Antimicrobial activity of G-0 against bacteria and fungi isolated from cement lime roofs

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Recibido: 17 de noviembre del 2000. Aceptado: 24 de julio del 2001.

Palabras clave: actividad antimicrobiana, antibacteriana, antifúngica, biodeterioro, preservos.
Key words: antimicrobial, antibacterial and antifungal activities, biodeterioration.

RESUMEN. Las tejas de cemento constituyen una solución económica para los tejados de casas elaboradas con bajos recursos. Sin embargo, al poco tiempo de su instalación en climas tropicales, estas tejas experimentan el embate del biodeterioro, el cual las destruye parcial o totalmente. En estas tejas se han aislado bacterias y hongos responsables en gran medida de este daño. La posibilidad de emplear nuevos productos para la preservación de este tipo de material, requiere un estudio previo para evaluar la potencialidad antimicrobiana del posible preservo frente a las cepas microbianas, a fin de determinar las concentraciones mínimas efectivas para el conjunto de microorganismos que las atacan. El presente trabajo muestra los resultados de la determinación de las concentraciones mínimas inhibitorias (CMI) para bacterias y hongos, así como las bactericidas (CMB) y funguicidas (CMF) de un producto nacional nombrado G-0 para 17 cepas bacterianas y 12 de hongos, la mayoría de ellas aislada previamente de tejas deterioradas. Los géneros de bacterias empleados fueron: *Bacillus, Pseudomonas y Micrococcus* y los de hongos: *Aspergillus, Penicillium, Cladosporium, Curvularia, Fusarium, Monilia, Aureobasidium and Tritirachium.* Se comprobó que el G-0 es capaz de inhibir el crecimiento microbiano en todos los cultivos estudiados (tanto de las bacterias como de los hongos) y que posee actividad biocida, y aunque ejerció un efecto funguicida sobre la totalidad de las cepas usadas, su poder bactericida sólo se manifestó sobre el 82,3 % de las cepas bacterianas, por lo que posee mayor efectividad como antifúngico.

ABSTRACT. The cement's lime roofs are an economic solution for the popular buildings construction. In spite of this, when this cement pieces are installed in tropical weather, in a short time they suffer visible decay signs, which can destroy them partial or totally. Recently, fungal and bacterial strains responsible for its biodeterioration have been isolated. The possibility to use new antimicrobial products for covering, protecting and enlarging the life time of this cement lime roofs requires a microbiological study to determine the antibacterial and antifungal action of the possible preservative agent against different microorganisms and the minimal active concentration of this product. In the present work, the antibacterial and antifungal activities of a new Cuban product named G-0 was studied against seventeen bacterial and twelve fungal strains principally isolated from similar lime roofs with evident signs of biodeterioration in order to establish its Minimal Inhibitory, Bactericidal and Fungicidal Concentration (MIC, MBC and MFC, respectively). Bacterial strains belonging to the *Bacillus*, *Pseudomonas* and *Micrococcus* genera and fungal strains of *Aspergillus, Penicillium, Cladosporium, Curvularia, Fusarium, Monilia, Aureobasidium and Tritirachium* genera were used. These results demonstrate the inhibitory properties of G-0 against all microbial cultures tested. The MIC, MBC and MFC values of all sensitive microorganism strains have been found. The fungicidal effects of this product were evident in all the fungal cultures used, but bactericidal properties have been observed only in 82.3 % of the bacterial strains tested. It was obvious that the antifungal action of G-0 is bigger than its antibacterial activity.

INTRODUCTION

Microorganisms have a great metabolic variability that makes them able to form colonies and to subsist in the most diverse habitats.[1] This variability enable them to be responsible for a great part of the biodeterioration of the most diverse materials.[2] Fungi, bacteria and lichens are among the responsible agents for the deterioration of buildings and mural paintings.[3]

As the buildings composed of the most diverse materials are exposed to the rough weather, they shelter tiny amounts of organic matter that is placed on those buildings because of the dust and the wind. Those organic pollutants are essential to start the growth of the microorganisms arriving to those surfaces[4] and to start the biodeterioration process.

Several works have been performed with the purpose of inhibiting the microbial growth on the surface of very valuable monuments, such as the Egyptian pharaons' tombs[5] or colonial fronts of Mexico.[6]

In former works, Rojas *et al*. isolated bacteria and fungi from the inner part of pieces of deteriorated cement's lime roofs. Those authors identified 16 strains of bacteria from the *Bacillus, Pseudomonas* and *Micrococcus* genera, as well as 12 strains of fungi belonging to the *Aspergillus, Penicillium, Cladosporium*, *Curvularia, Fusarium, Monilia, Aureobasidium* and *Tritirachium* genera.[7]

A solution to this problem would be to cover the lime roofs with spe-

cial paints, since they can contain antimicrobial substances in their composition.[8]

The possibility of using a paint containing a antimicrobial preservative agent appropriate to protect those materials would prevent their fast destruction, and would also allow to extend their life by preventing the growth of the pollutant microorganisms on their surface.

To be able to prepare this paint, the first step is the assessment of the *in vitro* antimicrobial activity of the compound devoted to be a preservative agent, in order to define its potentiality and action before preparing the painting that will contain it. The G-0 is a new product derived from sugar cane processed by the Chemical Bioactives Center of the Central University of Las Villas, Cuba. In spite of having an antibacterial and antifungal activity, this compound has a toxic effect that prevents its use for therapeutic purposes in humans, therefore, this would be an option for its practical use.

The purpose of this work is to assess the *in vitro* antibacterial and antifungal activity of G-0 and to determine its minimal inhibitory and biocidal concentrations against strains of bacteria and fungi isolated from deteriorated lime roofs.

MATERIALS AND METHODS

Microorganisms

Fourteen strains of bacteria and twelve of fungi isolated from cement's lime roofs and tree strains of phenol-resistant bacteria were employed (Table 1).

In order to assess G-0, it was dissolved in ethanol 96 %, it was taken to final concentrations between 2.5 and 0.001 %.

The Minimal Inhibitory Concentration (MIC) was determined by the method of serial double dilutions,[10] by using nutrient broth for bacteria and glucose Sabouraud broth for fungi as culture media. As control, parallel tubes were prepared with the same culture broth and the G-0 solution was substituted by ethanol 96 % at the same ratio. A tube with culture broth without additives was inoculated as a positive control of the growth of each strain. All the tubes were inoculated with 0.1 mL of a suspension similar to the 3 McFarland tube ($9 \cdot 10^8$ cell/mL) for each strain.[11] The study was performed in parallel for each culture and three replicates for tube were made (for the product and for the control of ethanol).

The Minimal Biocide Concentration (Bactericidal or Fungicidal, MBC or MFC, respectively) was determined after incubation for 24 h of the tubes inoculated and incubated at 35 °C for the bacterial strains and at 25 °C for the fungi and then, reseedings were made on the surface of plates with nutrient agar or glucose Sabouraud agar medium, according to the type of microorganism. Those plates were incubated at the corresponding appropriate temperatures for fungi or bacteria.

RESULTS AND DISCUSSION

Although the ethanol used as solvent of G-0 has an inhibiting effect and, in a lesser degree, a bactericidal effect on the bacteria, it was confirmed that this effect is always lower than the one caused by the tested product (Table 2). An inhibition of growth was detected in some cultures, but not a biocidal effect, not even at the higher concentration (Tube 1 with 1.2 % of the product tested), both by the solvent and G-0.

The resistant strains are some of those belonging to the *Bacillus* genus tested, producing endospores, very resistant to physical and chemical agents, such as 8, 9 and 12. Generally, this product's MIC is in an interval of 0.001-0.03 %, while the bactericidal effect is in concentrations of 1.2 and 0.001 % for the group of bacteria.

It was shown that all the fungal strains were inhibited by effect of G-0, although ethanol also showed a fungistatic effect, on all the cultures (Table 3). The performance of the control series with ethanol made possible to detect the antifungal effect of G-0, independently of the inhibition due to the solvent. Meanwhile, the bactericidal effect of ethanol only affected seven strains (Table 2). Moreover, the 12 cultures of fungi showed sensitivity due to the fungal effect of G-0, since there was no subsequent growth in the reseeding in solid medium (Table 3).

The MIC of G-0 for the bacterial strains studied are from 0.001 to 0.03 %, The values found do not seem to have a relationship with the type or genus of bacterium, since a great variability was shown among the values of strains of the same taxonomic genus (Table 4).

All the strains, including the phenol-resistant strains used showed a delay in their growth due to the G-0 at concentrations higher than 0.03 %, so we could say that this is the Minimal Effective Concentration to achieve the inhibition of all the bacterial strains studied. Moreover, three bacterial strains were shown to be resistant to G-0 and did not show any lethal effect to any of the concentrations of the product employed, that because of their scarce solubility could not be used at higher

Table 1. Bacterial and fungal cultures used for the assessment of the antimicrobial activity of G-0.

Bacteria			
Key	Strain	Key	Strain
1	*Pseudomonas aeruginosa* *	10	*Pseudomonas pseudoalcaligenes*
2	*Escherichia coli* *	11	*Pseudomonas psudomallei*
3	*Staphylococcus aureus* *	12	*Bacillus sphaericus*
4	*Pseudomonas syringae*	13	*Bacillus lentus*
5	*Bacillus thurigiensis*	14	*Bacillus stearothermophilus*
6	*Micrococcus sedentarius*	15	*Bacillus circulans*
7	*Pseudomonas putida*	16	*Micrococcus varians*
8	*Bacillus brevis*	17	*Pseudomonas maltophila*
9	*Bacillus megaterium*		
Fungi			
1	*Curvularia lunata*	7	*Aureobasidium pullulans*
2	*Penicillium* sp.	8	*Cladosporium cladosporoides*
3	*Syncephalastrum racemosum*	9	*Fusarium* sp.
4	*Tritirachium album*	10	*Penicillium* sp.
5	*Aspergillus fumigatus* Fres	11	*Aspergillus tamarii*
6	*Aspergillus flavus* Link	12	*Monilia sitophila*

* Phenol-resistant strains.

Table 2. Comparison of the antibacterial effects of the control of ethanol and G-0 at concentrations between 1.2 and 0.001 % in front of the bacterial strains tested.

Strain	A/B	C/D	Strain	A/B	C/D
1	8/4	6/2	10	7/3	5/2
2	8/4	4/2	11	8/4	5/2
3	8/4	4/2	12	8/4	0/0
4	9/3	4/2	13	7/4	4/2
5	8/4	7/3	14	8/4	7/2
6	9/4	6/2	15	7/4	1/0
7	9/4	6/3	16	10/3	1/0
8	9/5	0/0	17	9/4	1/0
9	5/3	0/0			

Last tube of each series:
A and B: Bacteriostatic effect. C and D: Bactericidal effect.
A and C: Series with G-0 (*). B and D: Control series with ethanol (**).
(*) The numbers correspond to the last tube with the effect tested and the G-0 concentrations (%) were the following: Tubes 1 (1.2), 2 (0.6), 3 (0.3), 4 (0.15), 5 (0.07), 6 (0.03), 7 (0.01), 8 (0.005), 9 (0.002) and tube 10 (0.001).
(**) The numbers correspond to the last tube with the effect tested and the ethanol concentrations in [% (v/v)] them were: 1 (25.0), 2 (12.5), 3 (6.2), 4 (3.1), 5 (1.5), 6 (0.7), 7 (0.3), 8 (0.15), 9 (0.07) and 10 (0.03).

Table 3. Comparison of the antifungal effects of the ethanol control and G-0 at concentrations of 1.2-0.001 % in front of the fungal strains tested.

Strain	A/B	C/D	Strain	A/B	C/D
1	7/2	6/1	7	8/2	7/0
2	7/2	7/0	8	8/4	7/2
3	8/2	6/1	9	9/4	8/2
4	8/3	6/1	10	8/2	7/0
5	7/2	7/0	11	9/1	6/0
6	8/2	7/0	12	8/2	7/0

Last tube of each series showing:
A and B: Fungistatic effect. C and D: Fungicidal effect.
A and C: Series with G-0. B and D: Control series with ethanol.

Table 4. Minimal Inhibitory Concentrations (Bacteriostatic or MIC) and Minimal Bactericidal Concentrations (MBC) of G-0 to the bacterial strains tested.

Strain	MIC	MBC	Strain	MIC	MBC
	(%)			(%)	
1	0.005	0.05	10	0.010	0.01
2	0.005	0.03	11	0.005	0.01
3	0.005	0.03	12	0.005	R
4	0.002	0.03	13	0.010	0.30
5	0.005	0.02	14	0.005	0.03
6	0.002	0.05	15	0.010	1.20
7	0.002	0.05	16	0.001	1.20
8	0.002	R	17	0.002	1.20
9	0.030	R			

R: Strain resistant to the higher concentration of the product employed (1.2 %).

concentrations. Those were the 8, 9 and 12 strains, above mentioned, members of the *Bacillus* genus. In the other 14 strains, the bactericidal activity was present at G-0 concentrations placed from 0.01 to 0.3 % for the strains showing that effect.

The fungistatic activity of G-0 expressed as MIC (%) to the fungal strains employed was shown to be from 0.001 to 0.005 %, values lowest than the necessary to detect similar effects among the bacterial strains. In this case, the Minimal Fungistatic Concentration effective for the total of strains corresponds to 0.005 % (Table 5). Likewise, all the tested fungal strains showed a lethal effect to G-0 at the assessed concentrations and the Minimal Fungicide Concentrations were between 0.01 and 0.005 %, even the MIC and MFC values (2 and 5 strains) were similar, what could be owed to the fact that the changes produced in them by the studied product are so strong that cause lethal damages. All these results show that this product has a strong fungistatic and fungicidal activity against fungi.

The results of this study are not to be compared to other similar studies by its novelty, since this is the first time that the analysis of the antimicrobial activity of G-0 on pollutant strains with biodeteriorating activity is performed, without finding a former similar research.

According to the results, G-0 showed to have a strong antimicrobial action, both inhibitory and biocidal action on the bacteria and fungi with biodeteriorating activity employed in this study. A characteristic to be emphasized is its activity on the phenol-resistant strains, that are usually used to evaluate disinfectants and new products for this purpose.[9] Even though a biocidal effect could not be detected, not even at the highest concentrations employed, in three bacterial strains producing endospores, the assessment of higher concentrations (what can be achieved by solving them in other solvent) could modify its preservative activity and show a higher or a lower biocidal action. This offers the possibility of its incorporation in the formulation of products like protecting paints in the international markets devoted to cover the lime roofs and to improve their quality with an increased durability, additionally, by giving the painted lime roofs a better esthetics.

When assessing a new product as an antimicrobial preservative agent for industrial purposes, the first step is to determine the potentiality it has against the microbial cultures of interest. As the result of this first test is satisfactory, then other studies can be carried out, once the product is incorporated to the paint or formu-

Table 5. Minimal Inhibitory Concentrations (Fungistatic, MIC) and Minimal Fungicide Concentrations (MFC) of G-0 to the fungal strains tested.

Strain	MIC	MFC	Strain	MIC	MFC
	(%)			(%)	
1	0.005	0.010	7	0.002	0.005
2	0.005	0.005	8	0.002	0.005
3	0.002	0.010	9	0.001	0.002
4	0.002	0.010	10	0.002	0.005
5	0.005	0.005	11	0.001	0.010
6	0.002	0.005	12	0.002	0.005

lation devoted to its use, which would be subjected to new tests of effectivity.[12]

This product inhibited 100 % both the bacteria and the fungi employed and even though it showed a fungicidal effect on all the fungal strains studied, its bactericidal capacity was only demonstrated on 82.3 % of the bacterial strains.

CONCLUSIONS

G-0 has antimicrobial activity, both antibacterial and antifungal. Likewise, it showed properties as an inhibitor of the growth and as biocide on both bacterial groups.

ACKNOWLEDGEMENTS

The authors acknowledge the Center of Bioactive Products of the Central University of Las Villas for having supplied the G-0 used for this work.

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