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Interactions of fungi with concrete: Significant importance for bio-based self-healing concrete

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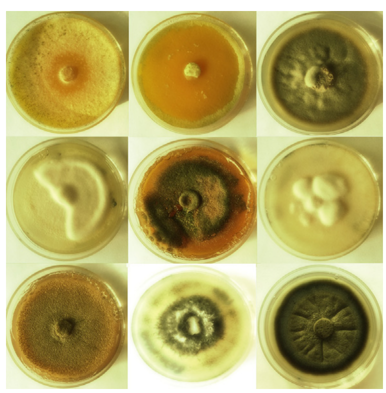
HIGHLIGHTS

- A new self-healing concept is explored, in which fungi are used fill concrete cracks.
- An initial screening of different species of fungi has been conducted.
- *Trichoderma reesei* was found to be able to grow equally well with or without concrete.
- *Trichoderma reesei* can promote the formation and precipitation of $CaCO_3$.

GRAPHICAL ABSTRACT



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ABSTRACT

The goal of this study is to explore a new self-healing concept in which fungi are used as a self-healing agent to promote calcium mineral precipitation to fill the cracks in concrete. An initial screening of different species of fungi has been conducted. Fungal growth medium was overlaid onto cured concrete plate. Mycelial discs were aseptically deposited at the plate center. The results showed that, due to the dissolving of $Ca(OH)_2$ from concrete, the pH of the growth medium increased from its original value of 6.5 to 13.0. Despite the drastic pH increase, *Trichoderma reesei* (ATCC13631) spores germinated into hyphal mycelium and grew equally well with or without concrete. X-ray diffraction (XRD) and scanning electron microscope (SEM) confirmed that the crystals precipitated on the fungal hyphae were composed of calcite. These results indicate that *T. reesei* has great potential to be used in bio-based self-healing concrete for sustainable infrastructure.

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1. Introduction

Concrete infrastructure suffers from serious deterioration [1,2], and thus self-healing of harmful cracks without high costs or onerous labor have attracted enormous amount of attention. As for how

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to endow cementitious materials with self-healing properties, many experimental studies and laboratory investigations have been conducted and generated many innovative strategies during the past decades [3–27].

To date, self-healing in concrete has been achieved primarily through three different strategies: autogenous healing, encapsulation of polymeric material, and bacterial production of $CaCO_3$. During the autogenous healing, cracks are filled naturally by means of hydration of unhydrated cement particles and carbonation of dissolved calcium hydroxide as a consequence of exposure to CO_2 in the atmosphere [3]. However, this autogenous healing is limited to small cracks (less than 0.2 mm) and requires the presence of water [16]. Encapsulation of polymeric material can fill the cracks in concrete by converting healing agent to foam in the presence of humidity. However, the chemicals released from incorporated hollow fibers behave quite differently from concrete compositions, and they may even cause to further propagate the existing cracks [6].

Due to these drawbacks, the use of the biological repair technique by applying mineral-producing microorganisms becomes highly desirable, as it provides a safe, natural, pollution-free, and sustainable solution to the serious challenge [8–28]. When a calcium source is present, $CaCO_3$, the most suitable filler for concrete due to its high compatibility with cementitious compositions, can be produced through various biomineralization processes. This microbial approach is superior to the other self-healing techniques owing to its excellent microcrack-filling capacity, strong bonding between filler and crack, high compatibility with concrete compositions, favorable thermal expansion, and sustainability [27].

Recent research has demonstrated that some ureolytic bacteria, such as *Bacillus sphaericus* and *B. pasteurii*, have the ability to precipitate calcium carbonate through urea hydrolysis and thus can be used as a powerful tool to heal the cracks [8–12]. However, for each carbonate ion two ammonium ions are produced, leading to excessive nitrogen loading to our environment. To avoid this drawback, metabolic conversion of organic compound to $CaCO_3$ has been proposed by Jonkers et al. [18–20] In this approach, aerobic oxidation of organic acids produces CO_2, then leading to the production of CO_3^{2-} in an alkaline environment. Then the presence of a calcium source results in the precipitation of $CaCO_3$. However, this approach requires high concentrations of calcium source [29], which could possibly lead to buildup of high level of salts in concrete. The third pathway to precipitate $CaCO_3$ is known as dissimilatory nitrate reduction [23]. Mineral production is promoted through oxidation of organic compounds through nitrate reduction by means of denitrifying bacteria. However, it has been shown that the efficacy of denitrification approach is much lower than ureolysis regarding the production of $CaCO_3$ [30].

2. Fungi-mediated self-healing concrete

While the term "microbe" defines a wide variety of organisms, studies on self-healing concrete are still limited to bacteria [8–27]. Of course, using bacteria has many advantages. For example, bacteria are easy to culture and handle in a laboratory setting and are typically harmless to humans [31]. Moreover, collection and isolation of bacteria are not very complex, as during the years numerous selective media have been introduced for direct isolation of bacteria [32]. On the other hand, however, bacteria do not generally possess sufficient resistance to survive the deleterious environment such as high pH, varied temperature, and dry condition of concrete. So far there has been little success with respect to the long-term healing efficacy and in-depth consolidation, mainly due to the limited survivability and calcinogenic ability of the bacteria. Furthermore, from the economical point of view, the production of bacteria-based self-healing concrete currently results in considerable costs due to the need of aseptic conditions to produce the microbial spores and the use of expensive growth media, making this approach unlikely to be applied in practical applications [33]. In summary, there are still huge challenges to bring an efficient self-healing product to the concrete market with the guaranty that this product can both attain legislative requirements and be cost-effective.

Due to the above-mentioned problems, further investigation on other types of microorganisms having the ability to catalyze calcium mineral precipitation becomes of great potential importance. The overarching goal of the current study is to explore a revolutionary self-healing concept in which bacteria are replaced by fungi to promote calcium mineral precipitation on cracks in concrete infrastructure. Fungi are the most species rich group of eukaryotic organisms after insects with the magnitude of diversity estimated at 1.5 M–3.0 M species [34]. Fungi have been investigated mostly due to their important role in organic matter degradation, and their relationship with inorganic matter has mainly been focusing on mineral nutrition via mycorrhizal symbiosis, production of mycogenic organic acids, and lichen bioweathering.

The current study is driven by the following three hypotheses. (1) It is hypothesized that some species of fungi can better adapt to the harsh conditions of concrete including high alkalinity, moisture deficit, and severe oxygen and nutrient limitation [35–50]. (2) It is hypothesized that some species of fungi can promote calcium mineralization in the harsh environment of concrete [51–61]. (3) It is hypothesized that using fungi in biogenic crack repair is more effective than bacteria due to their extraordinary ability to both directly and indirectly promote calcium mineralization [62–73]. The details of the three hypotheses are shown in the Appendix.

To test the hypotheses, this work presents a pilot study to investigate the feasibility of using fungi to promote calcium mineral precipitation to heal cracks in concrete infrastructure. Although many species of fungi have been reported to be able to promote calcium mineralization [71–77], they have never been investigated in the application of self-healing concrete, thus a wide screening of different species of fungi will be conducted.

3. Materials and methods

The following criteria will be used to select the candidates of fungi for self-healing concrete. (1) They should be eco-friendly and nonpathogenic, i.e., pose no risk to human health and are appropriate to be used in concrete infrastructure. Fortunately, fungi that are pathogens are usually pathogenic to plants, and there are comparatively few species that are pathogenic to animals, especially mammals. Among the 100,000 described species of fungi, a little more than 400 are known to cause disease in animals, and far fewer of these species will specifically cause disease in human. Many of the latter are superficial types of diseases that are more of a cosmetic than a health problem. (2) The matrix of young concrete is typically characterized by pH values approximately 13 due to the formation of $Ca(OH)_2$, which is after calcium-silica-hydrate (C—S—H) quantitatively the most important hydration product. Therefore, the fungi placed into the concrete not only have to resist mechanical stresses during the mixing process but also should be able to withstand the high-pH environment for prolonged periods of time. Most promising fungal agents thus should be alkaliphilic spore-forming fungi. The fungal spores, together with nutrients, will be placed into the concrete matrix during the mixing process. When cracking occurs, water and oxygen will find their way in. With enough water and oxygen, the dormant fungal spores will germinate, grow, and precipitate $CaCO_3$ to *in situ* heal the cracks. When the cracks are completely filled and ultimately no more water or oxygen can enter inside, the fungi will again form spores. As the environmental conditions become favorable in later stages, the spores could be wakened again. (3) It is preferred if the genomes of the fungi have been sequenced and are publicly available so that they can be genetically manipulated to enhance their performance in crack repair.

Besides genetically engineered fungi, alkaliphilic fungi could also be found in nature. Through their evolution over millions of years, fungi have developed different strategies to survive and prosper in unfavorable environments. Many species of fungi can grow in alkaline environments where the pH value can often be consistently at about 10 [78]. For example, alkaliphilic *Paecillomyces lilacimus* is able to grow well when pH value is between 7.5 and 11.0 [78]. *Chrysosporium* spp. isolated

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