World J Microbiol Biotechnol (2007) 23:363–366
DOI 10.1007/s11274-006-9232-3

ORIGINAL PAPER

Diversity of salt-tolerant culturable aerobic microorganisms on historic buildings in Southern Brazil

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Received: 18 January 2006 / Accepted: 28 June 2006 / Published online: 26 July 2006

Abstract Halotolerant and halophilic microorganisms may be expected to be present on the external walls of buildings, especially in dry and/or hot climates. They have been detected in Europe in various molecular biological studies. Using plating on specific salt-supplemented medium (10 and 15%), bacteria and fungi were detected in winter, spring and summer months on the discoloured external walls of 5 historic buildings in Porto Alegre, South Brazil. Microbial biodiversity in the samples was calculated using the Simpson Index. Halotolerant non-phototroph diversity was highest in the winter (September), when environmental conditions are less extreme. Some collections yielded organisms that grew on media with 15% added salt; diversity in this case was lower. Frequently, and in all winter collections, only one type of microorganism grew; this was a brown-pigmented fungus, probable genus *Cladosporium*. Greater diversity was found at 15% salt in 2 collections made in the summer month of January. It is suggested that this results from the selection of organisms able to resist the more extreme conditions of high temperature and lower relative humidity.

Keywords Biodeterioration · Biodiversity · Biofilms · Halotolerance · Historic property

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Introduction

There has been increased interest recently in the preservation of historic monuments; considerable effort is being employed in developing new methods for controlling weathering, and especially the biologically influenced deterioration, of these structures (Meira 2001). Concrete and stone structures can be attacked and destroyed by microorganisms. The problems caused by microbial growth are both structural and aesthetic (Gurtner et al. 2000).

The organisms associated with biodeterioration processes on buildings grow as biofilms containing a complex community of microorganisms, extracellular polymeric substances (EPS), particulates and a high percentage of water, forming, under suitable moisture conditions, a gelatinous film (Gaylarde and Morton 2002). Biofilms on internal and external surfaces of buildings contain a wide variety of microorganisms, whose physical presence and metabolic activities lead to degradation of the constructional materials (Gaylarde and Morton 1999; Shirakawa et al. 2002).

The main microorganisms involved in this biodeterioration are bacteria, fungi, algae/cyanobacteria and lichens. Fungi and the majority of bacteria use organic materials as nutrients, as they lack photosynthetic activity. Yeasts such as *Rhodotorula*, bacteria of the genera *Bacillus, Micrococcus* and *Pseudomonas*, and fungi such as *Ulocladium, Phoma, Alternaria, Penicilium* and *Cladosporium* are commonly found (Flores et al. 1997). This variety in the microbial community indicates the complexity of the populations influencing deterioration (Nica et al. 2000). Among the microorganisms growing on exposed surfaces of buildings, halophiles, or salt-tolerant organisms (Kushner 1978)

are important because of their ability to survive the desiccation occurring on these surfaces, particularly in the hotter parts of the world.

An increased understanding of the microbial diversity in biofilms on historic monuments is important for the development of correct conservation and restoration strategies. One problem that has recently been pinpointed in determining such diversity is that cultivation methods recover less than 1% of the total species of microorganisms present in environmental samples (Giovannoni et al. 1990; Ward et al. 1990; Gurtner et al. 2000). Some of these ''non-culturable'' organisms could be detected by the use of specific growth media which have not previously been employed routinely. With respect to microorganisms on external surfaces of historic stone buildings, the use of media with higher than normal concentrations of salts is an obvious strategy.

The aim of the present study was to investigate the culturable diversity of samples derived from five historic buildings at various times of the year, using growth medium containing various salt concentrations.

Materials and methods

Sampling

Samples were collected by scraping visible biofilms (discoloured areas) on the external walls of 5 historic buildings in Porto Alegre, in the south of Brazil, into sterile containers. Some of these buildings were in an advanced state of biodeterioration. All the buildings date from the start of the 20th century. They include 4 churches, Nossa Senhora das Dores (1903), São Geraldo (1940), Santo Antônio do Partenon (1932) and São Judas (1943), and the Office of the Vice-Governor of the State of Rio Grande do Sul. Three samples were collected from each building in September (winter), November (spring) and January (summer). In Nossa Senhora das Dores (north face), São Geraldo (north face) and the Office of the Vice-Governor of the State of Rio Grande do Sul (east face) the substrate collect was covered by paint, and in Santo Antônio do Partenon (south face) and São Judas (north face) samples were taken from stone without paint.

Cultivation

The samples (a total of 15 at each collection) were hydrated in 1 ml of distilled water and 0.1 ml of each sample was inoculated onto Thornton's medium (Cattelan and Vidor 1990) supplemented or not with 10% or 15% NaCl. The plates were incubated in an illuminated BOD incubator at 28°C for 15 days.

After 15 days, the colonies growing on all the plates were counted, differentiating colony morphology and coloration for bacteria, and colony structure and pigmentation for fungi. No species identification was attempted.

Calculation of diversity

The Simpson Index was used to calculate diversity. Diversity (D) is given by $D = 1 - \sum Pi^2$ where Pi is the proportion of individuals of the same species in relation to the total number of individuals. In this case, species were not identified, but the different colony types were differentiated for use in the formula. A Simpson Index near to one indicates high diversity.

Results and discussion

Total viable counts

Table 1 shows the total numbers of colonies formed on each medium inoculated with the samples collected at different times. Bacteria and filamentous fungi were detected. No cyanobacterial colonies were seen, although it is known that these organisms are present in high numbers in these biofilms (Crispim et al. 2004). This is readily explained by the use of Thornton's medium, which contains an organic nutrient source; on this medium, heterotrophic microorganisms rapidly outgrow the autotrophs.

Growth on medium with no added salt was generally confluent and uncountable. Samples were not diluted as this would have influenced the total diversity detected. Many of the bacteria growing on these plates have been shown to be of the genus *Bacillus*, although Gram-negative species were also found (Kiel and Gaylarde 2006). On salt-containing plates, countable colonies, both bacterial and fungal, were produced from all buildings, confirming the presence of halotolerant microorganisms. Salt-tolerant bacteria have been detected by both culture and molecular biological techniques on European monuments (Saiz-Jimenez and Laiz 2000; Heyrman and Swings 2001). Piñar et al. (2001) reported the presence on a 14th century Austrian castle of a small number of halophilic bacterial types able to grow on up to 20% salt, optimally on 5–10%. They were identified by DNA analysis as new members of the genus *Halobacillus*. Although we did not identify our colonies, this is the first report of halotolerant bacteria on historic buildings in Brazil. It

Table 1 Number of colonies growing on media with added salt after 15 days (numbers are totals of colonies growing on 3 plates for each sample)

Building	Winter	Spring	Summer
10% salt			
1 Nossa Senhora das Dores Church	275	29	81
2 Vice-Governor's Office	289	31	21
3 São Geraldo Church	37	164	0
4 Santo Antônio do Partenon Church	77	7	6
5 São Judas Church	3	752	18
15% salt			
1 Nossa Senhora das Dores Church	114	1	37
2 Vice-Governor's Office	1	0	16
3 São Geraldo Church	3	0	0
4 Santo Antônio do Partenon Church	2	0	2
5 São Judas Church	1	0	0

Table 2 Simpson Indices for samples collected in 3 different seasons from 5 buildings in Porto Alegre

Building	10%	15%
Winter		
1 Nossa Senhora das Dores Church	0.35	0
2 Vice-Governor's Office	0.99	0
3 São Geraldo Church	0.74	0
4 Santo Antônio do Partenon Church	0.77	0
5 São Judas Church	0.45	0
Spring		
1 Nossa Senhora das Dores Church	0.69	
2 Vice-Governor's Office	0.17	*
3 São Geraldo Church	0.012	*
4 Santo Antônio do Partenon Church	0.61	*
5 São Judas Church	0	*
Summer		
1 Nossa Senhora das Dores Church	0.32	0.38
2 Vice-Governor's Office	0.57	0.73
3 São Geraldo Church	*	*
4 Santo Antônio do Partenon Church	0.67	0
5 São Judas Church	0	*

* No growth

is also the first time that halotolerant fungi have been detected on historic buildings.

Biodiversity

Salt concentration affected the Simpson Index considerably. More organisms grew at the lower salt concentration and diversity was higher. The diversity values are shown in Table 2. The Simpson Indices varied considerably in 10% salt cultures, but were highest in the winter collections (mean value 0.66, compared with 0.30 in spring and 0.39 in summer). Spring and summer collections also contained several samples that produced no growth on salt-supplemented plates. Diversity was highest in the winter samples for all buildings except Nossa Senhora das Dores Church, where the Simpson Index value was highest in spring.

The climatic conditions in Porto Alegre are very different in the months during which samples were taken (Table 3). In the winter month of September, there is high rainfall and relative humidity (RH), while temperatures are lower than in other seasons. The summer month of January has the highest temperatures and, although RH is similar to the average in November, rainfall is lower in the latter month.

This data makes it easy to understand the higher levels of diversity in September samples; high humidity and rainfall increases the likelihood of survival of less resistant microorganisms on exposed walls and the lower temperatures allow walls to remain damp for longer periods. In addition, the average number of hours of sunlight per day are lower in September (5 h) than November and January (8 h), reducing the time for which cells are exposed to damaging u.v. irradiation. Although slightly higher total numbers of microorganisms were detected in spring samples, the diversity was less, suggesting that environmental conditions were becoming inappropriate for some types of microorganisms. Frequently, and in all winter collections, only one type of microorganism grew (zero diversity); this was a filamentous fungus, probable genus *Cladosporium*.

In medium containing 15% salt, the colonies, where present, were small, suggesting that, although capable of survival and slow growth, these organisms are not truly halophilic, but merely halotolerant. When growth occurred, the calculated Simpson Index was most frequently zero, indicating no diversity. In other words, just one type of microorganism from 4 spring and 2 summer collections was able to grow at this salt concentration. In all cases, this was a brown-pigmented filamentous fungus, probable genus *Cladosporium*. This fungal genus has previously been shown to be halotolerant (Shirakawa et al. 2002; Gunde-Cimerman et al. 2000) and, indeed, has been detected as the major

Table 3 Climatic data for Porto Alegre in the sampling months (monthly averages)

Month	Sunlight (h/day)	Temperature min–max (°C)	RH min–max (%)	Rainfall (mm)
September (winter)	5	12–21	63–89	132
November (spring)	8	16–27	55–79	79
January (summer)	8	19–31	55–79	89

colonizer of recently painted external walls in the city of Sao Paulo, Brazil (Shirakawa et al. 2002).

It is interesting to note that the highest diversity of organisms growing on 15% salt occurred in the summer. This may reflect the higher temperatures, which result in more rapid drying of walls and hence a relative increase in the salt concentrations at their surface. The more halotolerant organisms would be selected at the expense of the less tolerant.

Conclusions

1. Bacteria and fungi capable of growth on medium supplemented with 10% NaCl were detected in winter, spring and summer months on 5 historic buildings in Porto Alegre, southern Brazil, independently of orientation.
2. Halotolerant non-phototroph diversity was highest in the winter (September), when environmental conditions are less extreme.
3. Some collections yielded organisms that grew on media with 15% added salt; diversity in this case was less, although present in 2 of the 5 summer collections.

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