Heritage Microbiology and Science

Microbes, Monuments and Maritime Materials

Edited by

Eric May
University of Portsmouth, UK

Mark Jones
Mary Rose Trust, Portsmouth, UK

Julian Mitchell
University of Portsmouth, UK

RSC Publishing

The papers in this volume were presented at the conference Heritage Microbiology and Science: Microbes, Monuments and Maritime Materials held in Portsmouth 28 June – 1 July 2005.

The conference and, in part, this publication were made possible by a generous donation by The Coral Samuel Charitable Trust.

Special Publication No. 315

ISBN: 978-0-85404-141-1

A catalogue record for this book is available from the British Library

Published by The Royal Society of Chemistry,
Thomas Graham House, Science Park, Milton Road,
Cambridge CB4 0WF, UK

Registered Charity Number 207890

For further information see our web site at www.rsc.org

ANALYSIS OF BACTERIAL COMMUNITIES ON AN ANTIQUE STAINED GLASS WINDOW

M. Marvasi*, E. Vedovato, C. Balsamo, G. Mastromei, B. Perito

Department of Animal Biology and Genetics "Leo Pardi", University of Florence, Via Romana 17, 50125, Florence, Italy

1 INTRODUCTION

Microbial corrosion of glass causes problems on delicate antique glass samples, which may significantly interfere with its optical properties, or its use. Until now, the effect of microbial activity on corrosion phenomena has not been well documented and only a few studies have been published concerning the microflora growing on glass surfaces. Different microorganisms including lichens (*Diploica*, *Pertusaris*, *Lepraria* sp.), fungi (*Aspergillus* sp., *Penicillium* sp.) and bacteria (*Flexibacter* sp., *Nitrosospira* sp., *Arthrobacter* sp., *Streptomyces* sp., *Micrococcus* sp., *Frankia* sp., *Geodermatophilus* sp.) have been shown to grow on glass surfaces.[1,2,3,4,5] Moreover, it has been demonstrated that bacterial and fungal communities on biodeteriorated glass surfaces are much more complex than previously believed.[6,7] Physical-chemical mechanisms of deterioration are known[8] and the microorganisms could accelerate physical and chemical reactions leading to decay processes. Microorganisms can enhance the glass deterioration process by excretion of chemically aggressive substances or by physical attack and, furthermore, microorganisms can get the elements needed for growth from the deteriorating glass.[9] Due to the absence of organic nutrients, the glass is considered an extreme environment, where only specialised microorganisms are able to survive.[6] On glass exposed to the external environment (such as a stained window), pollen, bird faeces and fine organic matter can however deposit and could provide nutrients for microorganisms.[7]

The study of microbial communities on antique glass is important to understand the relationship between microorganisms and the glass surface, and to identify the more aggressive strains or the successive colonisations by different microorganisms. Studies on microbial communities are useful for monitoring microorganisms, after completion of restoration or to recognize those effective biocides that can eliminate microorganisms.[10,11] The present work is based on the characterization of cultivable aerobic bacteria isolated from the historical glass window "Natività" of the Florence Cathedral, designed by Paolo Uccello and made by Angelo Lippi between 1443 to 1444 (Figure 1). Microbial strains were sampled from four of the 25 panels of the "Natività" during a recent restoration due to the presence of various kinds of patinas and crusts. Isolated bacteria were submitted to

* Corresponding author's e-mail: massimiliano.marvasi@unifi.it

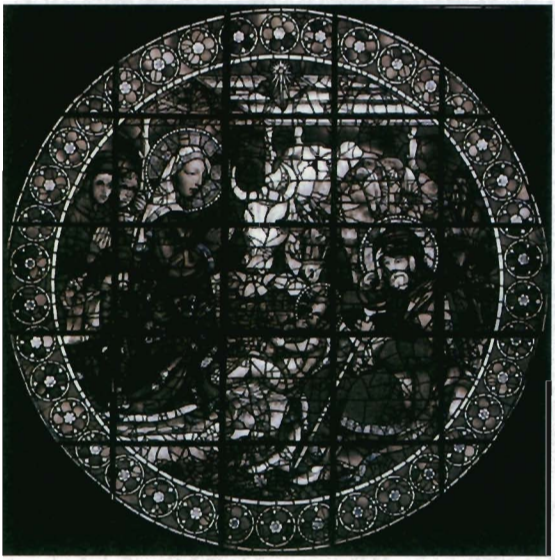

Figure 1 *Window of "Natività" of the Florence Cathedral. It represents the birth of Jesus*

morphological characterization and classified according to Gram stain. For sixteen strains, from different glass panels, the rDNA 16S gene was amplified and sequenced.

2 METHODS AND RESULTS

2.1 Deterioration on the window "Natività"

In Spring 2004, restorers (Studio Polloni) removed the window "Natività" to clean the stained glass, 50 years after the last restoration. Macroscopic crusts and patinas covered a large part of the glasses. The stained glasses, on the outside of the Cathedral, were deteriorated and presented various kinds of crusts: powder and hard crusts. Only pieces of green glasses were clean, with no visible deterioration phenomena. On the inside of the Cathedral the glasses were clean and no visible crusts were present.

2.2 Sampling, growth conditions and phenotypical characterisation

Contact plates filled with Nutrient Agar (OXOID) supplemented with 1% glucose were used for microbiological sampling. Four of the 25 panels of the window were sampled by 10 contact plates: panels 6, 14 and 17 on the outside of the window and panel 7 on the inside of the window. The plates were incubated at room temperature for 3 days. Colonies were isolated several times in order to obtain pure cultures on Nutrient Agar medium. One hundred microorganisms were isolated, 50% bacteria and 50% fungi. Bacteria were further characterized; isolates were named by a first number corresponding to the panel followed by a letter corresponding to the sampled area of the panel and a last number identifying the strain. Figure 2 shows the numbers of bacteria isolated from the panels. The green glass has the lowest number of isolated strains. Colonies were examined under the

stereomicroscope to characterize their shape. Cell morphology was observed in fresh samples with a phase contrast Nikon Alphaphot YS microscope at 400 and 1000 magnification. Observation with the phase contrast microscope showed that the most common cellular shape was bacillus (Figure 3). Lysis tests were performed with KOH 3% to classify the isolates into their Gram stain group.[12] The lysis test showed a preponderance of Gram-positives (Figure 3).

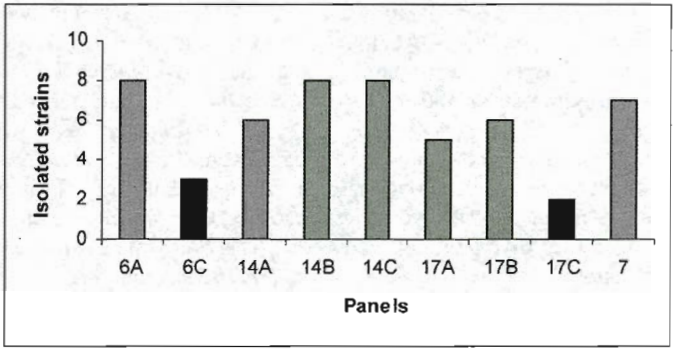

Figure 2 *Distribution of strains isolated from glass panels. X-axis: panel areas sampled. The number corresponds to the panel, the letter following corresponds to different areas of the same panel. Y-axis: number of strains sampled. Strains isolated from green glass are in black columns (6C, 17C)*

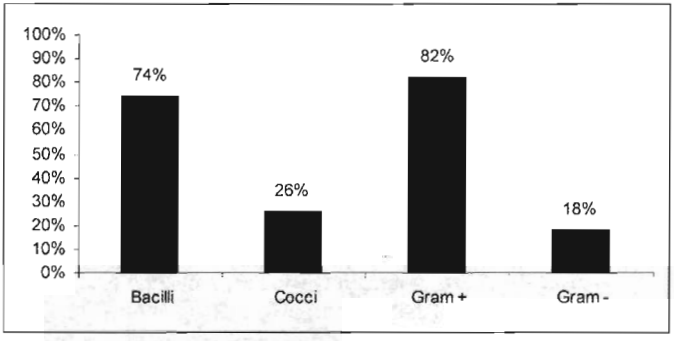

Figure 3 *Percentage of cocci, bacilli, Gram-positives and Gram-negatives*

2.3 Molecular characterization

For sixteen bacteria, isolated from the four panels, the 16s rDNA was analysed. To extract DNA, bacteria were grown on Nutrient Agar as a confluent lawn. DNA extraction was performed with FastDNA Kit (Q-BIOgene) according to the manufacturer's specifications. The 16S rDNA amplification was performed using primers P0 and P6, which anneal to positions 8–27 and to positions 1495–1515, respectively, of the *E. coli* 16S rDNA gene.[13] PCR products were purified (High Pure PCR Product Purification Kit, ROCHE) and sequenced. All sequences were analysed at the prokaryotic small subunit rDNA Ribosomal Database Project II (website: http://rdp.cme.msu.edu/index.jsp).[14] "Classifier" option was

used to assign them to genus. "Sequence Match" option was used to assign them to the nearest neighbour sequences contained in RDP II. Results are shown in Table 1.

Nine isolates sampled from panel 6 were characterised: one from green glass (6C-1) belonged to the genus *Arthrobacter*, while seven of the eight from deteriorated glass belonged to the genus *Bacillus* (6A-1, 6A-2, 6A-4, 6A-6, 6A-8, 6A-9, 6A-10) and one to genus *Peanibacillus* (6A-7). Among sequences classified as belonging to *Bacillus*, those of 6A-10 and 6A-9 are quite similar, with only 2 base differences. The two bacterial isolates from panel 14 belonged to genera *Peanibacillus* (14A-7) and *Arthrobacter* (14C-5). The isolate from stained glass of panel 17 belonged to the genus *Bacillus* (17B-6), while that from the green glass of panel 17 was identified as genus *Stenotrophomonas* (17C-6). Panel 7 was the only one sampled on the inside; the three bacteria characterised belonged to genera *Brevundimonas* (7-4), *Leucobacter* (7-5) and *Arthrobacter* (7-7). Almost all the isolates from dirty glass, except strain 14C-5, belonged to the Gram-positive low %G+C group of the Phylum Firmicutes. Among these, are all the isolates (eight) sampled from all panel 6, except the green glass. Nevertheless, bacteria from panel 6 related to *Bacillus* show a certain degree of divergence. Isolates 6A-9 and 6A-10 have the best match with *Bacillus megaterium*, 6A-8 with *Bacillus simplex*, 6A-6 and 17B-6 with *Bacillus thuringensis*, 6A-1 and 6A-2 with *Bacillus mojavensis* and 6A-4 with *Bacillus pumilus*. Isolate 6A-7 has the best match with *Paenibacillus pabuli*. The last isolate, 14A-7, of the low %G+C group has the best match with *Paenibacillus polymyxa*. Four isolates were related to representatives of the Gram-positive high %G+C group of the Phylum Actinobacteria, belonging to different species of the genera *Arthrobacter* and *Leucobacter*. 6C-1 and 7-7 have the best match with *Arthrobacter agilis*. Isolate 14C-5 has the best match with *Arthrobacter crystallopietes* and 7-5 with *Leucobacter komagatae*.

Two isolated Gram-negative bacteria were representatives of the Phylum Proteobacteria. Isolate 17C-6 has the best match with *Stenotrophomonas maltophila*, a γ-Proteobacteria, while 7-4 has the best match with *Brevundimonas subvibroides*, an α-Proteobacteria.

3 CONCLUSIONS

The present paper describes the characterization of bacterial strains isolated from the historical window "Natività". Classical techniques such as microscopical and physiological investigations showed that bacilli and Gram-positive bacteria were dominant. The genera assigned by molecular analysis are in agreement with the phenotypical data. Isolate 6A-7 resulted Gram-negative and should belong to the genus *Peanibacillus*. In the literature, *Peanibacillus* shows heterogeneity for Gram stain; there are Gram-positive species, Gram-negative and other species with a variable reaction depending upon growth stages.[15,16] The majority of the isolates belonged to Phyla Firmicutes and Actinobacteria. Almost all strains isolated from degraded glass (black circles, Table 1) belong to the Firmicutes group, with the genus *Bacillus* the most represented. Firmicutes were only isolated from crusts on the outside of the Cathedral. Therefore, a possible relationship between crusts and spore-forming bacteria belonging to Firmicutes seems to emerge. Almost all bacteria isolated from clean glass (green glasses and glass inside the Cathedral) belong to the Phyla Actinobacteria and Proteobacteria (white circles, Table 1).

Strain	Cell morphology	Gram group assigned by lysis test[11]	16S rDNA sequence (bp)	Genus assignment[a]	Best match (similarity score[b])
• 6A-1	Short rods	+	1283	*Bacillus*	(1.000) *Bacillus mojavensis*; IFO15718; AB021191 (0.916) *Bacillus licheniformis* (T); DSM 13; X68416
• 6A-2	Short rods	+	1350	*Bacillus*	(0.982) *Bacillus mojavensis*; IFO15718; AB021191 (0.953) *Bacillus vallismortis* (T); DSM11031; AB021198
• 6A-4	Short rods	+	1427	*Bacillus*	(0.982) *Bacillus pumilus*; WN697; AY260859 (0.874) *Bacillus vallismortis* (T); DSM11031; AB021198
• 6A-6	Streptobacilli	+	1326	*Bacillus*	(0.983) *Bacillus thuringiensis* (T); ATCC10792; AF290545
• 6A-7	Short rods	-	1393	*Paenibacillus*	(0.981) *Paenibacillus pabuli*; HSCC 473 (NRRL BD-537)AB045104 (0.969) *Paenibacillus amylolyticus* (T); NRRL NRS-290T; D85396
• 6A-8	Rods	+	1293	*Bacillus*	(0.965) *Bacillus simplex*; LMG 21002; AJ628745 (0.853) *Bacillus simplex* (T); DSM 1321; X60638
• 6A-9	Streptobacilli	+	1386	*Bacillus*	(0.999) *Bacillus megaterium*; MO31; AY553118 (0.939) *Bacillus megaterium* (T); DSM 32; X60629
• 6A-10	Streptobacilli	+	1396	*Bacillus*	(0.998) *Bacillus megaterium*; GSP10; AY505510 (0.940) *Bacillus simplex* (T); DSM1321; D78478
○ 6C-1 (green glass)	Diplococci	+	1374	*Arthrobacter*	(0.972) *Arthrobacter agilis* (T); DSM 20550; X80748
○ 7-4 (inside)	Little rods	-	1294	*Brevundimonas*	(0.937) *Brevundimonas subvibrioides* (T); LMG 14903T; AJ227784 (0.914) *Brevundimonas variabilis* (T); ATCC 15255 (T); AJ227783
○ 7-5 (inside)	Cocci	+	1226	*Leucobacter*	(0.983) *Leucobacter komagatae*; IFO15245T; AJ746337 (0.953) *Leucobacter komagatae* (T); JCM 9414; D45063
○ 7-7 (inside)	Cocci	+	1363	*Arthrobacter*	(0.948) *Arthrobacter agilis* (T); DSM 20550; X80748
• 14A-7	Rods	+	1448	*Paenibacillus*	(0.971) *Paenibacillus polymyxa*; GBR-27; AY359615 (0.954) *Paenibacillus kribbensis* (T); AM49; AF391123
• 14C-5	Cocci	+	1357	*Arthrobacter*	(0.984) *Arthrobacter crystallopoietes* (T); DSM 20117; X80738
• 17B-6	Streptobacilli	+	1363	*Bacillus*	(0.981) *Bacillus thuringiensis* (T); ATCC10792; AF290545
○ 17C-6(green glass)	Streptobacilli	-	1420	*Stenotrophomonas*	(0.959) *Stenotrophomonas maltophilia* (T); ATCC 13637T; AB008509

Table 1 *Phenotypical and molecular characterisation of 16 bacterial isolates from four panels of the "Natività". [(a)] Genus assignment by the Classifier option. [(b)] Similarity score assignment by the Sequence Match option: the number of (unique) oligomers shared between query sequence and a given RDP sequence divided by the lowest number of unique oligos in either of the two sequence. (•) Black circles represent isolates obtained from glass with macroscopic crusts; (○) white circles represent isolates obtained from glass without macroscopic crusts (inside the cathedral and on green glass).*

References

1 E. Müller, U. Drewello, R. Drewello, R. Weissmann and S. Wuertz. *J. Cultural Heritage*, 2001, **2**, 31.
2 E. Mellor, *Nature*, 1923, **112**, 299.
3 A.A. Gorbushina and K. A. Palinska, *Aerobiologia*, 1999, **15**, 183.
4 R. Drewello and R. Weissmann, *Appl. Microbiol. Biotechnol*, 1997, **47**, 337.
5 J.P. Kaiser, S. Trümpler and P. Raschle, in *Microbially Influenced Corrosion of Materials*, Springer, Berlin, 1996, p.353.
6 S. Rölleke, C. Gurtner, U. Drewello, R. Drewello, W. Lubitz and R. Weissmann, *J. Microbiological Methods*, 1999, **36**, 107.
7 Schabereiter-Gurtner, G. Piñar, W. Lubitz and S. Rölleke, *J. Microbiological Methods*, 2001, **47**, 345.
8 M. Verità, in *Le vetrate artistiche: struttura, composizione, proprietà chimico-fisiche dei vetri*, Ed. Edipuglia, Bari, 1998, p. 53.
9 I.H. Thorset, H. Furnes and O. Tumyr, *Chem. Geol.*, 1995, **119**, 139.
10 A. Bianchi, M.A. Favali, N. Barbieri, M. Bassi, *Int. Biodeter. Bull.*, 1980, **16**, 45.
11 S. Tayler and E. May, *Mater. Organismen*, 1994, **28**, 265.
12 E.M. Powers, *Appl. Env. Microbiol.*, 1995, **61**, 3756.
13 F. Di Cello, M. Pepi, F. Baldi and R. Fani, *Res. Microbiol.*, 1997, **148**, 237.
14 J.R. Cole, B. Chai, R.J. Farris, Q. Wang, S.A. Kulam, D.M. McGarrell, G.M. Garrity and J.M. Tiedje, *Nucleic Acids Res.* 2005, **33**(Database Issue), D294-D296, doi: 10.1093/nar/gki038.
15 A.S. Rosado, F.S. de Azevedo, D.W. da Cruz, J.D. van Elsas and L. Seldin, *J. Appl. Microbiol.*, 1998, **84**, 216.
16 M. Aguilera, M. Monteoliva-Sánchez, A. Suárez, V. Guerra, C. Lizama, A. Bennasar and A. Ramos-Cormenzana, *Int. J. Syst. Evol. Microbiol,* 2001, **51**, 1687.