Characterization of biodeteriorating microorganisms in buildings in Bucaramanga, Colombia

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Abstract. The action of the microorganisms upon the integrity of the constructing material is termed Biodeterioration, concrete resistance to the action of the microorganisms is considered an indirect measurement of its durability and could be used as a marker of the integrity of the structure. In Colombia, the studies considering this parameter are rare. The objective of this study was to isolate and characterize the microbial communities present in areas with evident deterioration in the selected buildings. To accomplish this, isolation, culturing and molecular identification of the isolates was performed. Results showed that Cladosporium spp, Aspergillus spp, Mucor spp, Penicillium spp, Penicillium spp, Rhizopus spp, Fusarium spp, Geotrichum spp, and bacterial genera such as Bacillus spp and Amphibacillus spp, coexist within the biofilms sampled. This study is a description and a starting point to deepen the characterization of these communities and to understand the role they perform in the integrity of the building materials considering the climatic and environmental conditions.

1. Introduction
Concrete is a material widely used in the construction of structures in the urban landscape, it is a mixture of coarse, and fine sand or crushed stone generally, it is aggregated with portland cement most cases and water. This matrix is subjected to biogenic deterioration because of the microorganisms present; this phenomenon is usually referred as biodeterioration [1]. It describes the progressive process caused by different chemical, physical, mechanical and biological agents, in the properties of a given material, as a consequence of the irreversible adherence of different macroscopic and microscopic communities [2]. The nature of this phenomena may be of biogeochemical nature, such as the bio solubilization that leads to the bioleaching of the substrate or bio geophysical nature, with the consequent formation of crusts or patinas on the surface of the material [3].

Deterioration depends on the release of nutrients, available to the microorganisms and plants; it can be initiated by airborne nutrients captured on the surface of the structure, metabolisms products and decay of the populations present in/on the building material or by the degradation of building materials themselves [4], the growing of biofilms on the surface, production of acids by diverse type of microorganisms are also determinants in the deterioration of the construction [5]. Deterioration of
concrete is found in structures exposed to aggressive environments, such as, those promoting sulphate attack, chloride ingress generating severely modifications on the physic-chemical properties of the compounds [6].

The biological diversity living on reinforced concrete includes a variety of macroscopic and microscopic communities: bacteria (such as cyanobacteria and actinomycetes), fungi, algae, archaeabacteria, lichens, protozoa and plants [2] Fungi are the most characterized by their ubiquity [7], followed by bacteria [8], and lichens [1].

The main types of bacteria associated with the carbonation of concrete and corrosion of steel are sulphate-reducing, nitrifying, iron oxidizing, manganese and sulphur. Sulphate-reducing bacteria (BRS) are involved in the corrosion of copper, steel and ferrous alloys [8]. Likewise, nitrifying bacteria produce nitric acid, responsible for the degradation of carbonate and solubilization of the stone [9]. On the other hand, fungi excrete organic acids, causing the dissolution of the substrate [8].

Biodeterioration have been studied in coastal constructions, architectonic landmarks, museums, underground structures, sewage systems, at-sea structures and waste water treatment systems [10]. The monetary impact, biodeterioration generates, usually is underestimated, since microorganisms accelerate process that occur in their absence at a lower rate [11], in this scenario biodeterioration related structural problems cost billions of dollars a year in maintenance and repair [12]. In Colombia, especially in Bucaramanga, there is no record of the characterization of the biodeteriorating microorganisms involved and this parameter is not considered when assessing deterioration upon buildings.

This work seeks to characterize the microorganisms (fungi and bacteria) present in points with evident deterioration of the buildings of Universidad de Santander (UDES), Bucaramanga, Colombia as a pilot work; the results of this study, would be a first approach in order to identify the microorganisms present in the buildings, identify them and try to understand if the geographical conditions influence the diversity present in the structures, when contrasted with the literature.

2. Materials and methods
The sampling points were selected according to the evident presence of deterioration, established by physicochemical and mechanical methods, through the trial of phenolphthalein and sclerometer. Three buildings within UDES campus were sampled Point I: Carare building, Point II: Chibcha building and Point III: Arhuaco building.

The biofilms visible in the selected buildings were detached from the surface by aseptic scraping, using stainless steel spatulas and placing the sample in petri dishes, all the material used was sterile. The samples were packed, labeled and transported to the molecular biology laboratory of the UDES, Bucaramanga, Colombia. Refrigerated at 4 °C until their respective processing.

The sample was macerated and homogenized, sowing by central puncture in potato dextrose agar (PDA) medium, incubating for 5-7 days at 30 °C. Isolation of the different fungal strains that grew on the plates was carried out. For the macroscopic characterization, the following identification criteria were taken into account: shape, size, color, texture, appearance and consistency. Subsequently, for identification to genus, fresh preparation was made with lactophenol using the taxonomic key as described by [7].

The sample was macerated, homogenized and weighed 0.001 mg, immersed in a tube containing 1 mL of nutritive broth. It was stirred at intervals for 5 minutes, and then incubated at room temperature with stirring at 200 revolution per minute 8 rpm until it reached a turbidity equivalent to No. 5 on the McFarland scale. Serial dilutions were made, inoculating 250 μl of 1/10³ and 1/10⁸ dilutions in nutrient medium and incubating for 24 hours at room temperature. The samples that presented countless colonies were seeded 1/10⁵ and 1/10⁸ dilutions. Each sample was processed in triplicate. Next, the colony forming units (CFU) per mL of sample were quantified to establish the number of viable bacteria in the sown sample.

Isolation was made of the different colonies that grew on the plates where CFUs were quantified, by sowing by exhaustion in nutrient medium. Once purified, the bacteria were preserved in 20% glycerol and kept in freezing at -80 °C. For the macroscopic identification, the following identification criteria
were taken into account: size, shape, surface, edge and consistency. Finally, for the determination until gender, Gram stain was performed in addition to differential staining of endospores and biochemical tests; catalase, voses proskauer, motility and TSI [13].

A colony of each bacterial isolate was transferred to a tube with sulfite polimixin sulfadiacin (SPS) medium and nitrated broth, for the identification of sulphate reducing and nitrifying bacteria, respectively. It was incubated for 48 hours at room temperature. For the interpretation of the test results: the appearance of black colonies due to the precipitation of iron sulphate in the SPS medium indicates the reduction of hydrogen sulphate. On the other hand, the appearance of a red color on the surface of the nitrated broth, after revealing, indicates a positive reaction.

The isolates with presumptive deteriorating characteristics were incubated in 5 mL of luria bertani (LB) broth at room temperature for 30 hours. Deoxyribonucleic acid (DNA) extraction was performed, using the wizard genomic DNA extraction kit (Promega®) following the manufacture indications. The concentration of DNA was measured at 260 nm in NanoDrop 2000 spectrophotometer. To determine the quality of the DNA obtained, the ratio of the absorbances measured at 260 and 280 between range of 1.6-2.3 was consider as a good extraction. Subsequently, verification was performed by 0.8% agarose gel electrophoresis, using the molecular weight marker 100 bp/plus DNA Ladder (GeneRuler™). The extracted DNA was used as a template for the amplification of the small subunit od the ribosome (16S rRNA gene), using 500 ng of genomic DNA, with the primers B1512R (5'-AAGGAGGTGATCCANCRCRA-3') and B27F (5'-GAGTTTGGATCTGCTAG-3'), the alignment was performed at 55 °C [14]. The reaction was carried out in the Applied BiosystemsTM SimpliAmp™ thermocycler. The amplification products were visualized by electrophoresis in agarose gel according to the protocol described in the laboratory.

The different PCR products were purified on silica columns following the protocol of the QIAquick PCR Purification Kit (QIAGEN®). The fragments were sequenced in MacroGen® (Seoul, Korea). The sequences were analyzed through the BLASTn tool of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results and discussion

Thirty filamentous fungi were isolated, and 8 genera were identified in the Carare, Chibcha and Arhuaco buildings, such as: Cladosporium spp, Aspergillus spp, Mucor spp, Penicillium spp, Rhizopus spp, Fusarium spp and Geotrichum spp.

The fungi with higher percentages in the samples, were in order: Cladosporium spp, being located in 75% (n: 18) of the Carare building samples; in 60% (n: 3) of the Chibcha building samples, and in 33.3% (n: 3) of the Arhuaco building samples. Mucor spp, was found in 4.2% (n: 1) of the Carare building samples; it was not registered in the Chibcha building, and in 33.3% (n: 3) of the Arhuaco building samples. Penicillium spp, was found in 4.2% (n: 1) of the Carare building samples; it was not registered in the Chibcha building, and in 33.3% (n: 3) of the Arhuaco building samples (Figure 1).

![Figure 1. Percentage of genera of filamentous fungi isolated in the Carare, Chibcha and Arhuaco buildings of the UDES, Bucaramanga, Colombia.](image-url)
The found fungal findings correlated with that reported by the literature, where the participation within the biodeterioration of the *Cladosporium* genera is pointed out, mainly, followed by *Alternaria, Penicillium, Aspergillus*, among others [15]. *Cladosporium spp.* were reported as involved in the deterioration of concrete by Ghafoori and Mathis, 1997 [1], it is a saprophytic fungus, colonizer of soil and plants, encompassing 500 species [16]. Most of their species are pathogenic in humans, considering themselves important allergens, after inhaling their spores or conidia. These fungal genera are common in indoor or outdoor environments, producing pigments in the substrates, as a result of the different types of proteases and organic acids they excrete (oxalic, fumaric, acetic, gluconic, glucuronic), establishing a barrier between the material and the adjacent medium [17].

The number of bacteria per unit of volume present in the biofilms of the 3 buildings was established. The concentrations of total bacteria in CFU/mL common in the three buildings ranged from 0 CFU/mL to 693 CFU/mL, showing the minimum concentrations in the Carare and Chibcha buildings and the maximum concentrations in the Arhuaco building (Table 1).

**Table 1.** Minimum, average and maximum concentrations in CFU/mL of total bacteria present in the Carare, Chibcha and Arhuaco buildings of the UDES, Bucaramanga, Colombia.

| Bacteria quantification | Mean (CFU/mL) | Minimum (CFU/mL) | (%) | Maximum (CFU/mL) | (%) |
|-------------------------|---------------|------------------|-----|------------------|-----|
| Carare                  | 179           | 0                | 0.00| 547              | 5.1 |
| Chibcha                 | 86            | 0                | 0.00| 627              | 12.1|
| Arhuaco                 | 319           | 7                | 0.03| 693              | 3.6 |

A total of 86 pure bacterial strains were isolated, however, after the morphological characterization, it was observed that most of them were similar, so finally, 36 strains of the Carare, Chibcha and Arhuaco buildings were isolated and identified. The Gram stains performed corresponded entirely to Gram positive bacilli. The results of the biochemical tests allowed to identify the genera, in order of predominance, *Bacillus* (n: 33) and *Amphibacillus* (n: 3). These genera were identified with the differential staining of endospores and biochemical tests, catalase, voges proskauer, TSI and motility.

The genus *Bacillus* was catalase, voges proskauer and TSI positive, which means that it ferments three carbohydrates (glucose, lactose and / or sucrose). Also, they presented variable mobility, being a differentiation between the species of the genus [18]. On the other hand, the strains identified within the genus *Amphibacillus*, presented the same biochemical characteristics of *Bacillus spp.*, except for being catalase negative and motile, main characteristics that differentiates it from other sporoforming genera of Gram positive bacilli [2], Table 2.

**Table 2.** Biochemical tests for the bacterial identification of isolated samples from the Carare, Chibcha and Arhuaco buildings of UDES, Bucaramanga, Colombia.

| Isolation number | Catalase | Endospore | TSI | VP | Motility | Microorganism          |
|------------------|----------|-----------|-----|----|----------|------------------------|
| 1, 4-5, 6, 9, 10-12, 14, 16-20, 22-23, 27-28, 30, 34-35 | Positive | Positive | Ac/Ac | Positive | Positive | *Bacillus spp*          |
| 2, 7-8, 13, 21, 24-26, 29, 31, 33, 36 | Positive | Positive | Ac/Ac | Positive | Negative | *Bacillus spp*          |
| 3, 15, 32 | Negative | Positive | Ac/Ac | Positive | Positive | *Amphibacillus spp* |

For the visualization of bacterial endospores, the strains were inoculated in a liquid medium described by Beltrán [19], being incubated for 5 days at room temperature. Massive seeding was carried out in nutrient medium and the cultures were incubated for 5 days at the same temperature to limit the nutrients. Subsequently, malachite green staining was performed, using the Shaeffer-fulton technique [20]. All of the strains were sporoforming. Sporulation corresponds to a defense mechanism to resist
adverse environmental conditions, a fundamental characteristic of the genera *Bacillus* and *Amphibacillus* [19].

From the genus Bacillus, 77 recognized species have been described [19]. Most of the members of this genus are saprophytes and are widely distributed in nature. It is commonly found in soil, water and plants, where it has an important participation in the carbon and nitrogen cycle [21]. This genus has been documented as a biodeterioration participant in different studies. Within the species, they have been described mainly: *Bacillus subtilis* [17], *Bacillus circulans* [22] and *Bacillus megaterium* [5].

The sulphate and nitrate production was determined to the 36 bacterial strains identified, as indicators of biodeterioration. The sodium sulfite present in the SPS medium was not reduced to hydrogen sulfide (H₂S), therefore, no strain is sulphate-reducing [19]. These results correlate with the literature, where it is reported that *Bacillus spp* and *Amphibacillus spp* are not reducing sulfates. BRS bacteria are strict anaerobic organisms, using sulphate as the final electron acceptor [1] (Table 3).

On the other hand, 6 strains were positive in the nitrate broth, observing a red coloration when adding the developer, reducing nitrates to nitrites. The rest of the strains were confirmed negatively by adding a small amount of zinc powder and retaining the same color as the reaction. Nitrifying bacteria are associated with the biodeterioration process, since they produce nitric acid, responsible for the carbonation of concrete, solubilization of the stone and corrosion of steel [1].

Table 3. Indicators of biodeterioration of bacterial strains isolated from the Carare, Chibcha and Arhuaco buildings of the UDES, Bucaramanga, Colombia.

| Microorganisms indicators of deteriorating activity | Strain number | SPS medium | Nitrated broth |
|-----------------------------------------------------|---------------|------------|----------------|
| 1-4, 7-14, 16-17, 19, 21-30, 32-36                   | Negative      | Negative   |
| 5-6, 17, 18, 20, 31                                  | Negative      | Positive   |

The 6 bacterial strains that showed nitrate-reducing activity, associated with the biodeterioration process, were molecularly identified. Traditional PCR was performed, using genomic DNA, based on the analysis of the 16S rRNA gene. The analysis by electrophoresis showed a common band pattern, with a molecular weight of 1500 bp, well defined and without degradation, being located in the third band of the molecular weight standard. Subsequently, PCR products were purified for their respective sequencing. (Figure 2).

![Figure 2](image-url) Electrophoresis in 0.8% agarose gel. The amplification products are shown, by PCR, of the 16S rRNA genes of the 6 bacterial strains. Lane MP: molecular weight marker 100 bp / plus DNA ladder (GeneRuler™). Lane 1: strain 5. Lane 2: strain 6. Lane 3: strain 17. Lane 4: strain 18. Lane 5: strain 20. Lane 6: strain 31. Source: Molecular Biology Laboratory, UDES, Bucaramanga, Colombia.
4. Conclusions
In the development of the present investigation, it was accomplished to identify the microbiota that causes the deterioration in the buildings of the University of Santander, Bucaramanga headquarters.

Thirty-eight filamentous fungal isolates were obtained, with a preponderance of *Cladosporium spp*. And around 36 bacterial isolates, where *Bacillus sp*. The results from biofilms located in the buildings Carare, Arhuaco and Chibcha of the University of Santander, Bucaramanga campus, determined that, in the same, different genera of filamentous fungi, such as: *Cladosporium spp*, *Aspergillus spp*, *Mucor spp*, *Penicillium spp*, *Penicillium spp*, *Rhizopus spp*, *Fusarium spp*, *Geotrichum spp*, and bacterial genera such as, *Bacillus sp* and *Amphibacillus spp* coexist. These genera have been widely reported in biodeterioration processes.

The microorganisms cultured and identified in the present study, are the starting point for assessing their deteriorating activity. However, the fact that this microorganism, among bacteria and fungi, have grown correlates with the reported literature but requires further assessment.

References
[1] Wei S, Jiang Z, Liu H, Zhou D, and Sanchez-Silva M 2013 Microbiologically induced deterioration of concrete - A review Brazilian Journal of Microbiology 44(4) 1001
[2] Menguano Chaparro V M, Pérez Castiñeira J R, and Sameño Puerto M 2013 Estudio de microorganismos causantes de biodeterioro mediante técnicas de biología molecular en el IAPH Revista PH 84 174
[3] Kobetičová K and Černý R 2019 Terrestrial eutrophication of building materials and buildings: An emerging topic in environmental studies Science of the Total Environment 689 1316
[4] Yakovleva G, Sagadeev E, Stroganov V, Kozlova O, Okunev R, and Ilinskaya O 2018 Metabolic activity of micromycetes affecting urban concrete constructions The Scientific World Journal 2018(8360287) 1
[5] Romani M, Carrion C, Fernandez F, Intertaglia L, Pecqueur D, Lebaron P, and Lami R 2019 High bacterial diversity in pioneer biofilms colonizing ceramic roof tiles International Biodeterioration & Biodegradation 144 104745
[6] Ding L, Weiss W J, & Blatchley E R 2017 Effects of concrete composition on resistance to microbially induced corrosion Journal of Environmental Engineering 143(6) 1
[7] Guerra F L, Lopes W, Cazarolli J C, Lobato M, Masuero A B, Dal Molin D C, Vainstein M H 2019 Biodeterioration of mortar coating in historical buildings: Microclimatic characterization material and fungal community Building and Environment 155 195
[8] Bertron A 2014 Understanding interactions between cementitious materials and microorganisms: a key to sustainable and safe concrete structures in various contexts Materials and Structures 47(11) 1787
[9] Vupputuri S, Fathepure B Z, Wilger G G, Sudo E, Nasrazadani S, Ley T, and Ramsey J D 2015 Isolation of a sulfur-oxidizing Streptomyces sp. from deteriorating bridge structures and its role in concrete deterioration International Biodeterioration and Biodegradation 97 128
[10] Kip N and Van Vee J A 2015 The dual role of microbes in corrosion ISME Journal 9(3) 542
[11] Ahmed T, Usman M, and Scholz M 2018 Biodeterioration of buildings and public health implications caused by indoor air pollution Indoor and Built Environment 27(6) 752
[12] Sanchez-Silva M, & Rosowsky D V 2008 Biodeterioration of construction materials: State of the art and future challenges Journal of Materials in Civil Engineering 20(5) 352
[13] Garcia J C 2016 Microbiologia aplicada: Una herramienta para la conservación del patrimonio cultural Conservar Patrimonio 24 23
[14] Lebuhn M, Achouak W, Schloter M, Berge O, Meier H, Barakat M, and Heulin T 2000 Taxonomic characterization of Ochrobactrum sp. isolates from soil samples and wheat roots, and description of Ochrobactrum tritici sp. nov. and Ochrobactrum grignonense International Journal of Systematic and Evolutionary Microbiology 50(6) 2207
[15] Rojas T I, Aira M J, Batista A, Cruz I L, and González S 2012 Fungal biodeterioration in historic buildings of Havana (Cuba) Grans 51(1) 44
[16] Páramo Aguilara L, Narváez Zapata J, and De la Cruz E 1970 Aislamiento e identificación de microorganismos en biopelículas provenientes del Castillo de Chapultepec Ciudad de México Nex Revista Cientifica 24(2) 83
[17] Encinas C, Magariños M, Corrales M, Borda C, and Wayar Á 2013 Identificación de microorganismos causantes del deterioro en pinturas al óleo del Museo Universitario Colonial Charcas Revista Ciencia, Tecnologia e Innovacion 13(14) 793
[18] Larrea-Izurieta I, Falconí Borja C, and Arcos-Andrade A 2015 Aislamiento y caracterización de cepas de Bacillus con actividad contra Tetranychus Urticae Koch en cultivos comerciales de rosas Revista Colombiana de Biotecnología 17(2) 140

[19] Liu Z, Zhang Y, Zhang F, Hu C, Liu G, and Pan J 2018 Microbial community analyses of the deteriorated storeroom objects in the Tianjin Museum using culture-independent and culture-dependent approaches Frontiers in Microbiology 9 1

[20] Rajendhran J, and Gunasekaran P 2011 Microbial phylogeny and diversity: Small subunit ribosomal RNA sequence analysis and beyond Microbiological Research 166(2) 99

[21] Cuervo Lozada J P 2010 Aislamiento y caracterización de bacillus spp como fijadores biológicos de nitrógeno y solubilizadores de fosfatos en dos muestras de biofertilizantes comerciales (Colombia: Pontificia Universidad Javeriana)

[22] Reyes J, Silva I, Pérez T, Corvo F, Martinez W, Alonso E M, and Quintana P 2012 El deterioro del Baluarte de San Pedro, un estudio de caso Revista Alconpat 2(3) 161