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# Time-lapse evaluation of *Aureobasidium pullulans* growth on selected lignin derivatives and chitosan

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## ABSTRACT

Wood, often referred to as the "building material of the 21st century", has gained recognition as an attractive alternative to several traditional building solutions. To enlarge the application of wood, several properties including biotic and abiotic degradation resistance need to be improved. Consequently, new solutions are available on the market that ensure expected properties and functionality over extended service life and reduce the risk of product failure. The latest trends are driven by biomimicry, an approach that captures and exploits properties that have evolved in nature. This was a strong motivation for the development of an alternative nature-inspired coating system using the yeast-like fungus *Aureobasidium pullulans* as a living component. *A. pullulans* is widely found in nature and has unique properties, such as forming biofilms or digesting various nutrients. The majority of research on biofilms focuses on preventing its formation while leaving their protective applications unexplored. Understanding the mechanisms of fungal growth, their ability to utilise different nutrients and form biofilms, are necessary to develop a technically applicable, controlled, and optimised biofilm that effectively protects the substrate surfaces.

This study investigated the growth and biofilm formation of *A. pullulans* on different lignin derivatives, namely, vanillin, phenol, 4-hydroxybenzoic acid, and p-coumaric acid. The ability of the fungi to utilise lignin derivatives in poor, synthetic nutrient media and rich malt extract media was determined, and the amount of biofilm produced was quantified. Fungal growth was visualised using a transmitted light imaging system (EVOS M7000, ThermoFisher Scientific), which captured projection images at defined time intervals. The acquired images were used for evaluating the morphological characteristics of the developing fungal biofilm. Furthermore, they served as quantitative data for the analysis of various morphological and growth parameters of *A. pullulans*. The combination of time-lapse microscopic observation, with mathematical analysis, offers valuable insights into the first steps of fungal growth that are crucial for our understanding of biofilm development. This knowledge can be used for developing alternative living protective biofilms as well for understanding bioreceptivity of materials and growth mode of early fungal colonisers.

**Keywords:** *Aureobasidium pullulans*, biofilm, microscopy, lignin derivates, chitosan

## 1. INTRODUCTION

*Aureobasidium pullulans* (*A. pullulans*) is a black yeast-like fungus known for its biotechnological significance (Zalar *et al.* 2008). Its main potential is the production of biodegradable extracellular polysaccharide pullulan (poly-α-1,6-maltotriose), a promising biomaterial used in packaging foods (Singh *et al.* 2008), and its ability to produce a variety of hydrolytic enzymes such as amylase, protease, lipase, cellulose, xylanase, mannose, and transferases with diverse enzymatic activity (Zalar *et al.* 2008, van Nieuwenhuijzen 2014). *A. pullulans* is a ubiquitous and widespread oligotroph (Zalar *et al.* 2008). It is present on diverse organic and inorganic outdoor materials, such as phylloplanes, soil, wood, marble, and water (Zalar *et al.* 2008). Alongside its high biotechnological potential, *A. pullulans* can be used as a natural living ingredient in various applications. *Aureobasidium* spp. can naturally form a black biofilm on oil-treated wood, protecting it from wood rot or UV degradation (van Nieuwenhuijzen 2014, Sailer *et al.* 2010). Alongside the production of various enzyme, *A. pullulans* has the ability to form biofilms, which has been thoroughly investigated for biocontrol against fruit rot agents (Zajc *et al.* 2020). Biofilms represent a microbial lifestyle that ensures a stable environment for microbial development, protection from environmental challenges, higher tolerance against antimicrobial factors, better communication, and efficient nutrient consumption (Flemming and Wingender 2010, Flemming *et al.* 2014). Biofilms have unique properties such as big structural complexity, metabolic heterogeneity, and the production of EPS. Biofilm formation is a natural process that occurs with many microorganisms in nature and has many advantages for their societies. Therefore, biofilms can be found on surfaces exposed to extreme pH, temperature, salt concentration, radiation, and/or pressure (O'Toole *et al.* 2000, Flemming 2008).

A particularly noteworthy aspect of *A. pullulans* is its ability to use various aromatic compounds associated with weathered wood as a sole carbon source (Schoeman and Dickinson 1996). Lignin is a highly heterogeneous polymer composed of different monomers that can be degraded by fungal heme-peroxidases and laccases (Sigoillot *et al.* 2012). *A. pullulans* exhibits capabilities in both oxidising and reducing lignin derivatives (Bourbonnais and Paice 1987). This unique feature not only contributes to the fungus's ecological role in nutrient cycling but also holds promise for applications in the utilisation and bioremediation of lignocellulosic biomass (Putra *et al.* 2022).
In addition to its natural abilities, *A. pullulans* activity can potentially be modulated through the incorporation of chitosan, a natural polymer derived from chitin. Chitosan serves as an additive with antimicrobial properties, making it a valuable tool for limiting the colonisation of competing fungi and microbes (Elieh-Ali-Komi and Hamblin 2017).

The mechanisms regulating the growth of *A. pullulans* remain insufficiently researched and still poorly understood, thereby creating an opportunity for further research. *A. pullulans* exhibit diverse morphology, including yeast-like cells, filamentous structures, and various colony morphologies (Andrews *et al.*, 1994). This versatility enables *A. pullulans* to adapt to different environmental conditions, showcasing its resilience and adaptability (Cheng *et al.* 2009). In recent years, the integration of computational modelling with live-cell imaging has gained popularity.

This approach is particularly valuable as it enables real-time observations in a laboratory environment without perturbations, providing a comprehensive tool for understanding microbial growth dynamics (Shroff *et al.* 2024). Monitoring the growth of the mycelial network through mathematical modelling plays a pivotal role in understanding the dynamics of fungal biofilm formation. By transforming microscopy images into mathematical graphs, we gain a quantitative understanding of the spatial and temporal evolution of the fungal biofilm. This modelling approach provides valuable insights into the intricate processes involved in fungal growth, such as the

colonization of substrate surfaces and the development of biofilm morphology (Heaton *et al.* 2012).

This research presents preliminary results on the utilisation of selected lignin derivatives by *A. pullulans* and its ability to form biofilms. Understanding the factors influencing its growth and the intricacy of morphological development holds the key to harnessing its full potential in applications as a living component of protective coating systems. Growth modelling for *A. pullulans* remains inadequately explored. In this study, we introduce the initial steps of image analysis and data interpretation that have the potential to provide insights into various applications, including monitoring the early stage of the deterioration process or design of engineered living materials. Three aspects of *A. pullulans* growth were investigated: biofilm production in both nutrient-rich and nutrient-poor liquid media with various additions; observation of fungal growth on the microscopic level in 24-hour intervals; initial steps of growth modelling along with evaluation on a solid agar surface.

## 2. EXPERIMENTAL METHODS

### 2.1 Fungal strains and media

Four strains of *Aureobasidium pullulans* were used: IN-7, IN-27, IN-29, and EXF-150. The first three strains were isolated from treated wooden materials exposed to natural weathering at InnoRenew CoE (Butina Ogorelec *et al.* 2023), and the last was obtained from the Culture Collection Ex of the Infrastructural Centre Mycosmo (Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia). Cultures of IN-7, IN-27, IN-29, and EXF-150 were grown on malt extract agar (MEA) plates at 25 °C for 7 days in the dark.

The media used in this study included:

- Yeast nitrogen base (YNB) liquid media composed of 1.7 g YNB (Sigma Aldrich, Missouri, USA), 5 g $(NH_4)_2SO_4$ (Sigma Aldrich, Missouri, USA), and 20 g glucose (Merck, Darmstadt, Germany) per 1 L.
- MEA (Merck, Darmstadt, Germany) prepared according to the manufacturer's instructions.
- Malt extract (ME) liquid media composed of 30 g malt extract and 5 g mycological peptone (both Merck, Darmstadt, Germany) per 1 L.
- Synthetic nutrient (SNgs) liquid media composed of 1 g $KH_2PO_4$, 1 g $KNO_3$, 0.5 g $MgSO_4$ x $7H_2O$, 0.2 g glucose, and 0.2 g saccharose (all Merck, Darmstadt, Germany) per 1 L.
- Synthetic nutrient agar (SNA) composed of 1 g $KH_2PO_4$, 1 g $KNO_3$, 0.5 g $MgSO_4$ x $7H_2O$, 0.2 g glucose, 0.2 g saccharose, and 20 g of agar (all Merck, Darmstadt, Germany) per 1 L.
- Synthetic nutrient (SN) liquid media without the addition of glucose and saccharose.

To 100 mL of ME, SNgs, and SN liquid media, 1 mL of lignin derivatives dissolved in 2 mM DMSO, namely vanillin (4-hydroxy-3-methoxybenzaldehyde), and phenolic acids (phenol, 4-hydroxybenzoic acid, and p-coumaric acid) (all Sigma-Aldrich, Missouri, USA), were each added from a stock solution of 50 mg/mL to achieve a final concentration of 0.5 mg/mL in media.

### 2.2 Biofilm production quantification

Biofilm quantification with crystal violet staining was done based on the protocol initially described by Christensen *et. al* in 1985, with the following modifications for yeast cultures. One loop of each culture was inoculated in 10 mL of liquid YNB and incubated at 24 °C with shaking at 180 rpm for approximately 24 hours. Cells were then resuspended in 1 mL of fresh liquid YNB

and optical density at $OD_{590}$ nm was measured. Microcentrifuges containing resuspended cells were centrifuged at 12,000 × g for 3 minutes, and the supernatant was removed. Cells were subsequently resuspended in prepared 18 types of media to optical density at $OD_{590}$ nm ranging from 0.15-0.2. Next, 100 µL of the prepared cell suspension was aliquoted in 96-well microtiter plates (Corning) in triplicate for each of the 18 media preparations containing lignin derivatives. Controls for each condition (medium without cells) were included. The prepared multi-well plates were incubated for 7 days at 25 °C without shaking. After the incubation period, 100 µL of 1% (w/V) Crystal violet solution (Merck, Darmstadt, Germany) was added to each well and incubated at room temperature for 30 minutes without shaking. The plates were then rinsed with distilled water, and 100 µL of 10% SDS solution (Sigma Aldrich, Missouri, USA) was added to each well. Biofilm growth was monitored at $OD_{590}$ nm using a microplate reader (Synergy Neo2, BioTek). Due to measuring limitations, an upper limit of 4 was set for all samples where biofilm production was too substantial to measure. All data are expressed as mean plus/minus standard deviation (SD) of the triplicate experimental data. Analysis was performed using GraphPad Prism version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

### 2.3 Microscopic biofilm formation observation

Five hundred µL of each medium were aliquoted into 48-well microtiter plates (Sarstedt, Germany). A cell suspension of cultures IN-27, IN-29, and EXF-150 was prepared and the optical density ($OD_{590}$ nm) was measured. The inoculation volume was calculated so that the final $OD_{590}$ in each well was 0.05 and seeded in triplicates. The plate was placed in an incubation chamber of EVOS M7000 (Thermo Fisher Scientific) at 25 °C and observed under a 60× objective at 24-hour intervals for 4 days. The scanned area size was 4.8 $mm^2$. Captured images were stitched together using Celleste6 software (ThermoFisher Scientific). The average number of cells at each timepoint was counted using the CellCount add-in in Fiji ImageJ software (Schindelin *et al.* 2012). An arbitrary boundary for overgrowth was implemented, considering cases where cells overlapped.

### 2.4 Time-lapse growth evaluation of *Aureobasidium pullulans*

MEA, SNA, and SNA with 0.7% chitosan in 1 M glycolic acid to a final concentration of 0.01% in 100 mL were prepared. 2 mL of each medium was aliquoted in 6-well microtiter plates in duplicates (Sarstedt, Germany) and inoculated with a culture of strain EXF-150 using a sharp needle point. The plate was transferred to the incubation chamber of EVOS M7000 (Thermo Fisher Scientific) at 25 °C and observed under a 10× objective at 3 and/or 6-hour intervals for 3 days. Captured images were stitched together and analysed using Celleste6 software (Thermo Fisher Scientific). Mathematical modelling on obtained images was done using software previously described by Heaton *et al.* 2012.

## 3. RESULTS AND DISCUSSION

### 3.1 Biofilm production quantification

The results from the microtiter plate analysis revealed a significant difference in biofilm production between nutrient-rich (ME) media and nutrient-poor media (SN, SNgs) with lignin derivatives (Fig. 1). All four strains exhibited the most abundant growth in ME, consistent with expectations due to the presence of multiple carbon sources (sugars) in the medium composition. Relatively robust growth was also observed in SN and SNgs media, although slightly less abundant given the limited availability of carbon sources (sugars) and the inclusion of only essential elements in medium composition. Notably, strain EXF-150 exhibited the least overall performance regarding the media or derivative addition, followed by strain IN-27. In contrast, the strains IN-7

and IN-29 demonstrated the highest biofilm production. The differences in biofilm production between strains and media persist despite similar morphology, as further demonstrated.

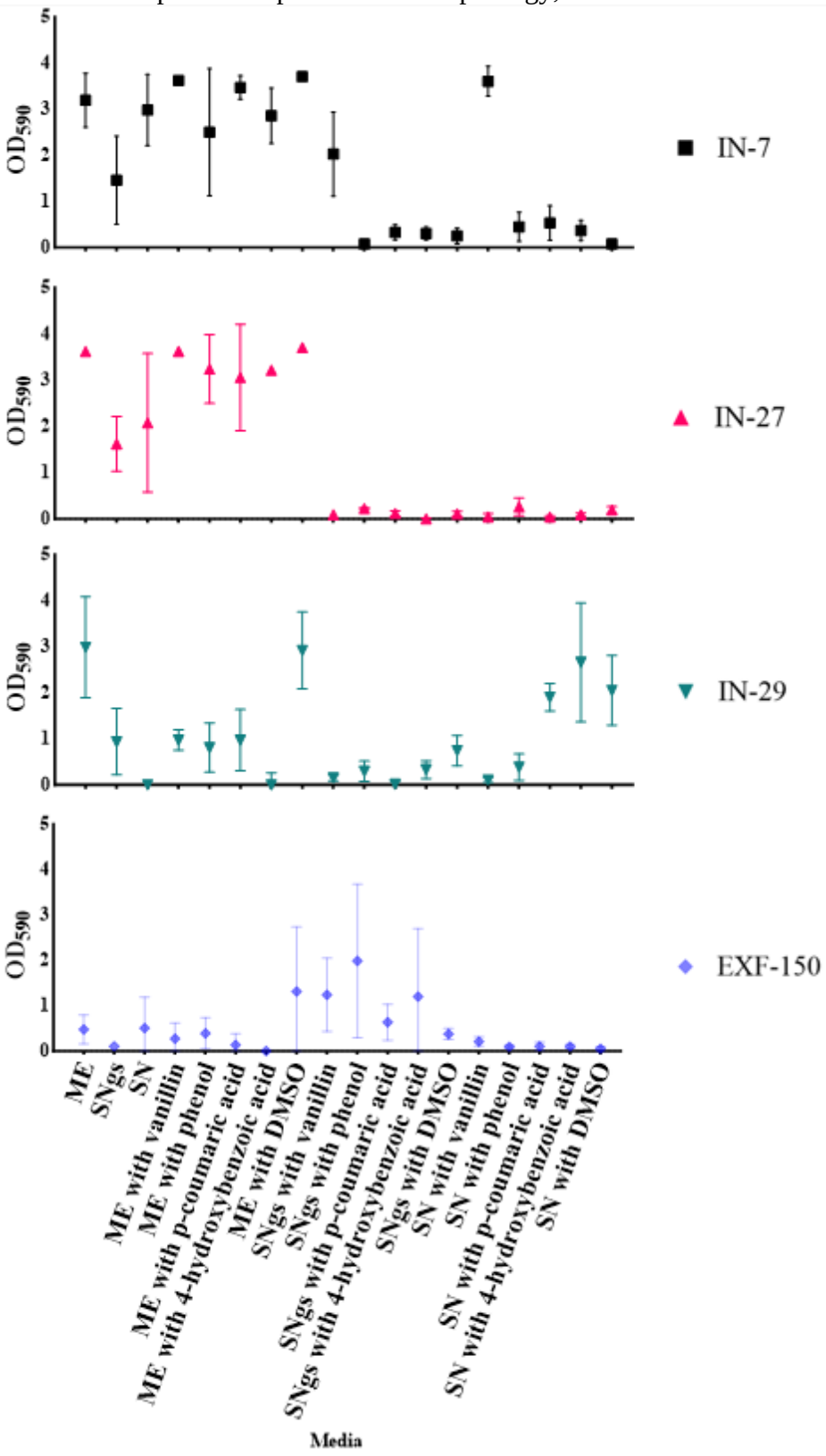


Figure 1: Quantification of biofilm production by optical density measurements $OD_{590}$ nm with plus/minus standard deviation for *Aureobasidium pullulans* strains IN-7, IN-27, IN-29, and EXF-150.

According to previous studies, *A. pullulans* can utilise organic substances, including some lignin derivatives, as the sole carbon source (Schoeman and Dickinson 1996). Photodegradation of lignin in the process of natural weathering following exposure to UV radiation is documented (Cogulet *et al.* 2016). *A. pullulans* is a known producer of various extracellular enzymes that are important for the degradation of the plant cell-wall material (Gostinčar et al. 2014). It can potentially utilise these molecules as nutrients and thrive on wooden surfaces. Fungi, known for their adaptability to environmental changes, can quickly adjust to new conditions (Gostinčar *et al.* 2019). The addition of certain lignin derivatives in low concentrations that do not impede growth, could benefit *A. pullulans* by facilitating contact and initiating mechanisms for utilisation. Such an adaptation might enable the fungus to outgrow other microbes present on the surface when exposed to such molecules in nature.

The observed high standard deviations are likely the result of a technique known to be less reliable. Despite its limitations, it still provides a reasonable estimation of biofilm production, as suggested by Shukla and Rao (2017) and Castro *et al.* (2022). It must be acknowledged that additional methods would be required for more precise conclusions.

### 3.2 Microscopic evaluation of biofilm formation

The microscopic observation of biofilm formation yielded results consistent with crystal violet staining. In the initial 24 hours, cultures of strains IN-27, IN-29, and EXF-150 overgrew the observed surface in multi-well plate (Table 1). In nutrient rich medium (ME) with added phenolic acids (phenol, 4-hydroxybenzoic and p-coumaric acid), cells exhibited a growth rate similar to that in media without derivatives, indicating that *A. pullulans* can grow in the presence of lignin derivatives when alternative carbon sources are available. Conversely, there was less and slower growth in SN with same concentrations of added derivatives, showing overgrowth between 24-48 hours. A similar trend was observed in SNgs media with vanillin and phenolic acids, with strain EXF-150 exhibiting the slowest growth, which aligns with biofilm quantification results. In SNgs and SN with addition of vanillin, growth was stunted, with cells appearing inactive and morphologically 'sick'. The inoculum size was consistent, suggesting it likely did not influence *A. pullulans* growth. Results suggest that *A. pullulans* is able to survive in the presence of lignin derivates, apart from vanillin. Natural phenolic substances are reportedly among the most antifungal active substances present in plant essential oils with high antifungal, antibacterial and insecticidal efficacy. Among phenolic acids, p-coumaric and 4-hydroxybenzoic acid possess little or no antifungal activity (Zabka and Pavela 2013); vanillin (a phenolic aldehyde), on the other hand, displayed antifungal action against several types of yeasts and moulds (Olatunde *et al.* 2022).

Distinct variations in speed of growth among strains were noticeable, with wood-isolated strains performing better in some cases. This suggests that cultures isolated from wood may be adapted to the presence of such molecules, making them promising candidates for further development in protective coatings. Often organisms colonising outdoor material surfaces face water shortages, which is a significant challenge in terms of microbial growth. They must depend on rainwater, which is seasonal and often in lower amounts. Strain EXF-150 was isolated from the hypersaline water in the Sečovlje salterns (Slovenia), where they are exposed to low water activity. Testing such organisms could provide an insight into their adaptation, survival mechanism, and their potential to form protective layers in similar environmental conditions. Thriving in such conditions is beneficial, and the ability to survive with limited input of nutrients and in the presence of harmful substances is crucial, especially keeping in mind the future application of developed coatings on building facades.

Table 1: Number of cells ($\times 10^4$) on 4,8 $mm^2$ surface at 24-hour time points of IN-27, IN-29, and EXF-150 strain

| Media/strain | IN-27 | | | | IN-29 | | | | EXF-150 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0h | 24h | 48h | 72h | 0h | 24h | 48h | 72h | 0h | 24h | 48h | 72h |
| ME | 4.5 | * | * | * | 1.6 | * | * | * | 3.5 | * | * | * |
| SNgs | 5.7 | * | * | * | 4.5 | * | * | * | 4.5 | * | * | * |
| SN | 5.6 | 14.8 | * | * | 4.7 | 16.2 | * | * | 5.5 | * | * | * |
| ME with vanillin | 5.4 | * | * | * | 4.8 | * | * | * | 6 | 8.5 | * | * |
| ME with phenol | 5.7 | 6.3 | * | * | 3.3 | * | * | * | 6 | * | * | * |
| ME with 4-hydroxybenzoic acid | 2.6 | * | * | * | 2.8 | * | * | * | 4.2 | * | * | * |
| ME with p-coumaric acid | 2.5 | * | * | * | 2.8 | * | * | * | 4.5 | * | * | * |
| ME with DMSO | 2.4 | * | * | * | 1.6 | * | * | * | 3.7 | * | * | * |
| SNgs with vanillin | 4.2 | 4.3 | 4.4 | 5.5 | 5.4 | 5.6 | 4.2 | 6.5 | 5.9 | 5.3 | * | * |
| SNgs with phenol | 4.2 | * | * | * | 4.8 | * | * | * | 4.3 | 4.7 | 4.5 | 3.7 |
| SNgs with 4-hydroxybenzoic acid | □ | □ | □ | □ | 5.4 | * | * | * | 4.5 | * | * | * |
| SNgs with p-coumaric acid | □ | □ | □ | □ | 6.2 | * | * | * | 3.9 | 7.4 | 8.3 | 9 |
| SNgs with DMSO | □ | □ | □ | □ | 5.4 | * | * | * | 4.5 | * | * | * |
| SN with vanillin | □ | □ | □ | □ | 4 | 5.5 | 5.2 | 4.8 | 4.5 | 4.9 | 5.2 | 5.3 |
| SN with phenol | □ | □ | □ | □ | 4.5 | 8.8 | 15.9 | * | 4.9 | 5.5 | 7.1 | * |
| SN with 4-hydroxybenzoic acid | □ | □ | □ | □ | 6.8 | 15.3 | * | * | 4.5 | 13.5 | * | * |
| SN with p-coumaric acid | □ | □ | □ | □ | 7.7 | 18.9 | * | * | 5 | 13.4 | * | * |
| SN with DMSO | □ | □ | □ | □ | 4.4 | 17.4 | * | * | 4.5 | 11.5 | * | * |

** overgrown, □ no data available.*

The cells in nutrient-rich media were large, oval, ranging from 13–16 × 8–12 μm, and exhibited rapid overgrowth within 24 hours (Fig. 2). Predominantly, liberated conidia transforming into budding cells and transformed conidium (yeast cell) exhibiting synchronous production of conidia were present as morphological features previously described by Zalar *et al.* 2008. Additionally, some dark brown conidia were present with a slightly thicker cell wall. The darker coloration is a result of melanin production, which is essential for high tolerance to both gamma irradiation and ultraviolet (UV) irradiation (Campana *et al.* 2022). In contrast, cells in nutrient-poor media appeared slightly smaller and more irregular in shape. Cells in media with added phenolic acids generally displayed similar morphology, except with vanillin, where cells exhibited a different shape, becoming smaller in size, seemingly not multiplying, and forming clumps. Short hyphae were observed after 24 hours only in strain EXF-150, cultured in ME with added phenol. Morphological changes as a stress response in *A. pullulans* are potentially regulated on the genomic level (Gostinčar *et al.* 2014). An example was reported by Gadd and Griffiths (1980) where the presence of copper during growth had a profound effect on *A. pullulans* morphology, stimulating melanin synthesis, and promoting the formation of filaments or hyphae. Apart from one example, the formation of hyphae was not detected in the present study. However, this does not mean that a certain stress response was not induced. For example, in *A. subglaciale*, a close relative of *A. pullulans*, enhanced trehalose biosynthesis was observed under radiation and heavy metal stress, even if cells seemed morphologically unaffected (Liu *et al.* 2017).

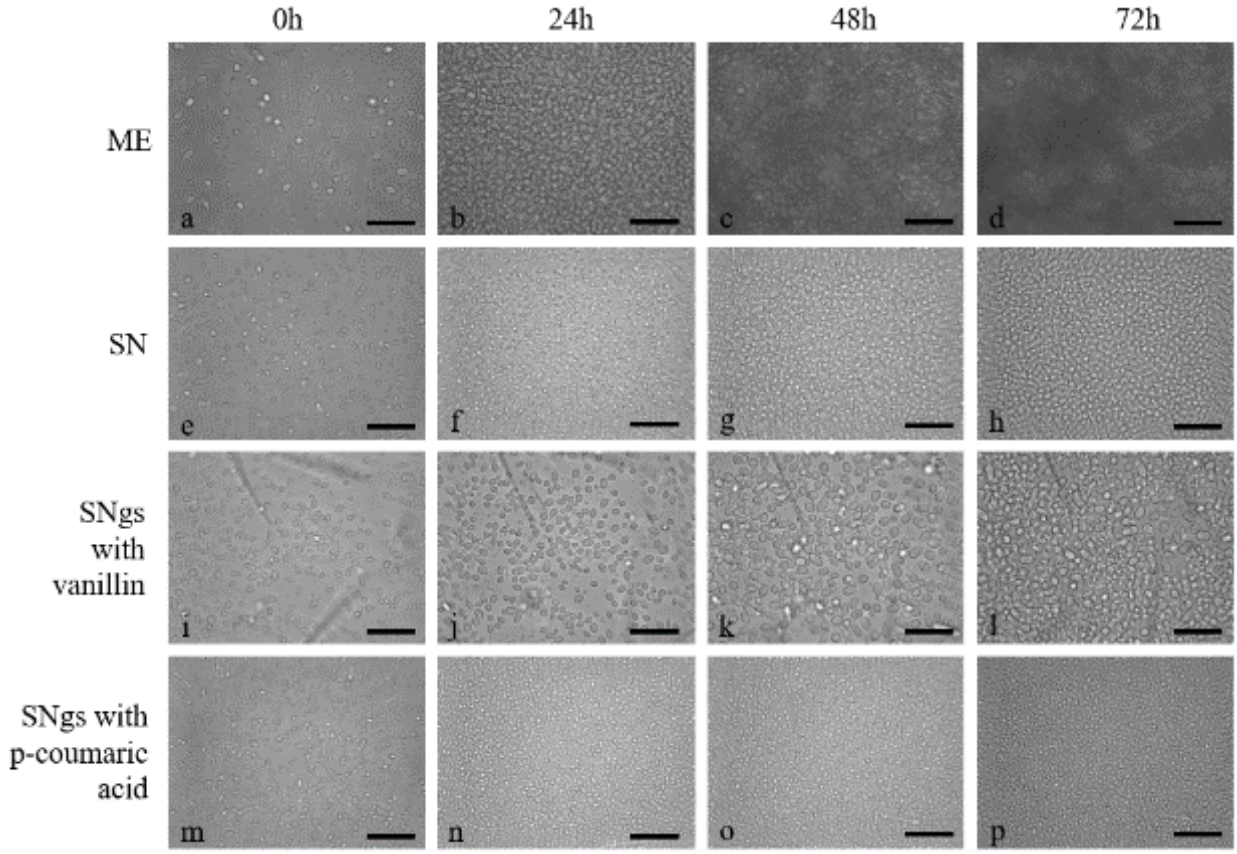


Figure 2: Time-lapse of *Aureobasidium pullulans* IN-29 growth in liquid media ME (a,b,c,d), SN (e,f,g,h), SNgs with vanilin (i,j,k,l) and SN with p-coumaric acid (m,n,op) at 0 hours (a,e,i,m), after 12 hours (b,f,j,n), 48 hours (c,g,k,o), and 72 hours (d,h,lp). Scale bar 50 µm.

### 3.3 Time-lapse growth evaluation on solid media

Differences in branching are evident in nutrient-rich and nutrient-poor media (Fig. 3). Nutrient rich medium (MEA) exhibits faster growth, less pronounced branching, almost exclusively hyphal formation, and higher coverage. The growth rate per day progresses from less than 2 mm on the first day to approximately 2.5 mm on the second day and exceeds 7 mm in 72 hours. On the other hand, the nutrient poor medium (SNA) displays more prominent hyphal branching, lower coverage, and a slower growth rate. Starting from 2 mm on the first day, around 3 mm on the second day, and less than 5 mm in 72 hours. The addition of chitosan to SNA results in visibly reduced branching and hyphal formation, accompanied by a significantly higher amount of immersed hypha with lateral accumulation of conidia, presumably because of its adaptive nature where it can enter hibernation mode and, under optimal conditions, resume thriving (Kocková-Kratochvílová *et al.* 1980). The growth rate per day is similar to SNA but with visibly less coverage, featuring more extended structures (Fig. 3-i, j, k, l). Similar growth pattern in SNA and SNA with addition of chitosan is potentially attributed to the lack of sugars.

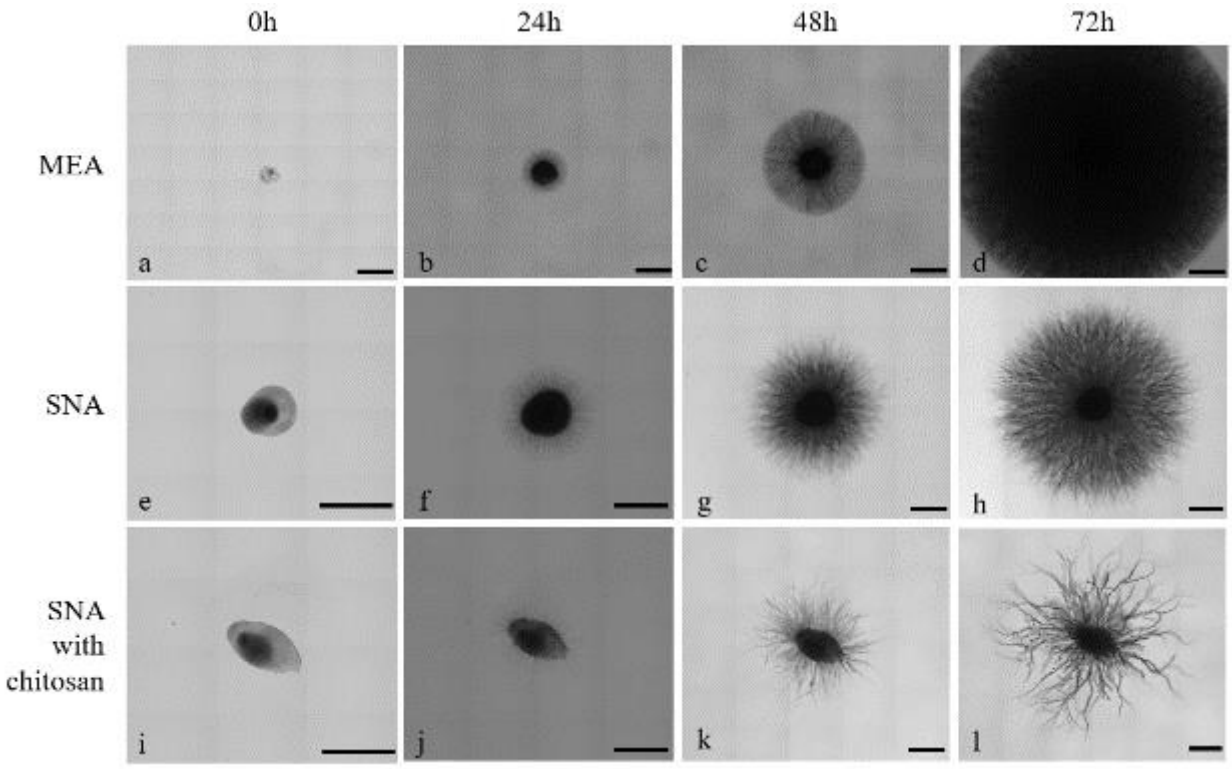


Figure 3: Time-lapse of *Aureobasidium pullulans* EXF-150 growth on solid media MEA (a,b,c,d), SNA (e,f,g,h), SNA with 0.01% chitosan in 0,1 M glycolic acid (i,j,k,l) at 0 hours (a,e,i), after 12 hours (b,f,j), 48 hours (c,g,k), and 72 hours (d,h,l). Scale bar 1 mm.

Chitosan exhibits high antimicrobial activity against pathogenic and spoilage microorganisms, including fungi, and both gram positive and gram negative bacteria. If *A. pullulans* can utilise it and grow in its presence it may modulate it colonisation and potentially inhibit growth of other microorganisms. Chitosan antimicrobial activity vary considerably with the type of chitosan and, occasionally, contradictory findings have been reported (Aider 2010). Since the structural scaffold of the fungal cell is composed of chitin and β-(1,3) glucan, fungi have many different chitinases that hydrolyse chitin and are, therefore, not only involved in exogenous chitin decomposition but also in fungal cell wall degradation and remodelling (Hartl *et al.* 2012) suggesting that they are potentially capable of chitosan utilisation.

Mathematical graphs derived from microscopy images allow for the precise tracking of individual loci and the analysis of their growth patterns over time. This enables quantification of key parameters related to fungal biofilm development, including growth rates, branching patterns, and spatial distribution of hyphae (Heaton *et al.* 2012). Such quantitative analysis facilitates the comparison of different experimental conditions and provides a basis for identifying factors that influence fungal growth and biofilm formation. However, the assessment of accuracy between the original image and encoded graph remains a challenge. Presented here are the initial results from the integration of microscopical observations and mathematical modelling. Fig 4. illustrates the coded graph overlayed on top of the original image for SNA with chitosan. The software was able to detect a total of 1068 nodes, and 1504 edges with an average degree of 2.8165. However, visual estimation alone pointed to some inaccuracies in detecting the tips of the hype. Further exploration of such quantitative analyses has the potential to contribute to a deeper understanding of variables that impact fungal growth and biofilm formation under different experimental settings.

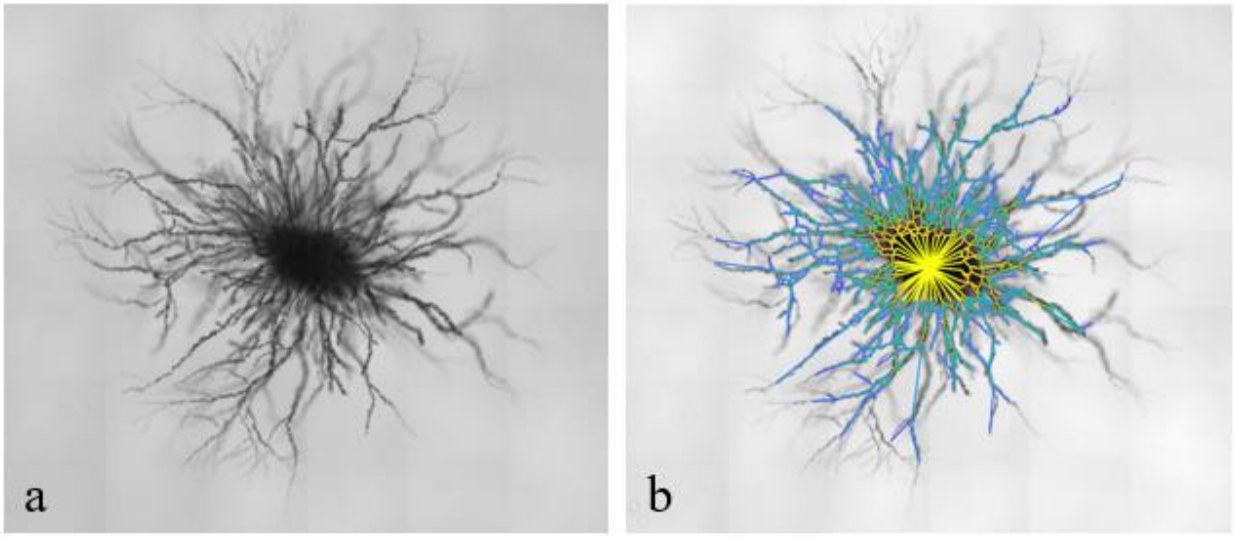


Figure 4: Source image of SNA with chitosan used for encoding the graph (a), and the resulting image overlayed with the encoded graph (b)

## 4. CONCLUSIONS

Certain lignin derivatives, particularly phenolic acids, can be added to protective coatings. *A. pullulans* exhibits growth in the presence of phenol, 4-hydroxybenzoic acid, and p-coumaric acid in both nutrient-rich and nutrient-poor environments, but its growth is evidently stunted in the case of the latter. The addition of chitosan can be beneficial in suppressing other microbes, due to its antimicrobial properties allowing colonisation of *A. pullulans* on the surface of building materials. The modelling of fungal growth might provide insights regarding infestation behaviour on solid surfaces that are advantageous for their initial colonisation. Moreover, modelling can aid in predicting timelines relevant to the proper estimation of coverage on materials surfaces.

## 5. ACKNOWLEDGEMENT

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