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Bioinspired Living Coating System for Wood Protection: Exploring Fungal Species on Wood Surfaces Coated with Biofinish during Its Service Life

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Abstract: Biofinish is an innovative wood protection system inspired by biological processes. It enhances the hydrophobicity of wood through oil treatment, resulting in improved dimensional stability. Living cells of the fungus *Aureobasidium pullulans* effectively protect wood from deterioration caused by other decaying fungi. The melanin pigment produced by the fungus provides an appealing dark surface and additionally protects the wood substrate against UV radiation. The significant advantage of Biofinish is its remarkable self-healing ability, which distinguishes it from conventional wood protection methods. This research aimed to explore fungal species colonising surfaces exposed to natural weathering and assess the survival of *A. pullulans* on wood surfaces coated with Biofinish during its in-service period. This study was performed on a facade composed of European larch wood (*Larix decidua*) treated with linseed oil and coated with Biofinish at the InnoRenew CoE building in Izola, Slovenia, following a 9-month exposure period. The majority of the detected species belonged to the genera *Aureobasidium*. The results indicated the survival and effective antagonistic action of *A. pullulans*, the living and active ingredient of the coating, against other wood-decaying fungi.

Keywords: wood coatings; building material; fungal colonisation; self-repairing

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1. Introduction

The bioinspired living coating system for wood protection (Biofinish) takes inspiration from natural processes, particularly the interactions between microorganisms and surfaces found in nature, for the development of innovative wood treatment. The idea is inspired by a natural phenomenon where microorganisms, particularly *Aureobasidium pullulans* (*A. pullulans*), a polyextremophilic, black, yeast-like fungus, naturally form a protective biofilm on surfaces such as wood or other building materials. *A. pullulans* can colonise the surface of wood and produce various types of extracellular enzymes including laccase, cellulase, amylase, protease, lipase, cellulase, and xylanase [1–3]. These enzymes have the potential to degrade the chemical components of wood such as cellulose, hemicellulose, lignin, and extractives [4]. However, *A. pullulans* typically exhibits limited degradation of cellulose and hemicelluloses in wood cell walls [4]. Instead, this fungus primarily targets easily accessible nutrients such as sugars, carbohydrates, starch, proteins, fats, and extractives found in the ray parenchyma cells [5]. As a result, while *A. pullulans* may cause discolouration and aesthetic issues that can affect the visual appeal and perceived quality of wood products, it generally does not result in significant wood degradation [5,6].

The system typically involves impregnating the wood substrate with substances like linseed oil, which serves as a nutritional source for living microorganisms and simultaneously enhances the hydrophobicity of the wood, which improves the dimensional stability of the timber element exposed to the outdoors [7]. Linseed oil is a clear-to-yellowish oil derived from the dried ripe seeds of the flax plant (*Linum usitatissimum*) with a long history of use as a wood finish [8]. Its application is attributed to its remarkable capacity to enhance various properties of wood, for instance, durability against wood-decaying fungi [9], resistance to weathering and UV radiation [10], hydrophobic properties [9,11], and the dimensional stability of wood [11]. These qualities make linseed oil suitable as a pretreatment prior to the application of Biofinish coatings, thereby enhancing the effectiveness of wood protection. The living cells of *A. pullulans* play a crucial role in protecting wood against decay caused by fungal infestation. The melanin pigment produced by the fungus not only contributes to an aesthetically appealing dark surface but also provides protection against UV radiation [7]. A combination of linseed oil treatment and Biofinish coating resulted in superior performance in Scots pine (*Pinus sylvestris L.*) wood after one year of natural weathering. The wood exhibited negligible total colour changes and demonstrated enhanced hydrophobic properties [12]. Notably, the finished surface possesses a self-healing ability capable of gradually repairing minor damages [13,14]. The composition of the bioinspired living coating is based on natural substances, making it an environmentally friendly and sustainable wood treatment solution [7]. It is considered to have minimal environmental impact and low maintenance requirements.

Previous studies have explored the combined impact of wood species, oil types, and geographic locations on the development of a uniform biofilm [7,15–17]. It is noteworthy that the term "biofinish", as discussed in Van Nieuwenhuijzen et al. (2015) [15], Van Nieuwenhuijzen et al. (2017) [16], and Peeters et al. (2018) [17], denotes a beneficial homogenous type of fungal-based staining observed in specific wood species treated with oils that is associated with the term "biofilm". Findings suggest that while the wood species may have some influence on the development of biofilm, the type of oil used appears to be more critical. Specifically, Scots pine sapwood fully impregnated with raw linseed oil exhibited the most promising performance of biofilm [15]. *Aureobasidium* was predominant in biofilm on pine sapwood treated with raw linseed oil [7,16]. The presence of biofilm on pine sapwood treated with raw linseed or olive oil can be found regardless of the geographical location [15]. According to Peeters et al. (2018) [17], *Aureobasidium melanogenum*, formerly categorised as a variety of *A. pullulans* known as *Aureobasidium pullulans var. melanogenum*, has demonstrated its ability to utilise linseed oil and olive oil as a sole carbon source for growth. The study highlights the significance of the degree of cross-linking in vegetable oils for the growth of this fungus. Additionally, the colony exhibited a darker colouration on linseed oil compared to olive oil. However, certain aspects related to protection mechanisms against wood-infesting fungi and the survival and adaptation of *A. pullulans* within the coating during its in-service period remain inadequately understood.

This research aims to explore fungal species and assess the survival of *A. pullulans* on wood surfaces coated with Biofinish during its service life. This evaluation provides valuable insights into the protective properties against wood-infesting fungi and the dynamic interactions between *A. pullulans* in the coating matrix and climate conditions. This comprehensive examination lays the foundation for further improvements of the bioinspired living coating system, ensuring its sustained effectiveness for wood protection.

2. Materials and Methods

2.1. Sampling and Cultivation

This study was performed on the exterior facade of the InnoRenew CoE building in Izola, Slovenia (45°31′49.1″ N, 13°39′28.0″ E) following a 9-month exposure period (Figure 1). The facade, comprised of European larch wood (*Larix decidua*), was impregnated with linseed oil and coated with 3 layers of Biofinish (Xyhlo B.V. Deventer, The Netherlands),

according to the industrial procedure for coating application (https://www.fungiforce.com/, accessed on 15 July 2021), and was installed on the building in November 2021.

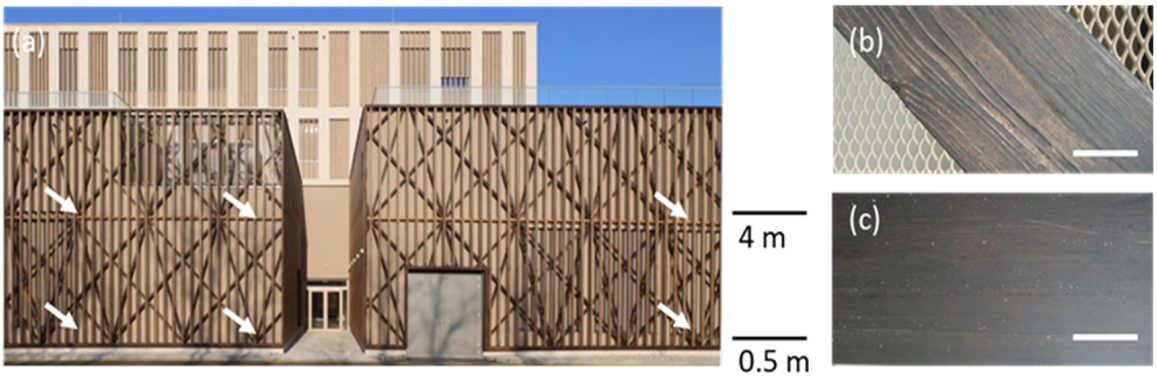


Figure 1. Exterior view of the InnoRenew CoE building with designated sampling points marked with arrows at heights of 0.5 and 4 m, strategically distributed to the left, middle, and right positions on the west-facing façade. (**a**) Close-up photos of the wood facade coated with Biofinish (**b**,**c**) Scale bar = 5 cm.

Hourly local weather conditions during the exposure period were obtained from the Historical Weather API (https://open-meteo.com/, accessed on 4 September 2022). The obtained weather data were used to interpret the fungal growth on the Biofinish-coated facade, providing valuable insights into how environmental factors such as temperature, humidity, precipitation, solar radiation, wind speed, and wind direction influenced fungal colonisation and proliferation.

The sampling and cultivation procedure involved using a wet sterile cotton swab to swab an area of approximately 25 mm × 25 mm on the surface of a wooden facade. The sampling points were strategically selected to ensure a comprehensive representation of the microbial community by including different elevations and lateral positions on the exterior facade. Samples were taken at heights of 0.5 and 4 m, and from the left, middle, and right, from all four cardinal directions of the building (Figure 1, Left). The swab was then streaked out on Malt Extract Agar (MEA, Biolife, Milan, Italy). The plates were then incubated in a growth chamber at 25 °C for 7 days. Following this incubation period, colony-forming units (CFUs) were counted. To standardise counts, the average number of colonies obtained from plates sampled from the left, middle, and right positions at the same height and cardinal directions was calculated. The average number of colonies was then categorised into four groups as follows: 0–25 colonies as 25 CFUs, 25–50 colonies as 50 CFUs, 50–75 colonies as 75 CFUs, and 75–100 colonies as 100 CFUs. It is important to note that a threshold of 100 colonies was established as the upper limit, and any plates containing counts exceeding 100 were recorded as having 100 CFUs. From each group of colonies displaying similar morphology, as determined by visual evaluation, one representative colony was selected and isolated to obtain a pure culture on an MEA plate. The pure cultures were then incubated at 25 °C in the dark in a growth chamber for further analysis.

2.2. Visualisation and Identification of Fungal Macro- and Micromorphological Features

The macro- and micromorphology of the selected cultures was assessed when the cultures were between 7 and 9 days old, with the exception of *A. pullulans*. For *A. pullulans*, 14-day-old cultures, when the margin became dark brown, were used, which allowed for sufficient development, enabling a more comprehensive assessment of its morphologies. Microscopic examination of the cultures was conducted using a Lacto Phenol Cotton Blue Teased Mount (LPCB-TM) technique. Briefly, small portions of colonies were extracted from the cultures using sterilised inoculation needles and transferred onto a microscope slide containing a drop of LPCB solution (Thermo Fisher Scientific, Walthum, MA, USA). The colonies were gently teased into tiny pieces using sterilised inoculation needles, and

the preparation was covered with a cover slip. The micromorphologies of the selected fungal species were then analysed using a Keyence VHX-6000 digital microscope (Keyence, Osaka, Japan). The macromorphology of pure cultures was visualised and imaged with an iPhone 15 Pro Max camera (Apple, Cupertino, CA, USA).

2.3. Molecular Identification

The genomic DNA of the isolates was extracted after mechanical lysis in CTAB buffer according to the modified protocol previously described by Gerrits van den Ende and de Hoog [18]. The majority of strains were identified by internal transcribed spacers 1 and 2, including the 5.8 S rDNA (ITS). These genes were PCR-amplified and Sanger-sequenced with the following primer sets: ITS1/ITS4 (ITS) [19]. The fungi were identified by comparing the sequences of the most similar types of strains and other closely related strains deposited in the non-redundant GenBank nucleotide database with the BLAST algorithm [20]. All DNA sequences of the isolated strains from this study were deposited in the GenBank database with accession numbers PP348129–PP348165.

3. Results

3.1. Weather Data

During the testing period, weather conditions exhibited distinct seasonal changes (Figure 2). Throughout the testing period, the hourly temperature ranged from as low as −1 °C to 33.4 °C, with a monthly average of 5 to 26 °C, showcasing significant variability (Figure 2a). Similarly, hourly relative humidity exhibited a wide range, fluctuating between 28% and 98%, with a monthly average of 58 to 78%. Hourly average precipitation varied from 0 to 5.3 mm (Figure 2c). Direct radiation levels gradually increased as the testing period progressed until the beginning of autumn 2022 (Figure 2d). The range of direct radiation varied substantially, from 0 during nighttime to a maximum of 795 W/m^2 during daylight hours. The intensity of direct radiation notably increased over time, particularly during the period from April to August 2022 (Figure 2d). This timeframe was characterised by consistently high levels of direct radiation, indicating a sustained elevation in solar irradiance during these months. Throughout the observational period, wind patterns were notably consistent, predominantly blowing from the southeast to the northwest from November to December 2021 and in January, March, May, July, and August 2022. In February, April, May, and June 2022, the wind direction shifted from predominantly south to north (Figure 2f). Hourly wind speeds fluctuated between 0 and 35.8 km/hour, with monthly average wind speeds between 8 and 12 km/hour, indicating variable but occasionally brisk air movement (Figure 2e). During the final two months of the exposure period, the wind direction notably shifted to the southeast (118° and 129°), suggesting a change in atmospheric circulation patterns. This shift in wind direction may have implications for the dispersion of airborne particles, pollutants, and microbial spores, potentially influencing environmental dynamics and microbial ecology in the study area.

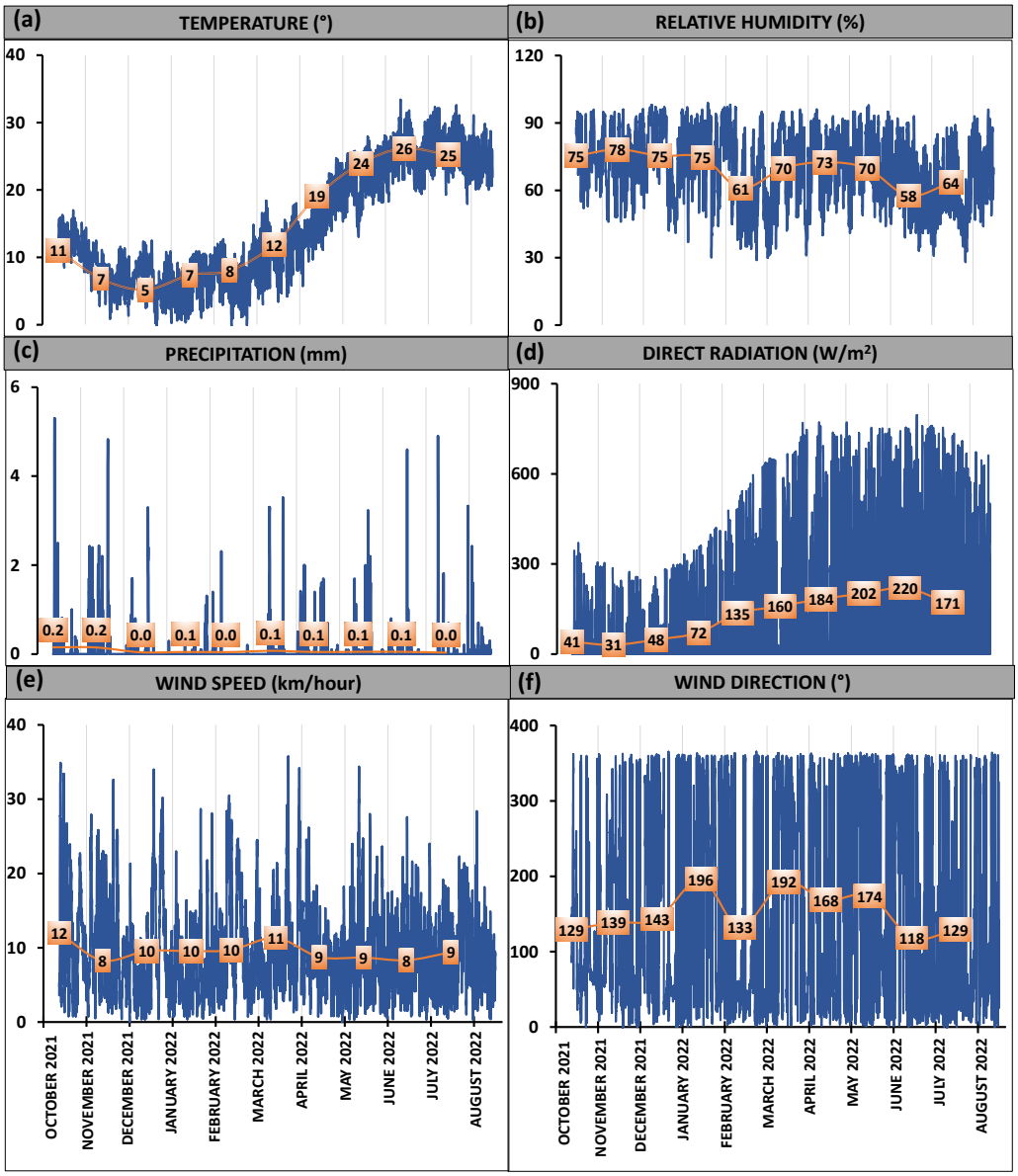


Figure 2. Hourly average weather parameters recorded throughout the testing period, including (**a**) Temperature, (**b**) Relative Humidity, (**c**) Precipitation, (**d**) Direct Radiation, (**e**) Wind Speed, and (**f**) Wind Direction. The labels represent monthly average values for each parameter.

3.2. Culturable Fungal Composition

Through DNA sequence analyses, we identified several fungal strains inhabiting the surfaces of facades coated with Biofinish after 9 months of exposure. These strains included *Aspergillus niger* (*A. niger*), *Aspergillus versicolor* (*A. versicolor*), *Aureobasidium melanogenum* (*A. melanogenum*), *Aureobasidium pullulans* (*A. pullulans*), *Cladosporium* sp., and *Penicillium crustosum* (*P. crustosum*). Notably, fungi within the *Aureobasidium* genus, particularly *A. pullulans*, which is the living and active ingredient of the coating, were predominant and consistently observed on wood surfaces exposed to all cardinal directions and heights (Figure 3). The highest total number of fungal colonies was observed on the facade exposed to the west at 0.5 m from the ground and to the north at 0.5 m and 4 m from the ground. The total number of culturable fungal colonies at a height of 0.5 m was higher than that at 4 m from the same direction, except for the east-facing facade. In terms of diversity, a greater number of fungal species were observed at a height of 4 m compared to 0.5 m within the same cardinal direction, except for the north-facing facade. Additionally, the west-facing facade exhibited the highest number of fungal species among all cardinal directions.

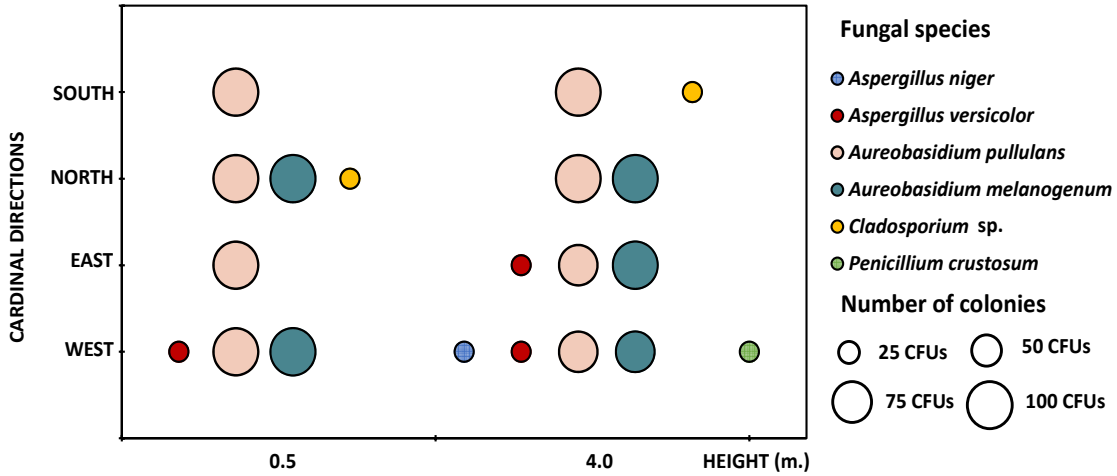


Figure 3. Culturable fungal colonies and fungal species identified on the Biofinish-coated facade after a 9-month exposure period at heights of 0.5 and 4 m from all four cardinal directions (north, south, east, west) of the building.

Representative macro- and micromorphologies of all species isolated from the Biofinish-coated facade are presented in Figure 4. *A. pullulans* colonies exhibited the characteristic smooth and slimy texture, with a pinkish colouration and a distinct dark edge on MEA at 25 °C after 14 days. Microscopic examination of slide preparations, obtained from the darker regions of the colonies, predominantly revealed the presence of dark-pigmented septate hyphae and conidia with thick walls. Additionally, hyaline ellipsoidal conidia and short septate hyphae were also observed. *A. melanogenum* colonies displayed the characteristic smooth and slimy texture, with a black centre and a yellowish-white margin on MEA at 25 °C after 7 days. Microscopic examination revealed dark brown, transversely septate, and thick-walled vegetative hyphae. Vegetative hyphae with hyaline, smooth, thin-walled, transversely septate characteristics and hyaline, one-cell conidia were present. It is important to note that the *A. melanogenum* colonies exhibited a dark appearance after only 7 days of incubation on MEA at 25 °C, whereas the dark appearance of *A. pullulans* colonies was observed after 14 days. *A. niger* colonies displayed a compact white or yellow basal felt, covered by a dense layer of dark brown to black conidial heads. Under microscopic examination, long, unbranched conidiophores were observed emerging from either the substrate or aerial mycelium. The colonies of *A. versicolor* exhibited a velvety or powdery texture, with a characteristic greenish colouration in the centre and a white edge. Long, unbranched, smooth stipes; subglobose vesicles; and conidia were observed under microscopic observation. *Cladosporium* sp. colonies on MEA exhibited a greenish colouration with a woolly texture. Microscopic examination revealed septate hyphae with conidiophores and conidia. Colonies of *P. crustosum* displayed shades of green and consisted of a dense felt of conidiophores with a powdery appearance. Under a microscope, typical conidiophores with phialides forming basipetal conidia were observed.

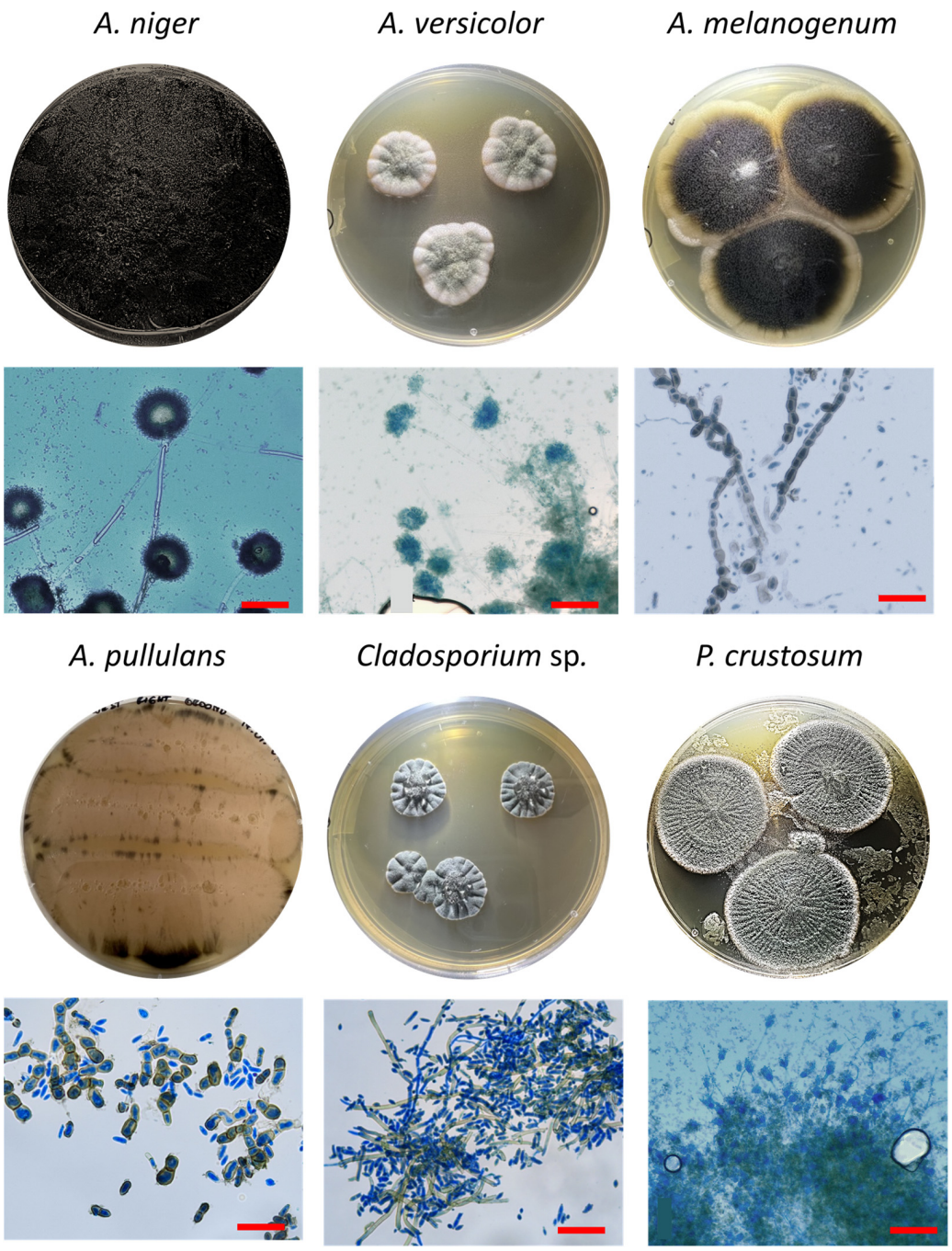


Figure 4. Macro- and micromorphologies of fungal species isolated from the Biofinish-coated façade. Scale bar = 50 μm.

4. Discussion

The identified fungi detected in this study, such as *A. niger*, *A. versicolor*, *A. melanogenum*, *A. pullulans*, *Cladosporium* sp., and *P. crustosum*, represented discolouration blue-stain and mould fungi [5,6], which are commonly found on wooden surfaces [4]. These fungi typically penetrate the wood by only a few millimetres, primarily utilising easily accessible nutrients such as proteins, lipids, and simple carbon compounds stored in the wood parenchyma, with limited degradation of cellulose. Consequently, they may cause discolouration and aesthetic issues, affecting the wood's appearance, while the overall strength properties of the wood remain largely unaffected [4–6]. The presence of *A. pullulans*, the living and active ingredient of the coating, across all cardinal directions of the facade following a nine-month exposure period highlights its remarkable survival and exceptional adaptability to

diverse ecological conditions. This adaptability was evidenced by its capacity to withstand significant environmental fluctuations, ranging from the relatively low temperatures and high relative humidity of winter to the contrasting conditions of high temperatures, low relative humidity, and intense solar radiation characteristic of Izola's summer climate. *A. pullulans* exhibits a remarkable ability to tolerate a relatively broad range of ecological conditions including UV radiation [21,22], cold environments [23–26], desiccation and high temperatures [4,22], and environments with fluctuating water activity [23]. One of the key characteristics of *A. pullulans* that enables it to thrive in diverse ecological niches and withstand a wide range of environmental challenges is its ability to exhibit phenotypic plasticity. This phenomenon involves the capacity of *A. pullulans* to respond to environmental signals by altering its morphology, physiological state, or behaviour [27–29]. In response to varying environmental conditions, *A. pullulans* can adjust its morphological features such as colony texture, colour, and size [28]. *A. pullulans* demonstrates varying degrees of melanin accumulation under different environmental conditions [28]. It can also modify its physiological state by regulating metabolic pathways, enzyme production, and energy utilisation to optimise growth and survival [30]. Additionally, *A. pullulans* may alter its behaviour by biofilm formation or by exhibiting antagonistic activity against competing microorganisms to enhance its adaptability to different ecological niches [27,28,31].

Solar radiation, particularly biologically damaging UV-B radiation within the wavelength range of 280 nm to 315 nm, can significantly impact the growth and survival of fungi. Studies have shown that exposure to UV-B radiation can inhibit fungal growth and disrupt cellular processes [32–34]. In response to the stress imposed by solar radiation and various other environmental factors, microorganisms, including fungi, have evolved various strategies for protection and survival. One such strategy involves the production of melanin—dark pigments known for their ability to absorb and dissipate UV radiation [35,36]. Melanin acts as a natural sunscreen, shielding cells from the harmful effects of UV radiation by absorbing and scattering photons, thereby reducing DNA damage and oxidative stress. Furthermore, melanin's properties extend beyond UV protection; they also contribute to thermal regulation, tolerance to desiccation, and protection against chemical and environmental stressors [35,36]. The presence of melanised fungi, such as *Aureobasidium*, *Aspergillus*, *Cladosporium*, *Alternaria*, and *Penicillium*, on the Biofinish-coated facade exposed to intense solar radiation and diverse weather conditions in Izola highlights the adaptive significance of melanin production in these fungi. By harnessing the protective capabilities of melanin, these fungal species can effectively thrive in challenging outdoor environments characterised by fluctuating temperatures, humidity levels, and solar radiation intensities.

However, the intensity of solar radiation varies significantly across different cardinal directions, influencing the survival and growth of fungi on building facades. South-facing facades, for instance, receive the most direct sunlight during the day, resulting in higher elevated temperatures and increased solar heat gain compared to other orientations. Conversely, the lowest solar radiation levels are typically found along the north-facing surface [37,38]. This discrepancy in solar exposure leads to pronounced variations in microclimatic conditions across different facade orientations, creating distinct ecological niches that could influence the colonisation and proliferation of fungi on the Biofinish-coated facade in this study.

Wind plays a crucial role as a vector in dispersing microorganisms to new habitats [39,40]. The higher number of fungal species observed on the facade facing north and west may be attributed to prevailing wind directions during the exposure period, which was primarily from the southeast and south. These prevailing wind patterns likely facilitated the transportation of fungal spores and other microorganisms towards the north- and west-facing surfaces of the building. As a result, these surfaces received a greater influx of airborne fungal species, leading to an increased diversity of fungi compared to other directions.

Apart from the other characteristics mentioned above, the dominance of *A. pullulans* on the facade can be attributed to its effective antagonistic action against a wide range of fungi including *Aspergillus niger*, *Alternaria alternata*, *Penicillium expansum*, and *Rhizopus stolonifer* [2,41–44]. These are well-known wood-infesting fungi that are commonly found on weathered wood surfaces [4]. This antagonistic activity serves as a natural defence mechanism that inhibits the growth of competing fungal species and allows *A. pullulans* to establish and proliferate on the substrate surface, further enhancing its effectiveness as a biocontrol agent in architectural coatings. Our previous study [45] examined fungal colonisation on Scots pine sapwood exposed to various climatic conditions. Interestingly, fungal strains such as *Cladosporium allicinum*, *Cladosporium pseudocladosporioides*, *Cladosporium crousii*, *Lithohypha guttulate*, *Phoma herbarum*, *Pseudotaeniolina globose*, *Stachybotrys* sp., and *Sydowia polyspora* were detected in Isola, Slovenia, but were not found in the current study. In addition to the effects of wood species and linseed oil treatment, the absence of these fungal strains in this current study could be attributed to the antagonistic activity of *A. pullulans* present on the facade coated with Biofinish. This antagonistic action may have inhibited the growth of these fungal strains. Moreover, linseed oil-impregnated Scots pine sapwood coated with Biofinish demonstrated remarkable durability against wood-destroying basidiomycete fungi. It achieved durability classes 1–2 according to European standard EN113 and durability class 2 in a field test to determine the relative protective effectiveness of a wood preservative in ground contact, as per European standard EN 252 [7,46]. This highlights the effectiveness of the treatment in enhancing the wood's resistance to fungal decay, attributable to the combination of the hydrophobicity of the oil and the presence of *A. pullulans* living cells in the coating matrix.

The higher total number of culturable fungal colonies observed at a height of 0.5 m compared to 4 m can be attributed to various factors influencing fungal distribution and growth along the vertical axis of the building facade. One contributing factor is the leaching of fungal spores by rainwater, which tend to concentrate near the lower regions of the building due to gravity. This phenomenon can lead to the increased deposition of spores and organic matter at lower elevations, providing a nutrient-rich environment conducive to fungal colonisation and proliferation. Additionally, the bottom part of vertically exposed samples, such as wood panels, may experience extensive accumulation of moisture due to the "moisture trap" effect and capillary uptake of water. Moisture can accumulate within the porous structure of the wood material, creating favourable conditions for fungal growth [45]. Moreover, the higher wind speed at the upper part of the facade can potentially contribute to the dispersion of fungal spores. As wind speed increases with height above ground level [47], particularly in unobstructed areas higher up on the building, there is a greater likelihood of the wind carrying spores away from the surface of the facade. The combination of the wind, moisture retention, and organic substrate availability in the lower portion of the building facade promotes the establishment of fungal colonies and enhances their viability over time.

The presence of different fungal species along with varying concentrations of *A. pullulans* on the facade highlights the complexity of fungal interactions and environmental factors influencing fungal diversity. While a trend of a higher number of other fungal strains was observed in areas with lower concentrations of *A. pullulans*, the relationship between the colony number of *A. pullulans* and the presence of other fungal species remains unclear. Indeed, the presence or absence of different fungal species on the facade is not solely influenced by the competitive interaction between fungal species and the concentration of *A. pullulans*. Rather, it is influenced by a combination of factors, including environmental conditions, competitive interactions among fungal species, and the concentration of *A. pullulans*. As mentioned above, environmental factors such as humidity, temperature, direct radiation, wind speed, wind direction, and the availability of nutrients create microhabitats with varying suitability for different fungal species, leading to spatial heterogeneity in fungal distribution across the facade.

5. Conclusions

In conclusion, the findings of this study highlight the promising potential of bioinspired living coating systems (*A. pullulans*-based Biofinish coatings) as an effective and sustainable solution for wood protection. The demonstrated ability of *A. pullulans* to tolerate significant environmental fluctuations and exhibit antagonistic action against a wide range of fungi, irrespective of orientation and height, indicates the versatility and resilience of Biofinish coating. This versatility makes Biofinish suitable for utilisation in various climates, offering a viable solution for wood protection in diverse environmental conditions. Further research and development in optimising the formulation and application methods of Biofinish coatings can enhance their efficacy and broaden their applicability, ultimately contributing to the advancement of sustainable construction practices.

Author Contributions: Conceptualisation, F.P.; methodology, F.P., A.G., K.B.O. and A.Č.; software, F.P. and A.G.; validation, F.P., A.G. and A.S.; formal analysis, F.P. and A.G.; investigation, F.P. and A.G.; resources, A.S.; data curation, F.P. and A.G.; writing—original draft preparation, F.P.; writing—review and editing, F.P., A.G., K.B.O., A.Č., J.S., L.R. and A.S.; visualisation, F.P.; supervision, A.S.; project administration, A.S.; funding acquisition, A.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The dataset used for the analysis in this study is available at https://zenodo.org/records/10685168 (accessed on 13 March 2024) Open Access depository [48].

Conflicts of Interest: The authors declare no conflicts of interest.

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