorganisms with specific nutrient requirements or with a defined metabolic capability.

It must be recognized that we are still far from a complete understanding of the physiological diversity of microorganisms and their interactions in the surface of stone monuments, but biodegradation of organic pollutants, largely neglected in cultural heritage studies, is envisaged as an important process in addition to biodeterioration.

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