Polish Journal of Microbiology
2008, Vol. 57, No 3, 213–220

ORIGINAL PAPER

Characterisation of Actinomycetes Isolated from Ancient Stone and Their Potential for Deterioration

HESHAM ABDULLA[1]*, ERIC MAY[2], MAGDI BAHGAT[1] and AHMED DEWEDAR[1]

[1] Botany Department, Faculty of Sciences, Suez Canal University, Ismailia 41522 – Egypt
[2] School of Biological Sciences, University of Portsmouth, Portsmouth, PO1 2DY, UK

Received 17 February 2008, revised 18 May 2008, accepted 20 July 2008

Abstract

Actinomycetes have been isolated from decayed and sound stone samples taken from a tomb site at Tell Basta, Zagazig City, Egypt. A total of 160 isolates have been characterised. The numbers and distribution of actinomycetes were studied during different seasons; during the winter months (18–20°C), actinomycete numbers ranged from 10^3 to 10^4 cfu/g; in the summer (28–38°C) lower counts were recorded. The actinomycete isolates were assigned to 4 different taxonomic groups: 54% belonged to the *Streptomyces* group, 26% to the *Nocardia* group, 14% showed the characteristics of the *Micromonospora* group, while the rest of the isolates analyzed (6%) were assigned to the sporangiate-type group of actinomycetes. The ability of the isolates to produce pigments as well as tolerance to high salinity were determined. It was shown that about 88% of the strains studied had the ability to produce extracellular pigments. Only 25% of the studied isolates showed tolerance to high salinity. The significance of actinomycetes to attack and degrade building stone was shown in laboratory experiments: actinomycetes recovered both from sound and decayed stones were capable of damaging stone under laboratory conditions as an up to 4% weight loss was recorded for some isolates.

Key words: actinomycetes, biodeterioration, stone monuments

Introduction

The harmful effects caused by biological activities of organisms growing on stone are well recognized (Webley *et al*., 1963). In fact, many cases showed that biodeterioration has proved to be dominant over physical and chemical agents, especially in places where high humidity and temperature favour the growth of these organisms (Jain *et al*., 1993).

The biodeterioration is a result of the action of different biological factors such as: algae, lichens, fungi, unicellular and filamentous bacteria (actinomycetes) on stone. Actinomycetes have long been recognised as a group of microbes that are distinct from other bacteria and eukaryotic fungi, and which play important functional roles in many natural environments. Actinomycetes occur in a multiplicity of natural and man-made environments. Most are strict saprotrophs, but some form parasitic or mutualistic associations with plants and animals. In nature, they play a crucial role in the decomposition of organic compounds and environmental pollutants (Lechevalier, 1981). Therefore, they are commonly believed to participate in the recycling of elements (Goodfellow and Williams 1983).

Actinomycetes have been isolated from rocks and stone by a number of workers (Webley *et al.*, 1963; Agarossi *et al.*, 1985; Groth *et al.*, 1999; 2007). However, little consideration has been given to the ecology, population structure and the diversity of this group of microorganisms in these extreme and nutrient-poor habitats. In addition, the effects of seasonal factors on numbers of microorganisms isolated from stonework have only been discussed in relatively few previous publications (Somavilla *et al.*, 1978; Tayler and May, 1991).

This paper deals with the occurrence of actinomycetes on ancient stone and considers the effects of climatic changes on their populations. The evaluation of the abilities of actinomycetes to cause damage to stone under laboratory conditions was also a target in this study. Direct measurements of weathering activity using laboratory experiments were done in order

* Corresponding author: H. Abdulla, Botany Department, Faculty of Sciences, Suez Canal University, Ismailia 41522 Egypt; phone: (20) 64 3230418; **e-mail: h.abdulla@scuegypt.edu.eg**

to identify a causal link between the presence of the organisms and stone decay, in addition to the study of different activities of actinomycetes, such as salt tolerance, pigment production, which may play a role in the biodeterioration process by this group of microorganisms.

Experimental

Materials and Methods

Study site and sampling procedures. The location of the tomb was Tell Basta (the site of the ruins of the old temple of Bast) which is south-east of the Nile Delta township of Zagazig, approximately 80 km North East of Cairo. The tomb was built from limestone blocks, with dimensions of 1 m depth × 1.5 m width × 2.5 m long that are suffering from different degrees of deterioration. The seasonal variation of actinomycetes was investigated on six sampling occasions, three during the winter months (January, February and March) and the other three during the summer months (July, August and September). Seven sampling sites from the tomb's blocks were chosen to represent different stages of deterioration. These sites were visually characterised as 2 sound, 3 moderately decayed and 2 severely decayed stones. Samples were taken once a month from these sites, which were fixed throughout the study.

Isolation media. Stone samples were crushed to a fine powder in sterile mortar and pestle. Stone powder was dispersed in sterile 1/4 strength Ringer's solution supplemented with 0.001% Tween 80 and shaken vigorously for up to one hour (Lewis *et al.*, 1985). Suspensions were then serially diluted in phosphate buffer. Spread plate method was applied for actinomycetes isolation where 0.1 ml of the appropriate dilution was spread evenly over the corresponding overnight dried medium plates. Four different isolation media known to support the growth of actinomycetes were used to select the best medium for actinomycetes recovery from stone: starch casein medium (Kuster and Williams, 1964), arginine glycerol salts medium (El-Nakeeb and Lechevalier, 1962), starch nitrate medium, M3 medium (Williams and Wellington, 1982).

Characterisation of actinomycete isolates. After incubation, actinomycete colony characteristics were examined. Each colony type was described and enumerated to give a total count. Representatives of each colony form were then picked off and streaked to obtain a pure culture. They were then streaked onto starch-casein slopes and stored at 4°C, and as spore suspensions in 20% glycerol at –20°C (Hopwood *et al*., 1985). A wide range of morphological, physiological and biochemical criteria were used to characterise these isolates (Table I). General details of the test methods are given by Williams *et al*. (1983).

Table I
Criteria used for the characterisation and identification of the isolates

Characteristics
1. Spore chain morphology
2. Other morphological features
3. Colour of aerial mycelia
4. Colour of vegetative mycelia
5. Pigmentation of vegetative mycelium (colony reverse).
6. Production of soluble pigments (on ISP5)
7. Melanin pigment production
8. Utilisation of organic compounds
9. Growth temperature and pH 4.3
10. Use of carbon sources (1.0% w/v)
11. Use of nitrogen sources (0.1% w/v)
12. Resistance to antibiotics
13. Diaminopimelic acid (DAP) in whole cell hydrolysate
14. Whole cell diagnostic sugars
15. Mycolic acid

Weight loss study to determine the potential of actinomycetes to cause damage to stone under laboratory conditions. A static culture with small intact limestone discs was used to investigate the decay potential of the actinomycetes isolated from stone over a time course. The procedures were according to Lewis *et al.* (1988), Small limestone discs (1 cm diameter) were sterilised by autoclaving (121°C, 15 min) and dried overnight at 120°C. Each disc was then accurately weighed and aseptically introduced into a 25 ml flask containing 10 ml sterile medium (0.5%, Oxoid peptone water containing 1% glucose, pH 7.2), previously inoculated with 5 µl spore suspension of each actinomycete. The flasks were then incubated at 28°C for 4 weeks. The stone discs were subsequently removed and placed in 3% (v/v) Decon 90 solution for 3–4 h to remove accumulated polysaccharides, which are known to affect the accuracy of the weight measurements (Tayler, 1991). The stones were then removed and rinsed in distilled water and dried overnight at 120ºC. The weight change in stone was compared to control discs incubated in sterile medium.The damage was assessed by means of stone weight loss, changes in pH of medium and levels of soluble calcium.

Salt tolerance assay. The effect of high salt concentrations on the growth of actinomycetes was evaluated. Sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) were used in this experiment; both are commonly found in limestone and well recognised as aggressive salt weathering agents. The ability of the isolates to grow on modified Bennett's agar medium supplemented with different concentration of NaCl and Na_2SO_4 was investigated. Four different concentrations were used: 4%, 7%, 10%, and 13%.

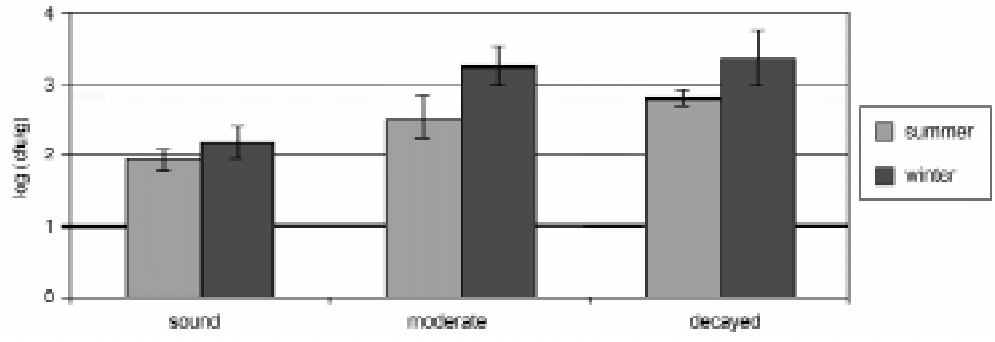

Fig. 1. Numbers of actinomycetes isolated from sound, moderately- and severely-decayed limestone on 1/10 strength starch-casein agar during summer and winter months

Results

Seasonal changes in actinomycete populations Numbers of actinomycetes were counted from sound, moderately- and severely-decayed limestone samples on 1/10-strength starch-casein medium, which support higher count and more diversity than the other tested media-using a spread plate, viable count method. Higher counts were recorded in the winter months on sound, moderately- and severely-decayed stone, with the mean counts being 1.8×10^2, 2.6×10^3 and 4.7×10^3 cfu/g respectively, while the mean counts in the summer were 1×10^2, 3×10^2 and 6×10^2 cfu/g respectively, as shown in Fig. 1. However, ANOVA for seasonal variations in actinomycete counts on sound and decayed stones showed no significant difference ($P = 0.11$). Counts from sound and decayed stones were found to differ significantly ($P = 0.001$). No significant difference was found between counts from moderately- and severely-decayed stones ($P = 0.8$).

Characterisation of actinomycetes isolated from the tomb. A total of 127 actinomycete isolates were recovered from sound and decayed stone of the tomb under investigation. These isolates were classified to the genus level according to Dietz and Thayer (1980). The criteria used in this primary identification were micromorphology, detection of diaminopimelic acid (DAP) isomers in the whole cell hydrolysate, whole cell diagnostic sugars, and presence of mycolic acids. Based on these criteria the isolates recovered from stone were found to belong to 15 different genera (Fig. 4). Those genera were *Streptomyces* (48%), *Micromonospora* (12%), *Nocardioides* (12%), *Nocardiopsis* (12%), *Nocardia* (4%) and the remaining 12% of the isolates were represented by other closely unidentified genera. The isolates were further classified to the species by morphological, physiological and chemotaxonomic features into 56 species belonging to the genera mentioned above. This identification was according to Bergey's Manual for Systematic Bacteriology (1989) and Bergey's Manual for Determinative Bacteriology (1995). The identification of *Streptomyces* species was performed using the key recommended by Szabo *et al*. (1975). The criteria used in this identification are listed in Table I.

Distribution of actinomycetes within the study sites The distributions of different genera isolated in this study are shown in Fig. 2. Members of the genus *Streptomyces* were the most widespread species all over the sampling sites. The sound stone harboured only 3 genera represented by 7 species while both moderately- and severely-decayed stones contained 15 genera assigned to 48 species. Two unknown isolates were recorded in this investigation; both were isolated from the moderately-decayed stone. Forty two species belonging to the genera, *Pseudonocardia*, *Kitasatosporia*, *Spirillospora*, *Saccharomonospora*, *Rhodococcus*, *Streptoalloteichus*, *Actinomadura*, and 2 unknowns could only be isolated from decayed stone and they were never recovered from sound stone. All the species found on sound stone were also recovered from decayed stone except for *Streptomyces* sp. 5.

Screening of actinomycetes isolated from stone for different salt tolerance. The effect of high salt concentrations on the growth of actinomycetes was evaluated. Sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) were used in this experiment, both are well recognised as aggressive salt weathering agents.

(i) NaCl tolerance. The ability of the isolates to grow on modified Bennett's agar medium supplemented with different concentrations of NaCl was investigated. Four different concentrations of sodium chloride were used: 4%, 7%, 10%, and 13%. The results showed that 89% of the actinomycetes recovered from stone were able to grow on the 4% concentration and 72% were able to tolerate salt concentrations up to 7%. Fifty-one percent of the isolates were found to grow on 10% concentration of NaCl, while only 25% could tolerate up to 13% NaCl. Fifty percent of the isolates tolerating the highest salt concentration (13%) were isolated only from decayed stone. These results are shown in Fig. 3.

(ii) Na_2SO_4 tolerance. The actinomycete tolerances to different concentrations of sodium sulfate were also

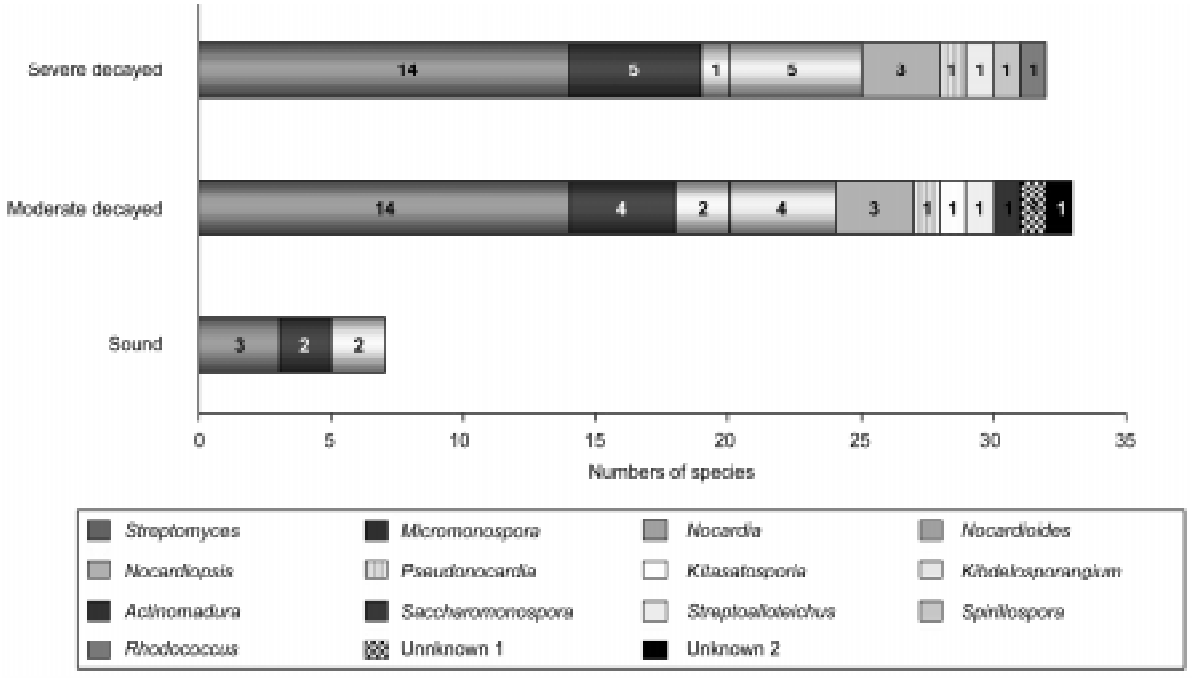

Fig. 2. Distribution of different actinomycete genera within limestone of Tell Basta tomb at different stages of decay. Numbers on bars indicate number of species

assessed using the same four concentrations: 4%, 7%, 10%, and 13%. Analysis of the data demonstrated that about 78% of the isolates were able to tolerate 4%, while 60% of the actinomycetes showed growth at 7% concentration. For the remainder, 40% and 3% of the isolates showed tolerance to 10% and 13% concentrations of Na_2SO_4 respectively. For this salt, 70% of isolates tolerating the 10% con-

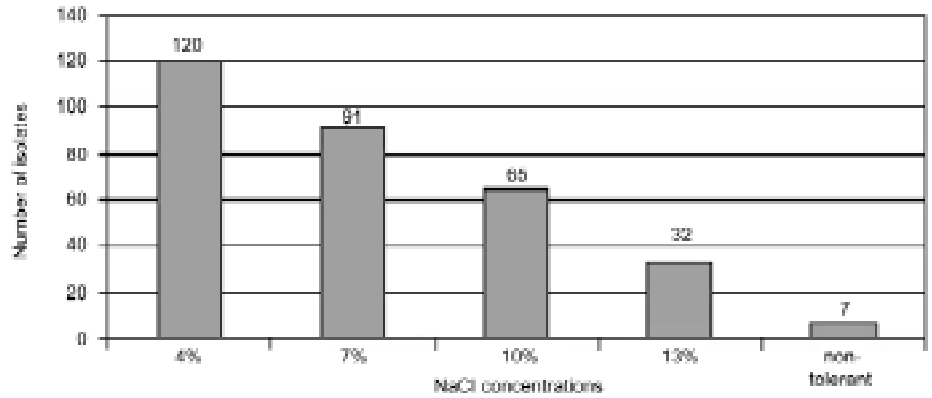

Fig. 3. Tolerance of actinomycetes isolated from limestone to different concentrations of sodium chloride

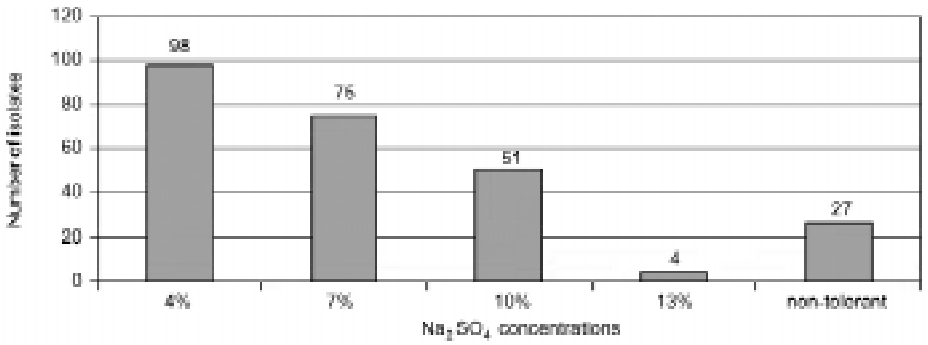

Fig. 4. Tolerance of actinomycetes isolated from limestone to different sodium sulphate concentrations

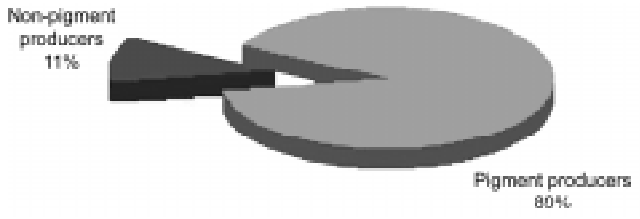

Fig. 5. Pigment production by actinomycetes isolated from limestone.

centration were recovered from decayed stone. These results are shown in Fig. 4.

Pigment production. The production of pigments of different colours by actinomycetes isolated from ancient stone was investigated in this study. As Fig. 5 shows, 89% of the isolates were found to be pigment producers. The majority of the pigments were brown in colour, although yellow and green pigments were produced by a more limited number of isolates.

Ability of actinomycete isolates to cause damage to limestone. In this study, a static liquid culture system was used together with small intact limestone discs to investigate the decay potential of the 127 actinomycetes isolated from stone over 4 weeks. The damage was assessed by means of stone weight loss, changes in medium pH and levels of soluble calcium. The results presented in Table II show that the majority of the isolates screened under these conditions were incapable of causing any significant weight loss in the stone. However, a significant proportion of the isolates (16%) was observed to cause a substantial weight loss (>2%) in the limestone discs, over a 4-week incubation period. In fact, 3 different species were observed to cause weight loss up to 4%, namely, *Nocardia brasiliensis*, *Micromonospora carbonacea* and *Pseudonocardia compacta.* In almost every case a large weight loss in the limestone was associated with low pH of the medium and an

Table II
Stone damage potentiality of actinomycetes species isolated from limestone of Tell Basta tomb

	% weight loss			
	<1	1–2	2–3	3–4
Streptomyces				
S. diastaticus			+	
S. cyaneus		+		
S. antimycoticus	+			
S. chromofuscus	+			
S. exfoliatus	+			
S. rochei		+		
S. albidoflavus	+			
S. anulatus	+			
S. roseflavus	+			
S. badius	+			
S. violaceus	+			
S. griseoflavus	+			
S. macrosporus	+			
S. phaeochromogenes	+			
S. griseostramines	+			
S. sp. 1	+			
S. sp. 2		+		
S. sp. 3			+	
S. sp. 4	+			
S.. sp. 5			+	
S.. sp. 6	+			
S. sp. 7	+			
S. sp. 8	+			
S. sp. 9	+			
Nocardioides				
N. fulvu	+			
N. luteus		+		
N. albus	+			
N. sp.1	+			
N. sp.2	+			
N. sp. 3	+			
N. sp. 4	+			
N. sp. 5	+			
Nocardia				
N. nova	+			
N. brasiliensis				+
N. sp. 1	+			
N. sp. 2	+			
Nocardiopsis				
N. alborubidus		+		
N.dassonivillei	+			
N. albus			+	
N. listeri		+		
N. sp. 1	+			
Micromonospora				
M. chalcae	+			
M. carbonacea				+
M. inositola	+			
M. halophytica	+			
M. sp.1		+		
M. sp. 2	+			
Other genera				
Spirillospora albida	+			
Rhodococcus sp.	+			
Streptoalloteichus sp.	+			
Actinomadura sp.	+			
Pseudonocardia compacta				+
Saccharomonospora caesia	+			
Kibdelosporangium philipinesis			+	
Kitasatosporia phosalacinea		+		
Unknown 1	+			
Unknown 2	+			

elevated concentration of soluble calcium, but these changes were not correlated statistically to weight loss (see May *et al.*, 2000).

Discussion

Interest in microbial communities present on stones and work of arts is mainly due to the fact that microorganisms affect cultural heritage (Monte and Ferrari, 1993). Actinomycetes are of special interest because of their versatile metabolic activities, and also their filamentous mode of growth.

In our study, the numbers of actinomycetes were higher in winter months than in summer, as a result of the summer higher temperatures, even in this semiarid climate. The higher numbers of actinomycetes and the greater variation in species composition for decayed stone (56 species belonging to 15 genera) than for sound stone (7 species belonging to 3 genera) may be due to low levels of competition between actinomycetes and other microorganisms. Since the nutrients available in decayed stone are likely to be complex organic remains, the actinomycetes may dominate this environment as they have remarkable abilities to utilise a wide range of more complex and recalcitrant polymers such as proteins, polysaccharides and lignocellulose (McCarthy and Williams, 1992). Lechevalier (1981) reported that actinomycetes usually begin digestion of organic matter when the numbers of other microorganisms are declining, so that actinomycetes are not found in large numbers on newly dead green plant, but rather are the most abundant on the older partially digested residues. Urzi *et al.* (1999) showed that bacteria isolated from weathered rock samples in the Mediterranean basin were, in the majority of cases, actinomycetes and other Gram-positive bacteria. The other possible reason for these high counts is the ability of actinomycetes to cope with dry habitats. They are frequently isolated from stone environments and many of their members exist for extended periods as resting arthrospores that germinate in the occasional presence of exogenous nutrients (Goodfellow and Williams, 1983).

A total of 127 actinomycete isolates were recovered from sound, moderately- and severely-decayed limestone from the tomb under investigation. These isolates were identified to species level. The genus *Streptomyces* comprised 48% of the total isolates from stone, and 20 different species have been identified. This was not surprising, since *Streptomyces* is undoubtedly the most widely distributed and most studied genus of actinomycetes (Williams, 1985). In their survey of a number of historical sites in Rome, Giacobini *et al.* (1988) isolated 200 strains of actinomycetes, most of which were identified as *Streptomyces.* Twenty-six per cent of the actinomycetes isolated from the rock surface of Altamira cave in Spain were identified as *Streptomyces* (Laiz *et al.*, 1999). Similar dominance of actinomycetes in the subterranean basilica of Porta Maggiore in Rome was observed by Agarossi *et al.* (1985).

In the present study, large numbers of genera other than *Streptomyces* were isolated and classified, whenever possible to species level. Some of these genera have been isolated from stonework by other workers, for example, *Nocardia*, *Nocardioides* and *Rhodococcus* by Groth *et al.* (1999) and *Micromonospora* by Urzi and Realini (1998). On the other hand, some other genera have not been reported elsewhere. Those were *Kitasatosporia Pseudonocardia*, *Kibdelosporangium*, *Spirillospora*, *Saccharomonospora*, *Streptoalloteichus* and *Actinomadura*. The results presented here revealed that the numbers of unidentified species of actinomycetes were about 20% of the total numbers of isolates, including two unknowns. This indicates the possibility of isolation of novel species or even genera from this poorly studied environment. Previous studies have suggested that many of the bacteria and fungi isolated from rocks are new species and genera, which have never been seen or described in any other environment (Krumbein, 1988; Groth *et al.*, 1999). Our study revealed that the actinomycetes population on the damaged stone consisted of the same species recovered from sound stone but 42 other species were found to be present. Furthermore, 10 of the identified genera were detected only on decayed stone and were never found on sound stone. Those were *Nocardioides*, *Nocardiopsis*, *Kibdelosporangium*, *Pseudonocardia*, *Kitasatosporia*, *Spirillospora*, *Saccharomonospora*, *Rhodococcus*, *Streptoalloteichu* and *Actinomadura*.

Such a high diversity of actinomycetes on decayed stone, as well as the obvious difference between the actinomycetes species composition on sound and decayed stones, may indicate selective succession between different genera of actinomycetes, and even between different species of the same genus. This may depend on the ability of these organisms to survive and compete in this extreme environment during different stages of stone deterioration. Such microbial succession on stone works of art was proposed by authors of previous research (O'Neill, 1988; De La Torre *et al.*, 1993). The screening of different activities associated with stone attack by actinomycetes was investigated to establish the possible involvement of actinomycetes in the actual decay process. The possible role of pure and mixed cultures of bacteria as causative agents of decay has been investigated under laboratory conditions in previous studies (Eckhardt, 1985; Tayler and May, 1991; Jimenez-Lopez, *et al*., 2007). Urzi *et al.* (1991) demonstrated that *Micrococ-*

cus sp. was able to cause severe damage to marble slabs under laboratory conditions. In the present work, 16% of the actinomycetes isolates were able to cause damage in stone discs measured in weight loss more than 2% over a short time course. Sodium chloride and sodium sulfate are the commonest salt-weathering agents to ancient stone (Arai, 1988). The relatively high tolerance to salt of the actinomycetes capable of causing damage to stone highlights their potential ability to persist and attack stone to augment the salt weathering process. Schostak and Krumbein (1992) reported that halotolerant bacteria play a significant role in the deterioration of plasters and wall paintings. Papida *et al.* (2000) showed that the combined effect of salt and microbial weathering was synergistic and could have serious consequences for the integrity of limestone in laboratory experiments. Most of the actinomycete genera isolated in the current study were pigment producers under laboratory conditions. Colour changes to rock monuments are a real threat to cultural heritage, not only from aesthetic point of view but also historically since some of these changes are irreversible. The role of pigment production by microorganisms in the biodeterioration of paintings and stone has been studied previously (Bassi *et al*., 1986; Gonzalez *et al*., 1999). Laboratory experiments have shown that fungi and actinomycetes are the most efficient producers of brown to black stains on rock surfaces (Krumbein, 1992). Actinomycetes were the most common microorganisms isolated from grey surface rocks in Sicily (Urzi and Realini, 1998).

The mechanical role of actinomycete hyphae was clearly evident from previous SEM studies, as their mycelia were extensively branched and penetrating the stone material, filling the pore system of the stone, as well as increasing the surface area for biofilm production (May *et al.*, 2003).

In conclusion, the ancient stone environment in this investigation supported the presence of actinomycetes at different stages of deterioration. The effect of seasonal variation on the composition on the stone was evident as more species were recovered in winter than in summer. Actinomycetes flourished and seemed to dominate stone in the later stages of deterioration, since the decayed stone harboured more genera and species than were present on sound stone. About 20% of the actinomycete isolates could not be identified to species level, which indicates that the stone environment could be regarded as a rich habitat for the isolation of new taxa of actinomycetes. The actinomycetes isolated from sound and decayed stones were capable of causing damage to stone under laboratory conditions but this ability was not correlated to acid production. Hence, the study suggests that the mechanical effect of actinomycete growth on stone may play an important part in the biodeterioration process.

Literature

Agarossi G., R. Ferrari and M. Monte. 1985. Microbial biodeterioration in the hypogea: the subterranean neo-pythagorean basilica of porta maggiore in Rome. pp. 597–605. In: G. Felix (ed.) *Proceedings of 5th International Congress on Deterioration and Conservation of Stone*, Lausanne, Switzerland, press Polytechniques Romandes.

Arai H. 1988. On organisms in the tomb of NEFERTARI. *Science for Conservation (Tokyo)* 27: 13–20.

Bassi M., A. Ferrari, M. Realini and C. Sorlini. 1986. Red stains on the Certosa of Pavia: A case of biodeterioration. *Int. Biodeterior.* 22: 201–205.

De La Torre M.A., G. Gomez-Alarcon, C. Vizcaino and T. Garcia. 1993. Biochemical mechanisms of stone alternation carried out by filamentous fungi living in monuments. *Biogeochemistry* 19: 129–147.

Dietz A. and D.W. Thayer. 1980. *Actinomycete Taxonomy*. Society for Industrial Microbiology, special publication no. 6, Arlington, Virginia, USA

Eckhardt F.E.W. 1985. Mechanisms of the microbial degradation of minerals in sandstone monuments, medieval frescoes and plaster. pp. 643–652. In: G. Felix (ed.) *Proceedings of 5th International Congress on Deterioration and Conservation of Stone*, Lausanne, Switzerland, press Polytechniques Romandes.

El-Nakeeb M.A. and H.A. Lechevalier. 1962. Selective isolation of aerobic *actinomycetes*. *J. Appl. Microbiol.* 2: 75–77.

Giacobini C., M.A. Decicco, I. Tiglie and G. Accardo. 1988. Actinomycetes and biodeterioration in the field of fine art. pp. 418–427. In: D.R. Houghton, R.N. Smith and H.O.W. Eggins (eds), *Biodeterioration 7*, Elsevier Applied Science, New York.

Gonzalez I., L. Laiz, B. Hermosin, B. Caballero, C. Incerti and C. Saiz-Jimenez. 1999. Bacteria isolated from rock art paintings, the case of Altamira shelter (south Spain). *J. Microbiol. Methods* 36: 123–127.

Goodfellow M. and S.T. Williams. 1983. Ecology of actinomycetes. *Annu. Rev. Microbiol.* 37: 189–216.

Groth I., R. Vettermann, B. Schuez, B. Schumann and C. Saiz-Jimenez. 1999. Actinomycetes in karstic caves of northern Spain (Altamira and Titobustillo). *J. Microbiol. Methods* 36: 115–122.

Groth I., G. Annie, J. González, L. Laiz, M. Carlsohn, B. Schütze, J. Wink and M. Goodfellow. 2007. *Amycolatopsis nigrescens* sp. nov., an actinomycete isolated from a Roman catacomb. *Int. J. Syst. Evol. Microbiol.* 57: 513–519.

Hopwood D.A., M.J. Bibb, K.F. Chater, T. Kieser, C.J. Bruton, H.M. Kieser, D.J. Lydiate, C.P. Smith, J.M. Ward, and H. Schrempf. 1985. *Genetic Manipulation of Streptomyces: A Laboratory Manual*. John Innes Foundation, Norwich, UK.

Jain K.K., A.K. Mishra and T. Singh. 1993. Biodeterioration of stone: a review of mechanisms involved. pp. 323–354. In: K.L. Garg, N. Garg, and K.G. Mukerji (eds), *Recent Advances in Biodeterioration and Biodegradation*. Naya Prokash, Calcutta.

Jimenez-Lopez C., C. Rodriguez-Navarro, G. Piñar, F.J. Carrillo-Rosúa, M. Rodriguez-Gallego and M.T. Gonzalez-Muñoz. 2007. Consolidation of degraded ornamental porous limestone stone by calcium carbonate precipitation induced by the microbiota inhabiting the stone. *Chemosphere* 68: 1929–1936.

Krumbein W.E. 1988. Biotransformations in monuments – a sociobiological study. *Durability of Building Materials* 5: 359–382.

Krumbein W.E. 1992. Colour changes of building stones and their direct and indirect biological causes. pp. 443–452. In: R. Delgado, F. Henriques and T. Jeremias (eds), *Proceedings of the 7th International Congress on Deterioration and Conservation of Stone*, Lisbon, Portugal, Laboratorio Nacional de Engenharia Civil.

Kuster E. and S.T. Williams. 1964. Selection of media for isolation of streptomycetes. *Nature* 202: 928–929.

Laiz L., I. Groth, I. Gonzales and C. Saiz-Jimenez. 1999. Microbiological study of the dripping waters in Altamira cave (Santillana del Mar, Spain). *J. Microbiol. Methods* 36: 129–138.

Lechevalier M.P. 1981. Ecological associations involving actinomycetes. pp. 159–166. In: K.P. Schaal and G. Pulverer (eds), *Actinomycetes*. Gustav Fischer Verlag. Stuttgart.

Lewis F., E. May and A.F. Bravery. 1985. Isolation and enumeration of autotrophic and heterotrophic bacteria from decayed stone. pp. 633–641. In: G. Felix (ed.), *Proceedings of the 5th International Congress on deTerioration and Conservation of Stone*, Lausanne, Switzerland: Press Polytechniques Romandes.

Lewis F.J., E. May and R.G. Greenwood. 1988. A laboratory method for assessing the potential of bacteria to cause decay of building stone. pp. 48–58. In: *Proceedings of the 6th International Congress on Deterioration and Conservation of Stone*. Nicholas Copernicus University press, Torun Poland.

McCarthy A.J. and S.T. Williams. 1992. Actinomycetes as agents of biodegradation in the environment – a review. *Gene* 115: 189–192.

May E., S. Papida, H. Abdulla, S. Tayler and A. Dewedar. 2000. Comparative studies of microbial communities on stone monuments in temperate and semi-arid climates. pp. 49–62. In: O. Ciferri, P. Tiano and G. Mastromei (eds), *Of Microbes and Art: Te Role of Microbial Communities in the Degradation and Protection of Cultural Heritage*. Kluwer Academic/Plenum Pub.

May E., S. Papida and H. Abdulla. 2003. Consequences of microbe-biofilm-salt interactions for stone integrity in monuments. pp. 452–471. In: R.J. Koestler. (ed.), *Art, Biology and Conservation*. Metropolitan Museum of Art, NY.

Monte M. and R. Ferrari. 1993. Biodeterioration in subterranean environments. *Aerobiologia* 9: 141–148.

Nuhoglu Y., E. Oguz, H. Uslu, A. Ozbek, B. Ipekoglu, I. Ocak and I. Hasenekoglu. 2006. The accelerating effects of the microorganisms on biodeterioration of stone monuments under air pollution and continental-cold climatic conditions in Erzurum, Turkey. *Sci. Total Environ.* 364: 272–283.

O'Neill T. 1988. Succession and interrelationships of microorganisms on painted surface. *Int. Biodeterior.* 24: 373–379.

Paine S.G., F.V. Linggod, F. Schimmer and T.C. Thrupp. 1933. The relationship of microorganisms to the decay of stone. *Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci.* 222: 97–127.

Papida S., W. Murphy and E. May. 2000. The use of sound velocity determination for the non-destructive estimation of physical and microbial weathering of limestone and dolomites. pp. 609–618. In: V. Fassina (ed.), *Proceedings of 9th International Congress on Deterioration and Conservation of Stone*, Venice, Italy, Elsevier Science.

Schostak V. and W.E. Krumbein. 1992. Occurrence of extremely halotolerant and moderate halophilic bacteria on salt impaired wall paintings. pp. 551–560. In: R. Delgado, Henriques F. and Jeremias T. (eds), Proceedings of the 7th International Congress on Deterioration and Conservation of Stone. Lisbon, Portugal, Laboratorio Nacional de Engenharia Civil.

Somavilla T.F., N. Khayyat and V. Arroyo. 1978. Comparative studies of the microorganisms present in the Aaltamira and La Pasieca caves. *Int. Biodeterior. Bull.* 14: 103–109.

Szabo M., M. Marton, I. Buti and C. Fernandez. 1975. A diagnostic key for the identification of species of *Streptomyces* and *Streptoverticillium* included in the international *Streptomyces* project. *Acta Bot. Acad. Sci. Hung*. 21: 387–418.

Tayler S. 1991. Composition and activities of bacterial populations found on decaying stone work. Ph.D. thesis Portsmouth University, UK.

Tayler S. and May E. 1991. The seasonality of heterotrophic bacteria on sandstone from ancient monuments. *Int. Biodeterior.* 28: 49–64.

Urzi C., M. Garcia-Valles, M .Vendrell and A. Pernice. 1999. Biomineralization process on rock and monument surfaces observed in field and in laboratory conditions. *Geomicrobiol. J.* 16: 39–54.

Urzi C. and M. Realini. 1998. Colour changes of notos calcareous sandstone as related to its colonisation by microorganisms. *Int. Biodeterior. Biodegrad.* 42: 45–54.

Urzi C., S. Lisi, G. Crises and P. Perince. 1991. Adhesion to and degradation of marble by a *Micrococcus* strain isolated from it. *Geomicrobiol. J.* 9: 81–90.

Webley D.M., E. Moira, K. Henderson and I.F. Taylor. 1963. The microbiology of rocks and weathered stone. *J. Soil Sci*. 14: 102–112.

Williams S.T. 1985. Streptomycetes in biodeterioration, their relevance, detection and identification. *Int. Biodeterior*. 21: 201–209.

Williams S.T., M. Goodfellow, G. Alderson, E. Wellington, P. Sneath and M. Sackin. 1983. Numerical classification of *Streptomyces* and related genera. *J. Gen. Microbiol.* 129: 1743–1813.

Williams S.T. and E.M.H. Wellington. 1982. Actinomycetes. pp. 969–987. In: A.L. Page (ed.), *Methods of Soil Analysis*, Part 2. *Chemical and Microbiological Properties Agronomy Monograph* no. 9, ASA-SSSA, Madison, USA.