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Potential of Fungi for Concrete Repair

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Abstract

Concrete is the most widely used construction material in the world being cement one of its main components. Cement production accounts for 5-8% of anthropogenic CO_2 emissions into the atmosphere. Most of the world's infrastructures are produced from reinforced concrete and cracking is one of the major drawbacks for its durability. The cracks in concrete reduce their resistance capacity and allow the entry of harmful agents both for their microstructure and for the reinforcements located inside the structure. Sustainable solutions aimed at reducing costs and environmental impacts for this problem have been researched. The bioscience of precipitation mechanisms with microbiologically induced calcium carbonate (MICCP) is an alternative to traditionally used methods and a way to mitigate the environmental impact of using more cement and polymers. Most of the biocementation studies present bacteria as microorganisms responsible for the $CaCO_3$ induction process. Fungi are potentially better for the biocementation process because they have more biomass and are filaments, which may aid in the mechanical behaviour of the formed bioconcrete. Thus, the present work proposes the development of a methodology to analyse the potential use of fungi present in concrete structures as biorepair agents.

Keywords: Bioconcrete; Microbiologically induced calcium carbonate precipitation (MICCP); fungi; concrete repair; cracks.

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1. Introduction

Cement is one of the most used building materials in the world, but its production generates considerable environmental impacts in all stages of manufacturing. Worldwide cement production is responsible for the consumption of about 10-15% of total industrial energy and 5-8% of anthropogenic CO_2 emissions. In order to minimize this negative effect, it is necessary to discover alternatives for conventional cement [1], [2], [3].

Recent research, with the introduction of biotechnology in the area of building materials, has enabled the development of the so-called bioconcrete produced through biomineralization mechanisms [4], [5], [6], [7], [8].

The biocement presents a more environmentally friendly alternative than conventional cement because it does not generates CO_2 in its manufacturing process and when it is produced, through the metabolic conversion of calcium salts, the CO_2 is consumed in the calcium carbonate ($CaCO_3$) mineralisation and these minerals can also promote the improvement of the mechanical properties and the durability of cementitious materials [9], [10], [11]. Studies on biocementation around the world use preferentially calcite precipitation by bacterial induction, applying different bacteria mainly from the *Bacillus* genus [12], [13], [14], [15], [16], [17], [18].

Filamentous fungi are also microorganisms and yet, so far, there is almost no research on biocementation using them. According to our best knowledge, only one work was found [19]. In addition to the potential $CaCO_3$ precipitation by urease-positive fungi, their mycelial structures, and higher biomass when compared to bacteria, indicates that this type of microorganisms can serve as a biological fiber to improve the resistance of bioconcrete [20].

The microbial precipitation of $CaCO_3$ by urea hydrolysis has been studied with application in soils, in the proposal of new building materials and in the repair of cracks of concrete as presented in Table 1. It proved to be better than many traditional technologies due to its eco-friendly advantage. The applicability of the microbial ureolytic mineral production to fill pores and cracks in concrete can be used as a surface treatment or as an integrated curative agent. This can be considered advantageous because over time this type of material will not need inspection, repair or energy. Also, this treatment promotes increased durability of the structure. Therefore, this work aims to identify the potential presence of filamentous fungi in concrete structures that can be used as biorepair agents of concrete.

Table 1. Bioprecipitation of calcium carbonate induced by bacteria and fungi.

Type of microorganism	Species	Precipitation Mechanism	Nutrient	Reference
Bacterium	*Bacillus sphaericus*	Ureolysis	Urea, calcium nitrate and yeast extract	[21]
Bacterium	*Bacillus sphaericus*	Ureolysis	Urea and calcium chloride.	[22]
Bacterium	*Bacillus megaterium*	Ureolysis	Urea, nutrient broth, and sodium chloride	[23]
Bacterium	*Sporosarcina pasteurii*	Ureolysis	Urea, yeast extract, calcium chloride, sodium chloride	[14]
Bacterium	*Bacillus cereus*	Ureolysis	Urea and tap water	[17]
Fungus	*Penicillium chrysogenum*	Ureolysis	Urea and calcium chloride	[19]
Fungus	*Neurospora crassa*	Ureolysis	Urea and calcium chloride.	[24]
Fungus	*Pestalotiopsis* sp. and *Myrothecium gramineum*	Ureolysis	Urea, calcium chloride and strontium chloride	[25]

2. Materials and methods

2.1. Identification of filamentous fungi present in apparent concrete constructions

The isolation and molecular identification of fungal strains present in concrete structures served as a basis for the study of the ability of these microorganisms to hydrolyse urea and subsequently to promote the $CaCO_3$ precipitation.

In this context an experimental research work was carried out. The concrete samples for fungal isolation were collected by direct scraping with sterilized spatulas and placed in sterile Petri dishes. A total of 6 samples were collected at three different points in the city of Guimarães, Portugal, shown in Fig. 1. Samples 1.1 and 1.2 were collected on the footbridge over João XXI Street, samples 2.2 and 2.3 were collected from the wall inside the

overpass of access to *Guimarães Shopping* and samples 3.1 and 3.2 were collected in the concrete bench of the *Plataforma das Artes*.

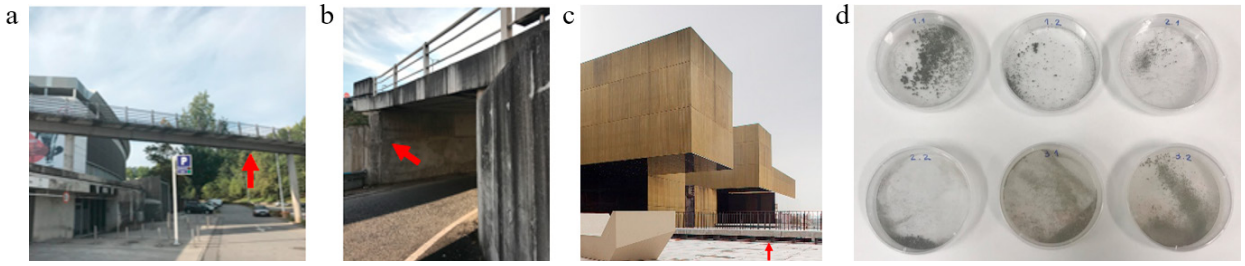

Fig. 1. (a) Footbridge over the street João XXI, (b) Overpass of access to Shopping Guimarães, (c) Concrete bench of the Plataforma das Artes and (d) Collected material.

Each sample was placed in a 10 mL solution containing 0.5% of peptone, homogenised and 100 µL were spread in DRBC medium (Dicloran Rose-Bengal Cloranfenicol Agar). The plates were kept in the dark at 25 °C in an incubator. The inoculated plates were observed every 24 hours and at the first visible growth the colony was removed and re-inoculated into a new plate containing PDA medium (Potato Dextrose Agar). All isolates were obtained after 12 days. Figure 2 shows sample solution 1.1 inoculated in DRBC and the colonies isolated from this solution. For the preservation of the isolated fungi, 5 mm diameter mycelium discs of 7 days old colonies were cut and transferred to sterile tubes with distilled water.

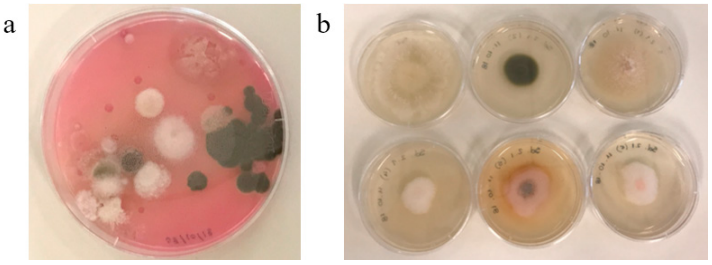

Fig. 2. (a) DRBC plate inoculated with solution 1.1 and (b) fungi isolated from the solution 1.1.l.

Molecular characterization: The isolates were subjected to DNA extraction using a method adapted from Rodrigues et al. [26]. DNA was dissolved in 100 µL of ultrapure water and stored at -20 °C. The amplification of the Internal transcribed spacer region (ITS) was done with the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') e ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [27]. For each final volume of 50 µL per reaction, 25 µL Master Mix, 1 µL ITS1 primer, 1 µL ITS4 primer and 50 ng extracted DNA was added. PCR was performed as follows: 1) 1 step: Initial denaturing at 95 °C for 5 min; 2) 30 cycles of the following three steps: Denaturing (60s at 95 °C) Annealing (45s at 56 °C), Elongation (90s at 72 °C); and 3) a final step: Final elongation of 10 min at 72 °C. Obtained amplicons were purified using the NZYGelpure kit (NZYtech) and sent for Sanger sequencing to Stab Vida Lda (Madan Parque, Caparica, Portugal). The resulting sequences were edited in Bioedit 7.0.5.3 software and compared to the NCBI (National Biotechnology Information Center) database using BLAST to determine fungal species that have similar DNA sequences.

2.2. Urease activities by isolated fungi

Christensen's agar medium is used on the determination of the presence or absence of the urease enzyme by visual identification of the medium colour if pH increases when the ammonia is produced by substrate degradation [28], [29].

The urease activity of the isolated fungi was determined according to the methodology suggested by Shingha et

al. [30], 20 mL of modified Christensen agar medium (1 g/L glucose, 1 g/L peptone, 12 mg/L of phenol red, 2 g/L disodium phosphate, 2 g/L sodium chloride, 15 g/L agar and 20 g/L urea) was poured into 90 mm diameter Petri dishes under aseptic conditions. A mycelial disc of the 5 mm diameter fungal isolates were then inoculated separately on the centre of the modified Christensen agar medium, keeping the mycelium surface attaching to the medium surface. Cultures were inoculated at 25°C in the dark.

Urease activity was evaluated as follows: strong activity (colour in Christensen medium was changed to deep pink), moderate activity (colour in Christensen medium was changed to pink), weak activity (colour in Christensen medium was changed to light pink), and no urease activity (colour in Christensen medium was not changed), it is inserted in yellowish-orange colour. Samples were observed on the third day of inoculation and on the sixth day to evaluate the intensity (colour) and the speed of production of the enzyme which will be classified as rapid production (3 days) and moderate production (6 days).

3. Results

3.1. Identification of fungal species

From the 6 samples collected at the selected locations, a total of 19 fungal colonies of different aspects and/or growth time were obtained. Strains 1 to 9 were isolated from samples 1.1 and 1.2 collected on the footbridge over João XXI Street, strains 10 to 15 were isolated from samples 2.1 and 2.2 collected from the wall inside the overpass of access to *Guimarães Shopping* and strains 16 to 19 were isolated from samples 3.1 and 3.2 collected on the concrete bench of the *Plataforma das Artes*. The figure 3 shows the strains isolated after 7 days growth at 25 °C in PDA medium.

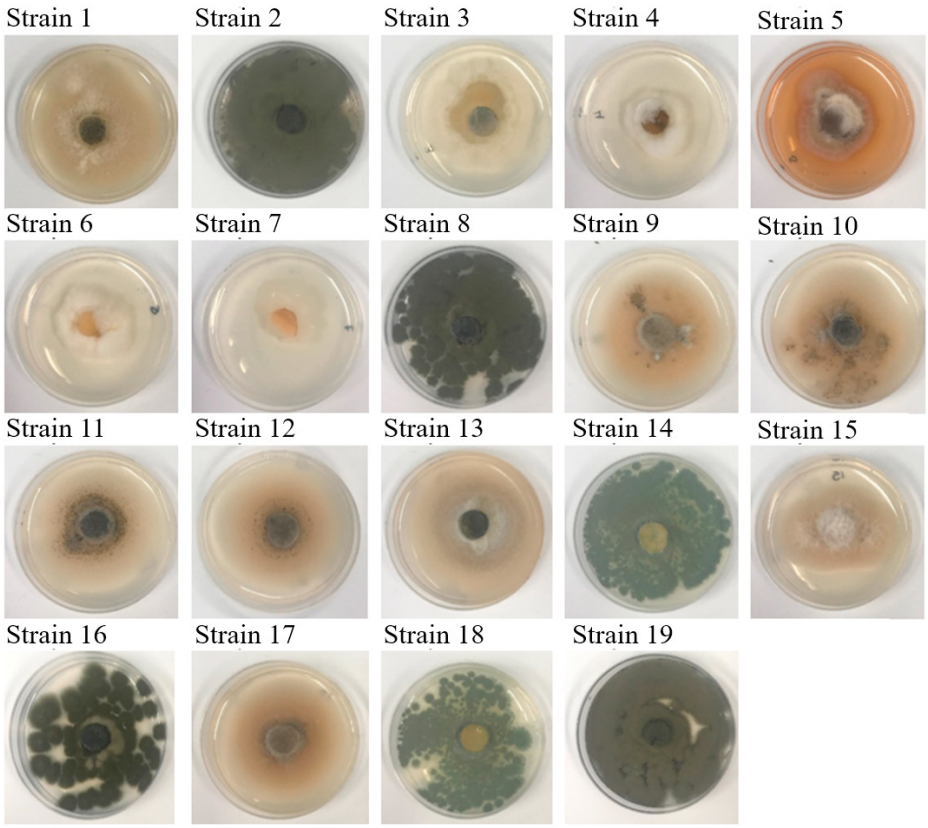

Fig. 3. Isolated strains of apparent concrete constructions in Guimarães.

Molecular identification of the isolates was performed by comparing the obtained nucleotide sequences with sequences already deposited in the GenBank database through BLAST. The isolates were identified by homology as shown in Table 2. The genus *Phoma* and *Cladosporium* are the most common isolates founded in the current work. These genera with *Alternaria* and *Fusarium* were also found in the outer wall of Lamego Cathedral [31].

Table 2. Fungal species identified in Guimarães buildings.

Strain	Identity	Sample	Location
1	*Phoma glomerata*	1.1	Footbridge
2	*Cladosporium cladosporioides*	1.1	Footbridge
3	*Valsa nivea*	1.1	Footbridge
4	*Fusarium lateritium*	1.1	Footbridge
5	*Phoma herbarum*	1.1	Footbridge
6	*Fusarium lateritium*	1.1	Footbridge
7	*Coniochaetaceae* sp.	1.2	Footbridge
8	*Cladosporium herbarum*	1.2	Footbridge
9	*Phoma aliena*	1.2	Footbridge
10	*Phoma saxea*	2.1	Overpass
11	*Phoma saxea*	2.1	Overpass
12	*Phoma saxea*	2.1	Overpass
13	*Alternaria alternata*	2.2	Overpass
14	*Penicillium brevicompactum*	2.2	Overpass
15	*Phoma herbarum*	2.2	Overpass
16	*Cladosporium angustiherbarum*	3.1	Bench of concrete
17	*Phoma saxea*	3.1	Bench of concrete
18	*Penicillium brevicompactum*	3.1	Bench of concrete
19	*Cladosporium cladosporioides*	3.2	Bench of concrete

3.2. Urease activities

The urease activity was observed every 3 days as described in the methodology, regarding the developing colour of the culture plates. The strains 2, 3, 14 and 19 (21%) presented strong and fast urease activity and 21% of the strains showed strong urease activity but the reaction occurred at moderate speed. However, only strains 8, 14, 16 and 18 showed biomass production and strong urease activity.

4. Conclusions

The studies suggest the potential of urease-positive fungi isolated from concrete constructions in the city of Guimarães, strains 8 (*Cladosporium herbarum*), 16 (*Cladosporium angustiherbarum*), and 14 and 18 (*Penicillium brevicompactum*) as biomineralization agents of $CaCO_3$ in the biocementation processes, because they present strong urease activity and biomass growth which serve as biofibers in the material. According to Van Tittelboom et al. [6], the microorganisms used in the ureolytic precipitation of $CaCO_3$, for the repair of concrete, need to comply with two main requirements: 1) to be able to transport and 2) to have the ability to survive on the concrete environment. The urease-positive fungi isolated in this study met these two requirements.

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