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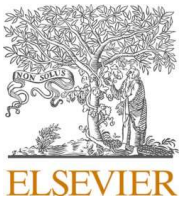

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Napyradiomycin-based coatings: Promising eco-friendly, sustainable and circular solutions for effective marine antibiofouling

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ABSTRACT

This study introduces a groundbreaking approach to marine antifouling by incorporating napyradiomycins extracts, meroterpenoids derived from marine actinomycetes, into biocide-free coatings, establishing the first comprehensive assessment of their dual antimicro- and antimacrofouling potential. By leveraging naturally derived bioactives, this work pioneers a sustainable alternative to copper- and ivermectin-based formulations, aligning with circular bioeconomy and green chemistry principles. At low concentrations (31.25 μg/mL), napyradiomycin-based coatings demonstrated effective macrofouling prevention with no detectable toxicity, while higher concentrations (10 mg/mL) rivaled conventional coatings in efficacy and induced lethality. Uniquely, ecotoxicological evaluations using oxidative stress biomarkers, supported by mussels' survival assays, revealed no significant adverse effects at the lower concentration, showcasing an environmentally friendly profile rarely achieved in antifouling solutions. Despite the promising results, limitations under current static laboratory conditions include no antibiofilm activity and the need for long-term field validation under dynamic marine environments. These challenges, however, offer valuable opportunities for future research in formulation refinement, release profile optimization, and biocide loading thresholds. By combining antimacrofouling efficacy with low ecological impact and scalability potential, these coatings represent a promising advance in marine coating technology, warranting clear path forward for enhancing sustainability in marine industry practices while protecting marine biodiversity.

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1. Introduction

Marine biofouling, usually defined as the accumulation of micro− and macro− organisms on submerged surfaces, represents a significant challenge to marine industries, impacting vessels, offshore infrastructures, and aquaculture systems worldwide (Magin et al., 2010). This complex process begins with the formation of biofilm by bacteria and diatoms, which facilitate the adhesion and settlement of larger organisms such as barnacles, mussels, and algae, eventually creating intricate biological communities (Abarzua and Jakubowski, 1995, Moumbock et al., 2021). The resulting biofouling causes a suite of operational and environmental issues, including increased surface roughness, fuel consumption, CO_2 and SO_2 emissions, and the potential spread of invasive species. These impacts not only threaten marine biodiversity but also impose considerable economic costs on the shipping industry due to heightened fuel demands and maintenance needs (Schultz, 2007, Schultz et al., 2011, Schultz et al., 2015, Yamaguchi et al., 2009).

Historically, antibiofouling coatings have been the primary strategy for combating biofouling. In 2023, the global antifouling coatings market was valued at USD 9.48 billion and is projected to grow steadily at a CAGR of 8.2 % through 2030, driven by demand from the shipping and offshore industries (https://www.grandviewresearch.com/industry-analysis/antifouling-coating-market; accessed April 14, 2025). Earlier solutions relied on toxic organotins, such as tributyltin (TBT) and tributyltin oxide (TBTO), which were banned due to their environmental persistence and harmful effects on non-target organisms (Almeida et al., 2007, Callow and Callow, 2011, Sonak et al., 2009). Most antifouling coatings, particularly those containing metallic, organo-metallic, or organic biocides, are known to pose ecological risks despite regulatory efforts (de Campos et al., 2022, Martins et al., 2018). Approved biocides still vary in environmental impact, and their continuous input into marine ecosystems represents a significant concern (Martins et al., 2018). The coating industry's resistance to adopting biocide-free alternatives is largely due to technical and economic challenges, including the lack of equally effective long-lasting non-biocidal options and cost-effectiveness (de Campos et al., 2022).

Despite the growing demand towards sustainable alternatives, copper-based antifouling coatings (such as metallic Cu, Cu_2O, $CuSO_4$, CuSCN) continue to dominate the market, accounting for 45.8 % of global revenue in 2023 (https://www.grandviewresearch.com/industry-analysis/antifouling-coating-market, accessed April 14, 2025). Their widespread use is due to their proven effectiveness in reducing biofouling, drag, and fuel consumption, as well as providing long-lasting protection and cost efficiency. While these copper coatings remain the industry benchmark for antifouling applications, they present significant water quality concerns, especially in marinas and other areas with restricted water circulation (Lagerström et al., 2020, Schröder et al., 2022). A suitable, cost-effective alternative for their replacement has yet to be found, driving research for sustainable environmentally friendly antibiofouling agents, worldwide.

Among the most promising antifouling innovative technologies are silicone-based foul-release coatings, which create smooth, non-stick surfaces (Selim, Azzam et al., 2024, Selim et al., 2025, Selim, Fatthallah et al., 2024); biomimetic coatings that imitate natural defenses like shark skin (Chen et al., 2021, Tian et al., 2021); and nanostructured superhydrophobic coatings that repel water and organisms (Chen et al., 2024, Shi et al., 2024, Tan et al., 2024). Other promising options involve natural biodegradable and non-toxic bio-based compounds from sea life, such as seaweed, sponges, or marine bacteria that mimic the chemical defenses of marine organisms to deter fouling (Culioli et al., 2008) and slippery hydrogel-based coatings that prevent organisms' adhesion (Bös et al., 2024). While many are still in development, hybrid approaches combining multiple technologies, such as silicone bases with biomimetic patterns or nanostructures, are gaining traction. Regulatory pressures, such as international bans on harmful biocides and tightening environmental standards, are accelerating the adoption of these innovations, but a stricter regulatory framework could drive the industry to more effectively develop and adopt sustainable and eco-friendly alternatives (Schröder et al., 2022). As green shipping initiatives gain momentum globally, the demand for sustainable antifouling solutions is growing stronger.

Our research focuses on exploring natural products from actinomycetes as environmentally friendly alternatives to traditional copper-based antifouling coatings. Actinomycetes metabolites, particularly those produced by *Streptomyces* species, have been reported to possess antifouling (antimacrofouling) and antibiofilm (antimicrofouling) activities (Morgan et al., 2023). For instance, the antibiofouling diterpene lobocompactol, produced by *Streptomyces cinnabarinus*, demonstrated activity against the macroalgae *Ulva pertusa*, the diatom *Navicula annexa*, and the bacterium *Pseudomonas aeruginosa*, making it a promising non-toxic antibiofouling candidate (Bavya et al., 2011, Cho and Kim, 2012). Similarly, 2,5-diketopiperazines, compounds produced by *Streptomyces* sp. were found to inhibit the barnacle *Balanus amphitrite* (Cho et al., 2012, Li et al., 2006, Xu et al., 2010). Quercetin, a flavonoid derived from *S. fradiae*, exhibited activity against the cyanobacteria *Anabaena* sp. and *Nostoc* sp., as well as the larvae of the mussel *Perna indica* (Gopikrishnan et al., 2016). Taxifolin isolated from mangrove-derived *S. sampsonii* PM33 inhibits biofouling bacteria, algal spore germination and mollusc foot adherence (Gopikrishnan et al., 2019). Another interesting compound is ivermectin, a modified chemical form of avermectin, a macrocyclic lactone derived from the actinomycete species *S. avermitilis*, which is known for inhibiting macrofouling (Pinori et al., 2011). Additionally, albofungin A isolated from *S. chrestomyceticus* BCC 24770, exhibit antimicrofouling and quorum-sensing inhibition (QSI) activities against fouling bacteria and antimacrofouling activity, preventing larval colonization of *Amphibalanus amphitrite* and *Bugula neritina* (She et al., 2022). Research conducted by our group has identified several meroterpenoids, namely napyradiomycins, madeirone and neomarinone, isolated from *S. aculeolatus* as potent antimacroufouling and antimicrofouling agents (Gaudêncio et al., 2018, Bauermeister et al., 2019, Pereira et al., 2020, Wissner et al., 2024).

Building on previous work in natural product-based antifouling strategies, this study presents the first investigation of napyradiomycins-based coatings, obtained from the marine-derived *Streptomyces aculeolatus* PTM-420, as active for marine biofouling. By incorporating napyradiomycins into biocide-free marine coating formulations, we evaluated their efficacy against both micro- and macrofouling organisms under controlled laboratory conditions, demonstrating their antifouling potential and low ecological impact. The study also aligns with the principles of the circular bioeconomy, proposing napyradiomycin-based coatings as a sustainable and

innovative alternative to traditional biocidal systems.

2. Results and discussion

2.1. Antimicrofouling evaluation

This study evaluated and compared the antibiofouling activity of various coatings, under static laboratory conditions, including commercially available Hempel® Antifouling Olympic (with high copper content, 36.2 %), AO-Cu_2O-362, Hempel® Antifouling Classic (with lower copper content, 9.7 %), AC-Cu_2O-97, a biocide-free coating produced by Hempel®, BF, identical in composition to AC-Cu_2O-97 but without copper, the same biocide-free coating incorporating ivermectin at a concentration of 10 mg/mL, BF-Iv-10, and napyradiomycin-based coatings, with the PTM-420 crude extract incorporated into the biocide-free coating at two concentrations: 31.25 μg/mL and 10 mg/mL, BF-PTM420–0.03125 and BF-PTM420–10, respectively (see Table 1). The micro- and macro-antifouling PTM-420 crude extract obtained from *S. aculeolatus* PTM-420 isolated from ocean sediments collected off the Madeira Archipelago, was previously characterized by our group, comprising majorly napyradiomycins in its composition (Bauermaister et al., 2019, Prieto-Davo et al., 2016). Twelve napyradiomycin derivatives from PTM-420 were isolated and characterized, as reported in Pereira et al. (2020), also revealing both micro- and macro- antifouling properties. In detail, Pereira et al. (2020) reported that napyradiomycins at 31.25 μg/mL from strain PTM-420 inhibited biofilm formation in various marine bacterial species, with rates between 25 %–60 % for *Marinobacter hydrocarbonoclasticus*, and up to 87–100 % against *Micrococcus luteus*. Bauermeister et al. (2019) also reported strong antibiofilm activity, with napyradiomycin extracts, including PTM-420, inhibiting *Staphylococcus aureus* biofilm formation by over 80 % (Bauermeister, et al., 2019). It is important to emphasize that both the antifouling PTM-420 extract and the pure napyradiomycins demonstrated activity without affecting the viability of the targeted species (Bauermeister et al., 2019, Pereira et al., 2020). In this study, we used the crude extract PTM-420 for coating incorporation instead of the pure compounds, following Hempel's recommendations, as it is more cost-effective and eliminates the need for purification steps. Unlike drug discovery, where the use of pure compounds is mandatory, the paint and coatings industry can utilize extracts, as environmental tests are needed rather than clinical trials.

The aim of our study was to evaluate whether napyradiomycins, when incorporated into coating formulations, could retain their antibiofilm activity. Developing environmentally friendly coatings with both antimicrofouling and antimacrofouling properties is particularly advantageous, as biofilms play a critical role in stabilizing and facilitating the development of more complex fouling communities (Almeida et al., 2007).

In our study, we used a 10-day exposure period to capture potential antibiofilm effects before the biofilm matures into a more complex and resilient structure. Several studies on biofilm formation under seawater conditions reported that marine biofilms typically exhibit exponential growth between approximately 6 and 18 days, depending on environmental factors. The selected 10-day period falls within this exponential-like accumulation phase, when bacterial biofilm growth is at its peak (Fischer et al., 2014, Grzegorczyk et al., 2018).

Under static laboratory conditions, all the biocides-based coatings (Table 1) were not effective in preventing the formation of complex biofilms involving multiple organisms (Fig. 1). Nevertheless, it is important to interpret these findings in the context of the tested conditions.

Interestingly, the copper-based coatings demonstrated the highest inhibition rates, with AC-Cu_2O-97 achieving 18 % and AO-Cu_2O-362 reaching 55 %, despite being commercialized primarily for macrofouling control and not specifically designed to target microfouling. Nevertheless, these values fall short of the $\geq$ 80 % threshold typically considered effective for industrial antibiofilm application (Bauermeister et al., 2019, Kwasny and Opperman, 2010, Sabotic et al., 2024). Although the coating that exhibited the highest antimicrobial activity (AO-Cu_2O-362) also contained the highest copper content, which is ca. 3.7 times greater than that of AC-Cu_2O-97, a direct comparison between the two is not straightforward. This is due to the distinct physicochemical properties of the matrices and their respective biocide release mechanisms: AC-Cu_2O-97 is a soluble matrix coating, whereas AO-Cu_2O-362 is a self-smoothing and self-polishing coating. In AC-Cu_2O-97, Cu_2O is released through diffusion, while in AO-Cu_2O-362 it is released via

Table 1
Coatings formulation. Nautical coatings and concentrations of the incorporated extracts.

Acronym	Nautical Coating	Compound/Extract incorporated	Concentration
AO-Cu_2O−362	Antifouling Olympic 86951 Hempel®	Cu_2O	36.2 % (m/v) or 362 mg/mL
AC-Cu_2O−97	Antifouling Classic 76110 Hempel®	Cu_2O	9.7 % (m/v) or 97 mg/mL
BF	Biocide-free (Antifouling Classic 76110 Hempel®, formulated without Cu_2O)	-	0 %
BF-Iv−10		Ivermectin	10 mg/mL
BF-PTM420–10		(Napyradiomycins) PTM−420 extract	10 mg/mL
BF-PTM420–0.03125		(Napyradiomycins) PTM−420 extract	31.25 μg/mL

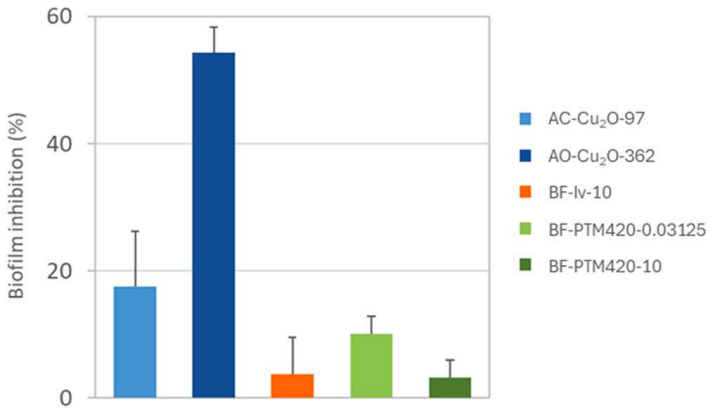

Fig. 1. Inhibition of biofilm formation. This figure displays the results of biofilm formation inhibition for all tested coatings: copper-based commercial coatings, AC-Cu_2O-97 and AO-Cu_2O-362, ivermectin-based BF-Iv-10 and napyradiomycin-based coatings, BF-PTM420–0.03125 and BF-PTM420–10, during a 10-day exposure bioassay. Mean values (n = 3) and ± standard deviations associated with each coating formulation are shown.

ion exchange. This latter process generates a hydrolysable activated layer that is gradually polished by the continuous movement of seawater, typically enhancing antifouling performance (Almeida et al., 2007). Therefore, a direct comparison between the two coatings is not possible.

The effectiveness of biofilm prevention under static laboratory conditions is often underestimated relative to dynamic marine environments, where loosely attached organisms are more readily dislodged by hydrodynamic forces (Krsmanovic et al., 2021, Yebra et al., 2004). Moreover, antifouling performance is influenced by several other factors, including the biocidal properties of the compounds, the chemical composition of the materials and additives used in the formulation, and the physical characteristics of the coating, such as porosity, thickness, among others (Chambers et al., 2006, Valkirs et al., 2003, Yebra, Kiil and Dam-Johansen, 2004). These factors collectively determine the efficiency of biocide release from the coating. As a result, antimicrofouling evaluations are most meaningful when performed on coatings with similar compositions and under consistent experimental conditions.

Coatings BF-Iv-10, BF-PTM420–0.03125, and BF-PTM420–10 were produced incorporating the antibiofouling agents (either ivermectin or PTM-420) into the same base coating, BF (similar to AC-Cu_2O-97 but without Cu_2O) and following the same protocol. Therefore, their biofilm inhibition ratios are comparable. Pinori et al. (2011, 2013) reported the incorporation of ivermectin into coatings at a concentration of 10 mg/mL, for comparison purposes we used this concentration for both ivermectin and napyradiomycins (Pinori et al., 2011, 2013). The ivermectin-based coating, BF-Iv-10, showed a biofilm inhibition of 2 %, highlighting its limitation in addressing microbial biofilms under these experimental conditions, despite reported macrofouling activity at the used concentration (Pereira et al., 2020, Pinori et al., 2011). Among the two napyradiomycin-based coatings tested, the coating BF-PTM420–0.03125 showed a biofilm inhibition of 10 %, while BF-PTM420–10 coating achieved 3 % inhibition (Fig. 1). Interestingly, the lower concentration (BF-PTM420–0.03125) resulted in higher biofilm inhibition. This inverse relationship suggests that higher loading does not necessarily translate into increased antibiofilm efficacy when the compound is embedded in a coating matrix. Possible explanation includes saturation effects, compound aggregation, or reduced diffusion efficiency from the coating, ultimately affecting surface availability of the active component (Callow and Callow, 2011, Yebra, Kiil and Dam-Johansen, 2004). Our results emphasize the need for antibiofilm coating optimization in terms of release profiles. It is worth noting that the microfouling inhibition achieved with BF-PTM420–0.03125 was 9 % higher than that obtained with BF-Iv-10 and only 1.8 % lower than that achieved with AC-Cu_2O-97, with all coatings sharing the same matrix composition and biocide release mechanism.

Additionally, it is important to note that the complexity of the microbial community used in this study, compared to simplified lab models is a factor to consider (Pereira et al. 2020, Bauermeister et al., 2019). Whereas previous studies reporting high antibiofilm efficacy used PTM-420 extracts, pure napyradiomycins and used monospecific bacterial cultures under optimized conditions, our study evaluated coatings with embedded biocides in a biocide-free base matrix, exposed to natural seawater, involving complex polymicrobial communities that are subject to seasonal variation, highly diverse and potentially composed of species with varying susceptibilities to napyradiomycins. These differences in microbial communities could also lead to different biofilm inhibition outcomes, as different bacterial species, even within the same genus, can exhibit variable biofilm-forming behaviours, seasonal variations, and resistance profiles (Valkirs et al., 2003). Given these considerations, direct comparisons between our results and previous napyradiomycin studies are not feasible. Nonetheless, by maintaining consistent coating formulations and testing conditions across all coatings, the relative comparison between coatings within this study remains valid.

To fully evaluate the potential of these coatings, future investigations should evaluate seasonal and dynamic environmental impacts, focusing on realistic maritime conditions, as well as matrix/formulation optimization.

2.2. Antimacrofouling evaluation

2.2.1. Evaluation of Mytilus galloprovincialis and Patella vulgata adhesion rate

The mussels *Mytilus galloprovincialis* and limpets *Patella vulgata* were exposed to the coatings described in Table 1, for 10 days under

static laboratory conditions, to assess their antimacrofouling activity, which relates to the organisms' ability to adhere to the coated surfaces or whether these coatings caused the animals to move away. Previous studies have demonstrated that using larvae, settled juveniles or adult mussels are suitable models for antimacrofouling testing (Kojima et al., 2016). Therefore, adult specimens were selected to evaluate settlement inhibition, simulating realistic macrofouling scenarios, where the settlement and persistence of adult organisms represents the final stage of successful colonization. Unlike *M. galloprovincialis*, limpets *P. vulgata* are not commonly considered suitable models for laboratory testing and are particularly challenging to handle (Curpan et al., 2022). For this reason, these organisms were not included in the subsequent analysis of survival and biochemical parameters, with the focus placed solely on their adhesive strength.

The aim of the exposure assay was to evaluate whether the nautical coatings incorporating napyradiomycins extract as additives exhibited the potent antimacrofouling activity of the reported pure napyradiomycins and PTM-420 extract while also ensuring environmentally safety (Bauermeister et al., 2019, Pereira et al., 2020). The results of the 10-day macrofouling assays, highlighting days 2 and 10 for description and comparison with the reference coatings are presented in Fig. 2. The results for days 4, 6 and 8, obtained with the non-commercial coatings, are presented in Figure S1, Supplementary Materials. Additionally, to ensure a clear presentation and facilitate comparison with the survival assays in Section 2.2.2, the results are illustrated in Fig. 3, presenting mussel adhesion rates over a 10-day period across the six different coatings (Table 1).

In the case of the BF coating, which serves as a reference, mussel adhesion remains consistently high, ranging from approximately 88 % to 100 % throughout the experiment. This indicates that coating alone does not inhibit settlement. In contrast, the two commercial copper-based coatings, AC-Cu_2O-97 and AO-Cu_2O-362, maintain a 0 % adhesion rate over the entire 10-day period, demonstrating the strongest antifouling effect among the tested treatments (lines overlapped in Fig. 3). This complete inhibition suggests either a sustained release of active biocidal agents or a strong deterrent effect prompting lethal effect. This result suggests that ivermectin may exert strong anti-settlement or potentially toxic effects over time. For BF-PTM420–10, a sharp and steady reduction in adhesion was also observed, decreasing from 78 % on day 1 to 0 % by day 4, thus presenting a similar level of performance to the reference copper-based coatings (AC-Cu_2O-97 and AO-Cu_2O-362) and the ivermectin-based formulation (BF-Iv-10), demonstrating effective antimacrofouling with no organism attachment, although possibly associated with lethal effects.

In contrast, the adhesion rates for BF-PTM420–0.03125 in this static laboratory experience fluctuated between 33 % and 55 % without showing a consistent downward trend. Furthermore, BF-PTM420–0.03125 allowed mussels and limpets to move freely across the panels without settlement (Fig. 2 and S1), thereby effectively preventing macrofouling. This outcome is highly promising, suggesting that BF-PTM420–0.03125 may represent a sustainable and environmentally friendly alternative to traditional copper-based

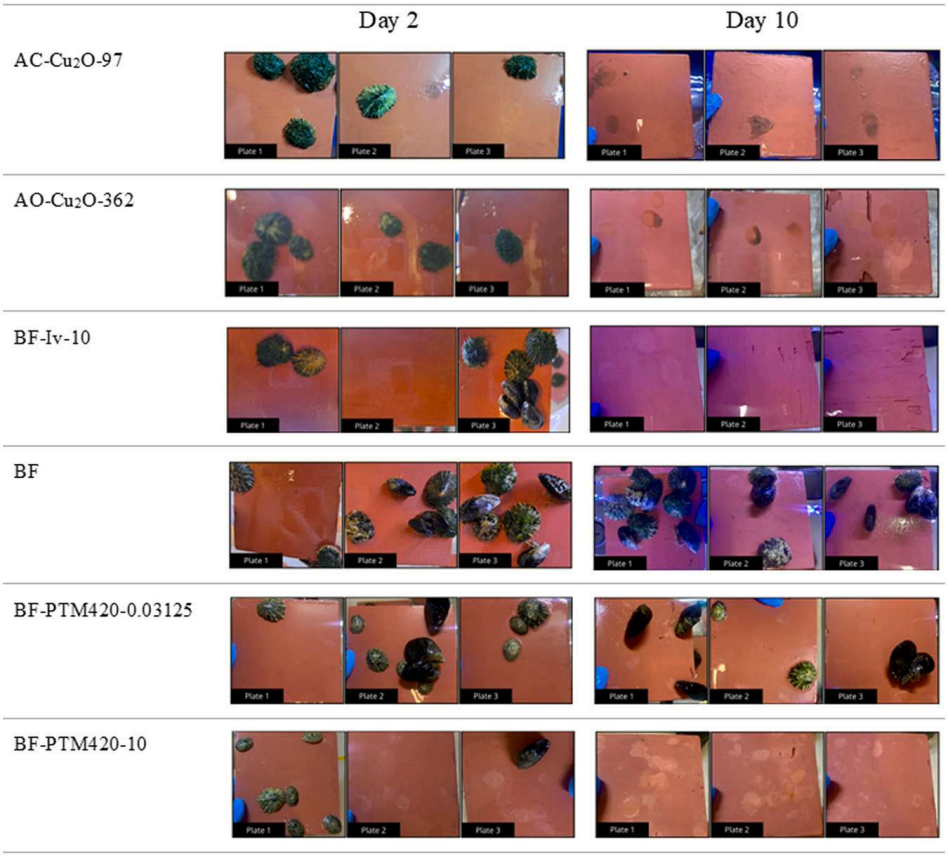

Fig. 2. Antimacrofouling assays with commercial nautical coatings (AC-Cu_2O-97 and AO-Cu_2O-362) and with the biocide free coating produced by Hempel® - either alone (BF), with ivermectin (BF-Iv-10,000) or napyradiomycin (BF-PTM420–0.03125 and BF-PTM420–10). The three test panels protected with each coating are presented on days 2 and 10.

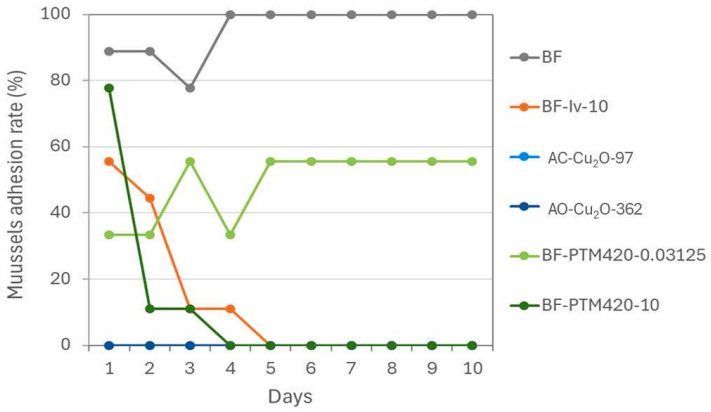

Fig. 3. Analysis of the adherence rate of mussels *M. galloprovincialis and P. vulgata* during the 10-day antimacrofouling assay with the copper-based commercial coatings (AC-Cu_2O-97 and AO-Cu_2O-362), biocide-free coating (BF), ivermectin-based (BF-Iv-10) and napyradiomycin -based coatings (BF-PTM420–0.03125 and BF-PTM420–10). A total of nine mussels were present in each assay.

antifouling coatings.

2.2.2. *Evaluation of Mytilus galloprovincialis survival rate*

The survival of *M. galloprovincialis* was monitored during a 10-days macrofouling assay, focusing on the toxicity of the various antifouling coatings, used as described in Table 1. For the evaluation of the survival rate, the same number (9) of *mussels* were placed in each tank.

Mussels' survival rate over a 10-day period for the various coatings is presented in Fig. 4. The findings provide a nuanced view of different antifouling coatings, emphasizing the balance between antimacrofouling effectiveness and environmental impact.

The biocide-free coating, BF, serves as a negative reference and maintains a consistent 100 % survival rate throughout the experiment, indicating no toxicity while confirming its environmentally benign nature (line overlapped with BF-PTM420–0.03125, in Fig. 4). BF coating exhibited no antimacrofouling properties (Fig. 3), showing the highest levels of macrofouling among all tested coatings. These findings underscore the challenges in achieving effective antifouling performance without the use of biocidal agents.

Copper-based coatings, on the other hand, were highly effective in preventing macrofouling, with no organism adhesion. However, they were associated with complete mortality of test organisms by day 6 (Fig. 4), underscoring the ecological risks posed by copper leaching into marine environments (Cima and Varello, 2023, Lagerström et al., 2020, Schröder et al., 2022). Coating AC-Cu_2O-97 survival rate begins at about 78 % but drops sharply, reaching 0 % by day 5. This demonstrates a very effective antifouling action, causing rapid mussel mortality and detachment. Similarly, AO-Cu_2O-362 starts at about 56 % and drops to 0 % by day 6. Although it begins with lower survival than AC-Cu_2O-97, the decline is consistent and complete, showing that it is also highly toxic. This is supported by studies that confirm copper's toxicity, causing significant harm to various marine species, including non-fouling organisms, and disrupting key biological processes in mussels, such as byssus production (Kojima et al., 2016, Ytreberg et al., 2010, Ytreberg et al., 2011). Although effective, copper-based biocides pose long-term environmental risks, indicating that while they provide strong antifouling action, their ecological drawbacks are substantial (de Campos et al., 2022).

Ivermectin-based coating, BF-Iv-10, leads to a gradual decrease in survival. Starting at 100 %, the rate drops steadily to approximately 10 % by day 9, where it stabilizes. This suggests that mussel detachment is due to mortality increase over time.While effective, the high mortality rate of ivermectin raises concerns similar to the copper-based coatings, emphasizing the need for less harmful

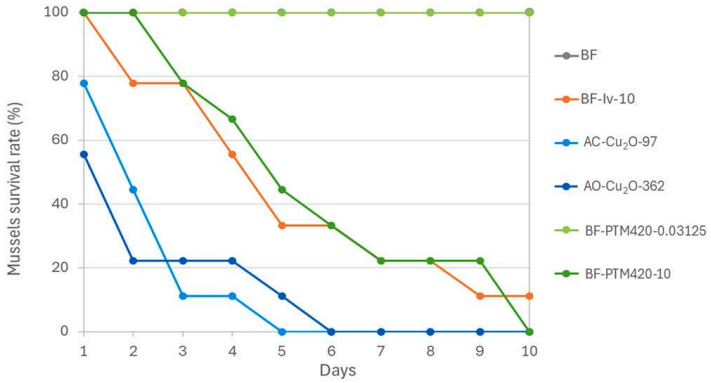

Fig. 4. Analysis of the survival rate of mussels *M. galloprovincialis* during the 10-day antimacrofouling assay with the copper-based commercial coatings (AC-Cu_2O-97 and AO-Cu_2O-362), biocide-free coating (BF), ivermectin-based (BF-Iv-10) and napyradiomycin -based coatings (BF-PTM420–0.03125 and BF-PTM420–10). A total of nine mussels were present in each assay.

alternatives in sensitive marine settings. This is corroborated with previous studies, reporting ivermectin's enviromental concerns to marine ecossystems (Davies et al., 1997).

Napyradiomycin-based coatings emerged as a potential alternative with varying antifouling effects and toxicity based on concentration. BF-PTM420–10 begins at 100 %, but shows a more defined decline, reaching about 22 % by day 7 and then dropping to 0 % by day 10. This coating presents a potent antifouling effect (Fig. 3), but with complete inhibition of mussel survival being as lethal as the commercial coatings, and suggesting that while napyradiomycin is effective, 10 mg/mL concentration may be too high for broad ecological applications. Interestingly, BF-PTM420–0.03125 maintains 100 % survival throughout the experiment. At 31.25 μg/mL, despite 56 % organisms remaining on the panels, no adhesion was detected during the 10-day laboratory static antimacrofouling test (Fig. 2 and S1), suggesting an avoidance behavior with no mortality, achieving 100 % survival rate for mussels. This concentration suggests a more balanced antimacrofouling efficacy with minimized ecological impact, though it remains less effective than the other biocide-based coatings under static tests. In comparison to commercial biocides, BF-PTM420–0.03125 offers a promising path for further development, potentially yielding a safer antimacrofouling agent with reduced toxicity. As napyradiomycins are relatively new antifouling agents, the promising results observed on (Pereira et al., 2020), which demonstrated antifouling efficacy without mortality at lower concentrations, emphasize the importance of future research under dynamic conditions to identify optimal, intermediate concentrations.

2.2.3. *Coatings adherence rate vs survival rate for environmental friendlyness assessment*

To compare the coatings performance under laboratory static conditions in terms of adhesion, mussel survival, and environmental friendliness, we analyzed Figs. 3 and 4 together and summarized the results in Table 2.

The BF coating maintains a high adhesion rate of around 100 % over 10 days, indicating poor antifouling performance. In contrast, BF-Iv-10 shows a sharp decline in adhesion, reaching 0 % by day 5, suggesting strong effectiveness in preventing mussel attachment. The copper-based coatings, AC-Cu_2O-97 and AO-Cu_2O-362, both maintain 0 % adhesion throughout the period, demonstrating excellent antifouling properties. Similarly, BF-PTM420–10 rapidly drops to 0 % adhesion by day 3. BF-PTM420–0.03125 also shows good antifouling performance, with adhesion stabilizing at around 55 % after an initial decline. Notably, marine organisms on this coating remain mobile rather than settled.

In terms of toxicity, the BF coating allows 100 % mussel survival, indicating it is non-toxic. BF-Iv-10 and BF-PTM420–10 both show a sharp decrease in survival, falling to around 10 % by day 10, suggesting significant toxicity. The copper-based coatings cause the most rapid mortality, with survival dropping to 0 % by days 5 and 6, respectively, highlighting their high toxicity. In contrast, BF-PTM420–0.03125 maintains 100 % survival throughout the test period, indicating it is non-toxic.

Considering both antifouling performance and toxicity, BF-PTM420–0.03125 emerges as the most environmentally friendly option. While it may not achieve the strongest antifouling effect visually, it offers a good reduction in adhesion without compromising mussel survival. Compared to commercial copper-based coatings and the ivermectin-based coating, it represents a better balance between efficacy and environmental safety.

Further studies, including marine field immersion panel tests under dynamic conditions and additional formulation research, are recommended to confirm and expand upon these findings.

The use of biocides produced by fermented microorganisms, such as actinomycetes, offer numerous advantages for sustainable paints and coatings production, as they are less harmful to humans and the environment. Their production methods are more sustainable and circular compared to the environmentally damaging practices associated with copper mining, which is resource-intensive and contributes to environmental degradation, or the use of synthetic petrochemical-based biocides, which require more energy and involve toxic chemicals (Díez et al., 2025). Furthermore, microbial fermentation is more cost-effective, enabling large-scale production with lower operational costs (Díez et al., 2025). Several microorganisms, as the case of *S. aculeolatus* that produced PTM-420 extract, grow in seawater, reducing the demand for freshwater, and the process further supports a circular economy by repurposing by-products. The integration of microbial-based paints and coatings in marine applications can significantly reduce ecological harm, as shown by the promising napyradiomycins PTM-420 extract assays, offering a safer, more sustainable alternative to traditional copper-based methods.

Table 2
Coatings comparison in terms of adherence rate vs survival rate.

Coating	Antifouling (Adhesion)	Toxicity (Survival)	Observation
BF	✗ Poor	✓ Safe	Not effective, but safe
BF-Iv-10	✓✓ Excellent	✗ Toxic	Effective, not friendly
AC-Cu_2O-97 / AO-Cu_2O-362	✓✓ Excellent	✗✗ Very Toxic	Not environmentally friendly
BF-PTM420-0.03125	✓ Good	✓ Safe	**Most environmentally balanced**
BF-PTM420-10	✓✓ Excellent	✗ Toxic	Effective, not friendly

2.3. Ecotoxicity evaluation of napyradiomycin-based coatings: oxidative stress biomarkers

The ecotoxicity evaluation of napyradiomycin-based coatings reveals valuable insights into their safety and effects on marine organisms, specifically mussels, by assessing biomarkers associated with oxidative stress. Oxidative stress biomarkers, including glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase CAT, total antioxidant capacity (TAC) and lipid peroxidation (LPO), were used to evaluate the toxicological effects of incorporating PTM-420 bioactive extracts containing napyradiomycins into biocide-free coating, BF-PTM420–0.03125.

For most of the biomarkers analyzed, no significant changes ($p > 0.05$) were observed between the marine organisms at arrival (T0, negative control, corresponding to mussels exposed solely to uncontaminated seawater) and the reference group (mussels exposed to panels with a biocide-free coating, BF). The exceptions were GST in the gills and SOD in both organs, which suggests that some stress occurred during transport and handling upon arrival.

Throughout the 10-day exposure period, stable water quality parameters (pH 8.05 ± 0.09, temperature 23.6 ± 1.3 °C, and salinity 32.4 ± 0.5 g/Kg) ensured that any biomarker changes observed were likely due to the napyradiomycin exposure rather than environmental stress. Furthermore, the mussels (3443.05 ± 1832.57 mg fresh weight; 34.41 ± 5.14 mm length) did not exhibit morphological signs of distress, such as tissue degradation or darkening, suggesting that napyradiomycins did not cause toxicity at the tested concentration. As the exposure bioassay with commercial biocide coatings, AC-Cu_2O-97 and AO-Cu_2O-362, and biocide-free coating ivermectin, BF-Iv-10, led to the mortality of all marine organisms, the analysis of oxidative stress biomarkers in the gills and glands was not possible to conduct. Therefore, the biomarkers' results presented below solely focus on the napyradiomycin-based BF-PTM420–0.03125 coating only, (Fig. 5). In this way, we aimed to determine whether this innovative coating formulation could disrupt enzymatic and metabolic pathways of exposed organisms through oxidative stress induced by ROS production to exposed organisms.

Although the precise molecular mechanism behind the antifouling activity of napyradiomycins remains undisclosed (Pereira et al., 2020), our findings suggest that these compounds induce a significant but manageable oxidative response in marine organisms, particularly mussels. In fact, exposure to the BF-PTM420–0.03125 coating led to an increase in GST activity in both the digestive glands and gills of mussels compared to the reference group (Fig. 5A). This enhanced GST response reflects an improved detoxifying process, as GST is a detoxification enzyme that promotes conjugation reactions, making compounds more polar and therefore facilitating excretion (Hernandez et al., 2018), but it can also aid in neutralizing reactive oxygen species (ROS) (Hayes and McLellan, 1999). Additionally, this increase in GST activity in both the digestive gland and gills suggests an upregulation of detoxification pathways, likely triggered by low levels of ROS generated upon contact with napyradiomycin-coated surfaces. This low-level ROS induction may contribute to the prevention of biofouling formation by disrupting key cellular processes in fouling organisms, without reaching a threshold of toxicity. Indeed, it has been shown that exposure to antifouling compounds can induce oxidative stress in mussels through ROS production, resulting in alterations in antioxidant enzyme activity, see (Parisi et al., 2022) for a review. For example, López-Galindo et al. (2010) reported a significant increase in GST levels and a decrease CAT activity in mussels exposed to antifouling agents (López-Galindo et al., 2010). Similarly, Gabe et al. (2021) observed elevated GST activity and reduced CAT levels in *Perna perna* mussels exposed to DCOIT (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one) (Gabe et al., 2021). Conversely, Gomes et al. (2011) found a significant increase in CAT and SOD activity in mussels exposed to copper-based antifoulants, reflecting a more toxic oxidative burden (Gomes et al., 2011).

In essence, the coating appears to prompt a beneficial adaptive mechanism that boosts the organism's natural defense. Although SOD activity, vital for neutralizing superoxide radicals, was significantly reduced ($p < 0.05$) in both digestive glands and gills (Fig. 5B), this adjustment may represent a strategic rebalancing of antioxidant defenses. Similarly, CAT activity decreased in the gills (Fig. 5C) without significant changes in the digestive glands, suggesting a localized response to oxidative challenges. Notably, the total antioxidant capacity (TAC) increased in the gills (Fig. 5D), while TAC remained stable in the digestive glands ($p > 0.05$). The observed decrease in SOD and CAT activities, along with the increase in TAC, suggests that the antioxidant defense system effectively responded effectively to neutralize ROS, thereby preventing oxidative stress. This response may involve the activation of other antioxidant enzymes, such as glutathione peroxidase (GPx) and thioredoxin, as well as non-enzymatic antioxidants like flavonoids, glutathione, and vitamins A, C, and E. These components could compensate for the reduced SOD and CAT activities, indicating a shift toward alternative antioxidant pathways that help mitigate oxidative stress (Yu et al., 2017). The localized increase in TAC further supports the presence of a compensatory response. Additionally, although lipid peroxidation (LPO), indicated by malondialdehyde (MDA) levels, increased in the digestive glands (Fig. 5F), MDA levels in the gills remained unchanged. This localized effect highlights that the overall impact on cellular membranes is limited, and that the organism is effectively managing the oxidative challenge. Notably, no tissue damage or mortality was observed, and LPO levels remained within acceptable limits, suggesting that the oxidative stress induced by napyradiomycin coatings does not overwhelm the organisms' defense mechanisms. Together, these results support the hypothesis that napyradiomycin-based coating BF-PTM420–0.03125 exerts antifouling effects not through direct toxicity, but by inducing sublethal oxidative stress that interferes with the metabolism or attachment mechanisms of fouling organisms. The observed antioxidative responses, with no correlation among oxidative stress biomarkers, suggest that the effects elicited by BF-PTM420–0.03125 occur independently rather than in a coordinated manner, demonstrating that mussels can adapt to the oxidative stress induced by the coating. While these findings highlight the promising antifouling potential of napyradiomycins-based coatings, they also emphasize the importance of performing long-term assessments to ensure their environmental safety in marine applications. Moreover, future studies should include quantification of biocide leaching into the exposure medium over time to establish more accurate correlations between chemical concentrations and observed biological responses (Valkirs et al., 2003).

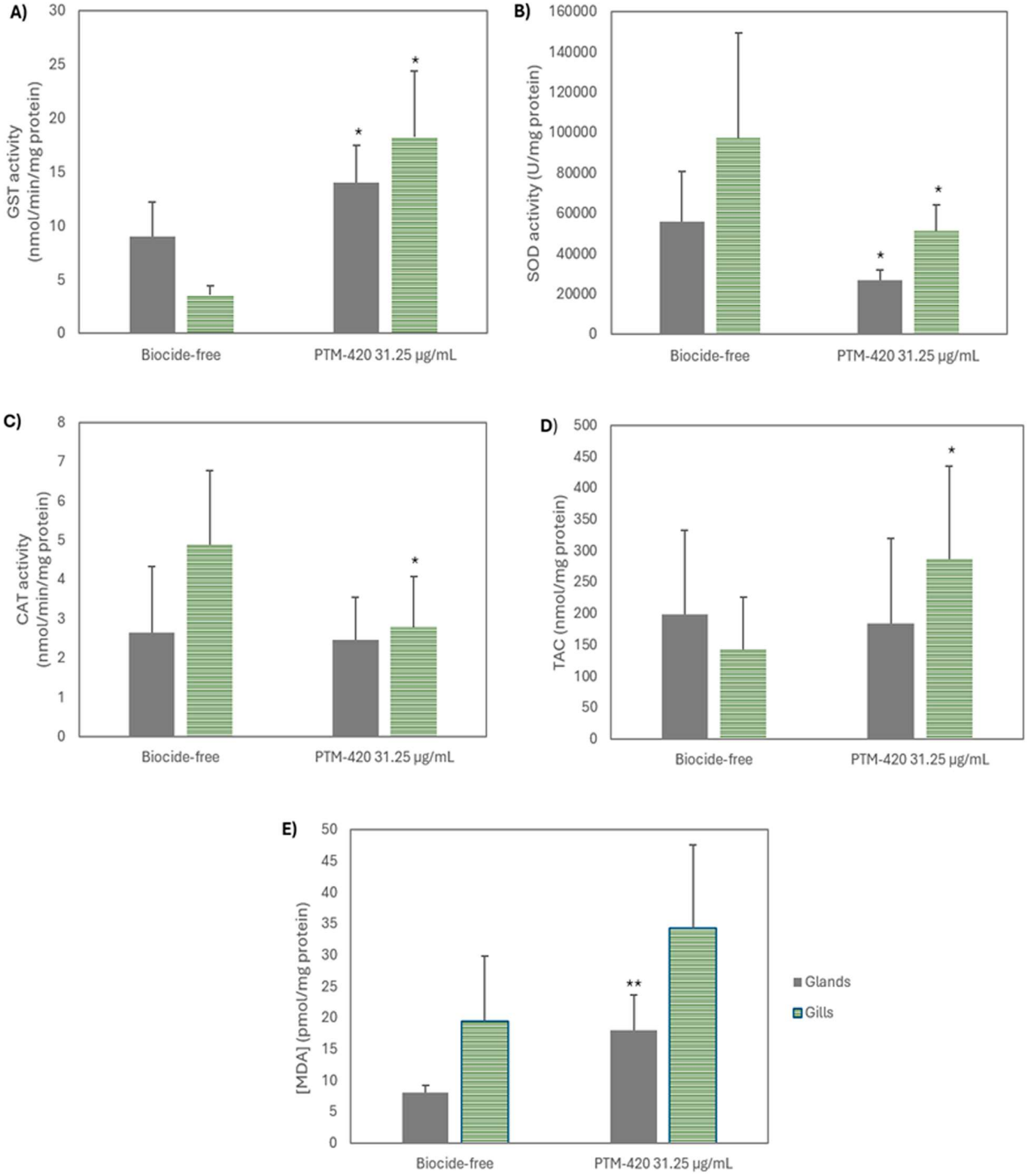

Fig. 5. Biomarkers activities measured in *M. galloprovincialis* digestive glands and gills. Bars represent mean ± standard deviation. Significant differences: * ($p < 0.05$), * * ($p < 0.01$), * ** ($p < 0.001$). A) GST; SOD B); C) CAT; D) TAC; E) LPO (measured by MDA).

3. Materials and methods

3.1. PTM-420 crude extract preparation

Streptomyces aculeolatus PTM-420, isolated from ocean sediments collected off the Madeira Archipelago as described by (Prieto-Davo et al., 2016) was cultured in 20 L of A1 medium (composed of 18 g agar, 10 g starch, 4 g yeast extract, and 2 g peptone) as described in previous studies from our group (Bauermaister at al., 2019, Pereira at al., 2020, Prieto-Davo et al., 2016). After a two-week incubation period, the culture media was extracted using ethyl acetate, and the extract was evaporated to dryness under vacuum conditions.

3.2. Marine antibiofouling coatings

Three distinct coating formulations supplied by Hempel® were used as received: Hempel® Antifouling Classic (AC-Cu_2O-97), Hempel® Antifouling Olympic (AO-Cu_2O-362), and biocide-free Hempel® Classic (BF).

The active ingredient in both AC-Cu_2O-97 and AO-Cu_2O-362 is copper (I) oxide (Cu_2O), which has demonstrated antifouling activity against macrofouling organisms. The main differences between the two coatings lie in their copper content and matrix types. According to the manufacturer, AC-Cu_2O-97 and AO-Cu_2O-362 have 9.7 % and 36.2 % Cu_2O content (m/v), respectively, volume solids of 54 ± 2 % and 52 ± 2 %, and specific gravities of 1.5 kg/L and 1.8 kg/L. While AC-Cu_2O-97 is classified as a soluble matrix antifouling coating ((https://www.hempel.com/en-gb/api/Hempel/DataSheet/PDS/Download/86951/en-GB, accessed April 15, 2025), AO-Cu_2O-362 is a self-smoothing and self-polishing coating (https://www.hempel.com/en-me/products/hempels-antifouling-classic-76110–76110, accessed April 15, 2025). The polishing mechanism of AO-Cu_2O-362 relies on ion exchange, leading to the formation of a hydrolysable activated layer. Both coatings are tin-free and comply with the International Convention on the Control of Harmful Antifouling Systems on Ships, adopted by the IMO in October 2001 (https://www.gc.noaa.gov/documents/afs-convention.pdf, accessed April 15, 2025). BF was developed by Hempel® exclusively for this study. It shares the same composition as AC-Cu_2O-97 (https://www.hempel.com/en-me/products/hempels-antifouling-classic-76110–76110, accessed April 15, 2025), except for the copper content, which is null.

Crude extract PTM-420 from marine-derived actinobacteria, containing napyradiomycins, was dissolved in acetone and incorporated into the BF coating for a final concentration, after drying the solvent, of 31.25 μg/mL and 10 mg/mL. For comparison, BF-Iv-10 was prepared using the same procedure as BF-PTM420–10 but with ivermectin as the active ingredient. For the antimacrofouling assays the coating formulations (Table 1) were applied by brushing onto three, 10 × 10 cm in length, h = 2 mm glass panels (Vidraria Ideal, Laranjeiro, Portugal), as previously described by (Valente et al., 2023). The same mass of coating after drying was used for each panel to ensure consistency. The same procedure was used for the antimicrofouling assays but brushing the three wells of a 6-well plate instead of glass panels.

3.3. Antibiofilm assays

To assess biofilm formation, five 6-well plates, each with three wells coated with all the different coating types listed in Table 1, were submerged in tanks with 8 L of filtered natural seawater from Raso Cape, Portugal, for a 10-day period. Natural seawater was supplied by the Portuguese Navy through the Vasco da Gama Aquarium, following an assessment of several quality criteria. Additionally, at the faculty's facilities, the seawater underwent further filtration using resin filters. On the final day, the plates were retrieved for immediate analysis of biofilm presence. To quantify biofilm accumulation on the submerged plates a previously reported method was used (Bauermeister et al., 2019, Wissner et al., 2024). Each 6-well plate was rinsed three times with distilled water to remove non-adherent bacteria, to ensure that only the firmly attached bacteria to the coating are measured, minimizing interference from unattached cells or those not yet integrated into the biofilm structure. After washing, the plates were left to air-dry at room temperature for 15 minutes, followed by an incubation at 60 °C for 1 hour to fix the biofilm. Each well was then stained with 0.06 % crystal violet by adding 15 mL of dye per well, with a five-minute incubation to fully stain all surfaces, including the side walls. Excess dye was rinsed off with two washes of distilled water, and the plates were left to dry at room temperature. Once dried, 1 mL of 30 % acetic acid was added to each well to thoroughly resuspend the biofilm, repeating this step twice. The resuspended biofilm solution was transferred to a 50 mL tube. For quantification, 20 μL of each biofilm sample was diluted 1:10 with acetic acid in a 96-well microplate, with duplicates prepared for all samples. Optical density was read at 600 nm using a SpectraMax 190 microplate reader (Molecular Devices) and analyzed with SoftMax Pro software (version 6.5.1).

3.4. Harvesting of marine organisms for antimacrofouling assays

All marine organisms utilized in this study were collected in September 2023 from Avencas Beach, a designated marine protected area, Cascais, Portugal. Upon collection, they were depurated and acclimated under controlled laboratory conditions using filtered seawater from Raso Cape, Portugal, prior to the start of the assays. In the laboratory, the adult mussels (*M. galloprovincialis*) and adult limpets (*P. vulgata*) were acclimated for 2–3 days in a 50-liter natural seawater aquarium equipped with filtration, aeration (maintaining dissolved oxygen levels above 6 mg/L), and a 12-hour light-dark cycle.

3.5. Antimacrofouling and mussels' survival assays

Each set of three glass panels, coated with the same material, was placed inside an aquarium containing 8 L of filtered natural seawater, prepared using an 80/20 blend of filtered seawater from Raso Cape, Portugal and synthetic saline water. Each aquarium housed 9 mussels (*M. galloprovincialis*) and 9 limpets (*P. vulgata*). The organisms were exposed for 10 days, during which they were fed every 48 hours with 2 mg of dry *Chlorella* algae (Shine, Portugal), dissolved in synthetic saline water. Key parameters, including pH, temperature, and salinity were monitored daily, and the number of deceased organisms was recorded to determine survival rates. All panels were photographed on days 2, 4, 6, and 10. At the end of the experiment, surviving mussels were promptly collected, weighed, and stored at -45 °C for subsequent biochemical analysis.

3.6. Biochemical assays

3.6.1. Sample treatment

Prior to the beginning of the macrofouling assays, mussels were sampled to analyse oxidative stress biomarkers at the arrival (T0, negative control corresponding to bivalves exposed solely to uncontaminated seawater). Following each assay, surviving marine organisms from each aquarium were collected, and their organs were removed and stored at -45 °C in 1.5 mL microtubes. For mussels, gills and digestive glands were carefully dissected using a scalpel and tweezers, weighed, and preserved. Subsequently, the samples were homogenized using a Tissue Homogenizer (Tissue Master 125, Omni, Kennesaw, GA, USA) in 2 mL of phosphate-buffered saline (PBS) containing 140 mM NaCl, 10 mM Na_2HPO_4, 3 mM KCl, and 2 mM KH_2PO_4, adjusted to a pH of 7.4 ± 0.2. The homogenates were stored at 4 °C before further processing. Samples were then centrifuged at 15,000 × g for 10 minutes at 4 °C using a VWR CT 15RE model centrifuge (Hitachi Koki Co., Ltd., Japan). The resulting supernatant was transferred to labelled microtubes and stored at -42 °C until biomarker analysis. All biochemical assay results were standardized to total cytosolic protein content, which was quantified using the Bradford method (Bradford, 1976).

3.6.2. Total protein content

Sample protein concentrations were measured using the Bradford method (Bradford, 1976). BSA (Bovine albumin, Fraction V, Nzytech, Portugal) served as the standard, with a concentration range from 0 to 4 mg/mL, prepared through serial dilution in PBS to create a calibration curve. Each sample was analyzed in duplicate by adding 20 µL of either the sample or BSA standard and 180 µL of Bradford reagent to the wells of a 96-well microplate (Greiner, Bio-One GmbH, Frickenhausen, Germany). Absorbance was recorded at 595 nm with a microplate reader (Synergy HTX, BioTek, USA). Data from subsequent biomarker assays were normalized based on the protein concentration of each sample (mg/mL).

3.6.3. Glutathione-S-transferase (GST) activity

Glutathione-S-transferase (GST) activity was measured using a modified version of the method by Habig et al. (1974) for 96-well microplates (Habig et al., 1974). This approach leverages GST's ability to conjugate reduced glutathione (GSH) to 1-chloro-2,4-dinitrobenzene (cDNB), which produces a steady increase in absorbance over time. A substrate mixture was prepared containing 200 mM GSH, 100 mM cDNB, and phosphate buffer (PBS), with all reagents obtained from Sigma-Aldrich (USA). In each well of a 96-well microplate (Greiner, Bio-One GmbH, Frickenhausen, Germany), 20 µL of sample was combined with 180 µL of this substrate mixture. Each assay was conducted in duplicate. Absorbance was measured at 340 nm every minute for 6 minutes using a Synergy HTX microplate reader (BioTek, USA) to determine GST activity. The increase in absorbance per minute was recorded, and the reaction rate was calculated using a cDNB extinction coefficient of 0.0053 μM^{-1}. Results were standardized against the cytosolic protein concentration of each sample, expressed as nmol/min/mg of cytosolic protein.

3.6.4. Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was assessed using the nitroblue tetrazolium (NBT) reduction method, modified from Sun et al. (1988). In this method, xanthine reacts with xanthine oxidase (XOD) to generate superoxide radicals ($O2^{\bullet-}$), which subsequently reduce NBT to formazan. Formazan formation is monitored spectrophotometrically at 560 nm. SOD inhibits this reduction by dismutating $O2^{\bullet-}$, competing with NBT. The level of SOD activity is reflected by the degree of inhibition observed. In each well of a 96-well microplate (Greiner, Bio-One GmbH, Frickenhausen, Germany), 10 µL of sample was combined with 230 µL of a buffer mixture containing 50 mM phosphate buffer (pH 8.0), 3 mM EDTA (Riedel-Haen, Germany), 3 mM xanthine (Sigma-Aldrich, Germany), and 0.75 mM NBT (Sigma-Aldrich, Germany). The reaction was initiated by adding 10 µL of XOD (Sigma-Aldrich, Germany). Absorbance at 560 nm was recorded every 2 minutes for a total of 20 minutes using a Synergy HTX microplate reader (BioTek, USA), allowing the calculation of the percent inhibition per minute due to SOD activity (as per the equation below). Results are expressed in SOD units per mg of protein.

3.6.5. Catalase (CAT) activity

Catalase activity was measured using a spectrophotometric method based on (Beers and Sizer, 1952), adapted for 96-well microplates (UVStar, Greiner Bio-One, Germany), following modifications by Cid et al. (2015). This approach tracks the decrease in absorbance at 240 nm as catalase decomposes hydrogen peroxide (H_2O_2). A substrate solution was prepared with 0.036 % (w/w) H_2O_2 in 50 mM potassium phosphate buffer (KH_2PO_4, pH 7.0 at 25 °C) from Sigma-Aldrich. In each well, 7 µL of sample was mixed with 193 µL of the substrate solution. Absorbance was measured at 240 nm every 15 seconds for about 3 minutes using a Synergy HTX

microplate reader (BioTek, USA). The catalase activity was calculated as the rate of absorbance change per minute ((ΔA240)/min), using the molar extinction coefficient of H_2O_2 (0.04 μM^{-1}). After normalization, catalase activity was expressed as nmol/min/mg of cytosolic protein.

3.6.6. Total antioxidant capacity (TAC)

The antioxidant capacity of the samples was evaluated using an adapted Trolox equivalent antioxidant capacity (TEAC) protocol (Kambayashi et al., 2009). This method uses ABTS (2,2′-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid)), a blue-green chromophore whose color intensity decreases in the presence of antioxidants. Myoglobin reacts with hydrogen peroxide to form ferrylmyoglobin, which oxidizes ABTS into a radical cation, detectable by spectrophotometry. Trolox and antioxidants in the samples inhibit this oxidation. A trolox calibration curve (0 to 0.660 nM) was prepared from a 1.5 mM stock solution (Aldrich, Russia). In each well of a 96-well microplate (Greiner, Bio-One GmbH, Frickenhausen, Germany), 10 µL of either sample or trolox standard was combined with 10 µL of myoglobin (90 µM in potassium phosphate buffer; Sigma, USA), 150 µL of ABTS (600 µM in MilliQ water; Alfa Aesar, Germany), and 40 µL of hydrogen peroxide (500 µM in MilliQ water; PanReac AppliChem, Germany). After a 5-minute incubation at room temperature, absorbance was read at 410 nm using a Synergy HTX microplate reader (BioTek, USA). The antioxidant concentration of each sample was calculated from the trolox calibration curve and expressed as µmol/mg protein, based on the sample's cytosolic protein concentration.

3.6.7. Lipid peroxidation (LPO)

The lipid peroxidation assay was performed using the thiobarbituric acid reactive substances (TBARS) method (Uchiyama and Mihara, 1978). This assay relies on the reaction between thiobarbituric acid (TBA) and malondialdehyde (MDA), a byproduct of fatty acid peroxidation. Under acidic conditions, MDA forms a colorimetric product with TBA, with trichloroacetic acid (TCA) added to accelerate the reaction. For each sample or standard, 5 µL of the solution and 45 µL of PBS were placed in 1.5 mL microtubes. Then, 12.5 µL of 8.1 % SDS (Sigma-Aldrich, Germany), 93.5 µL of 20 % TCA (Panreac, Spain), 93.5 µL of 1 % TBA (Sigma-Aldrich, Germany), and 62.5 µL of Milli-Q ultrapure water were added. The tubes were vortexed for one minute, then heated in a dry bath at 100 °C for 10 minutes with punctured lids. After cooling on ice, 62.5 µL of Milli-Q water was added, and the tubes were vortexed again. Then, 150 µL from each tube was transferred to a 96-well microplate (Greiner, Bio-One GmbH, Frickenhausen, Germany) for absorbance measurement at 530 nm using a Synergy HTX microplate reader (BioTek, USA). Each sample was analyzed in duplicate. Lipid peroxidation was assessed by quantifying MDA levels, using a calibration curve (Merck, Germany) with MDA concentrations from 0 to 0.100 µM. Results were normalized to the sample's total protein content, expressed as pmol MDA/mg cytosolic protein.

3.6.8. Statistical analysis

Statistical analyses were conducted using GraphPad Prism 9 software (version 9.3.0). The data are presented as mean ± standard deviation. For statistical comparisons, either one-way ANOVA (for parametric data) or the Kruskal-Wallis's test (for non-parametric data) was employed, followed by Dunnett's multiple comparison test, depending on whether the data met the assumptions of normality and homogeneity of variance were met. Normality was assessed using the Shapiro-Wilk and/or Kolmogorov-Smirnov tests, while heteroscedasticity was evaluated using with the Brown-Forsythe and Bartlett's tests. A significance level of $p < 0.05$ was considered statistically significant.

4. Conclusion

This study presents a novel, sustainable solution for marine biofouling mitigation by incorporating napyradiomycin-rich crude extracts from *Streptomyces aculeolatus,* through seawater fermentation, into biocide-free coatings. Under laboratory static conditions, the formulation BF-PTM420–0.03125 (31.25 µg/mL of PTM-420) demonstrated a balanced performance, reducing mussel adhesion to approximately 56 % (without settlement) while maintaining a 100 % survival rate over a 10-day exposure period. This contrasts with the higher concentration formulation, BF-PTM420–10 (10 mg/mL), which achieved 0 % adhesion by day 4, but resulted in 100 % mortality by day 10, similar to conventional copper-based coatings AC-Cu_2O-97 and AO-Cu_2O-362. Importantly, oxidative stress biomarker analysis for BF-PTM420–0.03125 revealed a significant increase in glutathione-S-transferase (GST) activity in both digestive glands and gills ($p < 0.05$), with no tissue damage or lethality observed, indicating a sublethal but non-toxic adaptive oxidative response.

While the napyradiomycin-based coatings achieved up to 10 % biofilm inhibition at lower concentrations under static laboratory conditions, low compared to the ≥ 80 % industrial benchmark, their potential lies in their biocompatibility and low ecological impact. The unique inverse relationship between concentration and biofilm inhibition suggests that matrix composition and release kinetics warrant further optimization. These findings highlight the originality and environmental promise of napyradiomycin-based coatings as an effective and safer alternative to traditional antifouling systems. Future efforts should focus on dynamic marine immersion trials, long-term ecotoxicological assessments, and refinement of release profiles to further enhance performance and commercial viability within the framework of the circular bioeconomy. Furthermore, by using natural extracts from marine-derived actinobacteria and minimizing reliance on toxic biocides, this approach aligns with circular bioeconomy principles, which emphasize sustainable production processes, waste reduction, and the reuse of biological resources. This research also supports key UN Sustainable Development Goals (SDGs), particularly SDG 14 (Life Below Water), by mitigating the environmental impacts of biofouling prevention strategies.

CRediT authorship contribution statement

Pereira Isabel: Writing – review & editing, Writing – original draft, Investigation. **Diniz Mario:** Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Funding acquisition, Formal analysis, Data curation. **Gaudêncio Susana P.:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Piçarra Susana:** Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis. **Sobral Rita G.:** Writing – review & editing, Visualization, Supervision, Methodology, Formal analysis. **Ferreira Inês:** Writing – review & editing, Visualization, Investigation. **Gonçalves Bárbara:** Writing – review & editing, Visualization, Investigation. **Macedo Helena:** Writing – review & editing, Writing – original draft, Investigation.

Ethical considerations

This study was developed in accordance with Portuguese legislation and regulations for the use of animals for experimental purposes. Following these standards ensures that research is conducted ethically and responsibly, promoting scientific advancement while respecting the rights of the involved marine animals.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT to improve English. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2025.104248.

Data availability

Data will be made available on request.

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