coatings

MDPI

Communication

Beyond Cultivation: Combining Culture-Dependent and Culture-Independent Techniques to Identify Bacteria Involved in Paint Spoilage

Mira Mutschlechner [1,*], Andreas Walter [1], Katrin Bach [2] and Harald Schöbel [1,*]

[1] Department of Biotechnology & Food Engineering, MCI—The Entrepreneurial School, Maximilianstraße 2, 6020 Innsbruck, Austria; andreas.walter@mci.edu
[2] Department of Food Technology & Nutrition, MCI—The Entrepreneurial School, Maximilianstraße 2, 6020 Innsbruck, Austria; katrin.bach@mci.edu
* Correspondence: mira.mutschlechner@mci.edu (M.M.); harald.schoebel@mci.edu (H.S.); Tel.: +43-51220703826 (M.M.); +43-51220703837 (H.S.)

Abstract: Due to globally increasing problems concerning biodeterioration of paints, it is worthwhile to enhance the determination of colony forming units (CFU) as a gold standard method via more rapid and culture-independent techniques. Here, we combined traditional culture-dependent techniques with subsequent sequencing, quantitative qPCR, and a serial quantification method (most probable number; MPN) to detect paint degrading bacteria in general and sulfate-reducing bacteria (SRB) in particular. During our investigation of three water-based paints that showed visible contamination, we found high bacterial counts of up to 10^7 CFU mL^{-1}. Subsequent sequencing allowed the identification of common paint degraders including *Bacillus* sp. and *Pseudomonas* sp., but less frequently detected bacteria such as *Rhodococcus* sp. and *Delftia* spp. were also found to be present. MPN, as well as *dsrA*-targeted qPCR to detect SRB, only showed positive results for two out of three samples. These results coincided with the inherent physicochemical properties of the paints offering suitable conditions for microbial growth or not. The MPN method can be used for a diversity of aerobic and anaerobic bacteria and is rapid and reproducible. A combination of culture-independent techniques such as qPCR or NGS can help to fully elucidate the bacterial diversity in spoiled paint by also recovering anaerobic and unculturable ones.

Keywords: CFU; coating; MPN; qPCR; sequencing; sulfate-reducing bacteria

Citation: Mutschlechner, M.; Walter, A.; Bach, K.; Schöbel, H. Beyond Cultivation: Combining Culture-Dependent and Culture-Independent Techniques to Identify Bacteria Involved in Paint Spoilage. *Coatings* **2023**, *13*, 1055. https://doi.org/10.3390/coatings13061055

Academic Editor: Robert J. K. Wood

Received: 5 May 2023
Revised: 1 June 2023
Accepted: 5 June 2023
Published: 7 June 2023

1. Introduction

Paint is defined as a unique homogeneous mixture with a thin liquid to semi-solid paste viscosity [1]. The three major components comprise the binder/additive that adheres paints to the surface, pigments that give paints coloration and prevent corrosion, and solvents that make paints more spreadable [2,3]. Modern household paints can be divided into two main categories, namely oil- or solvent-based paints (thinned with either organic solvents or mineral turpentine) and emulsion paints (water-based vinyl or acrylic paints) [1]. Some of these (biodegradable) components in paint formulations may serve as carbon sources for a variety of microorganisms, involving both aerobic and anaerobic bacteria as well as fungi [4,5]. Water-based paints (commonly acrylic-based) are particularly vulnerable to microbial deterioration and degradation, primarily due to cellulosic compounds used as thickeners in paint formulations [5]. Microbial contamination of paints can occur during manufacturing as well as during storage, causing stains, chalking, malodor, changes in properties (e.g., viscosity, pH), and visible surface growth [6]. The majority of contaminants during manufacturing derive from raw materials including the source of water, equipment vessels, and plumbing lines [6]. Certain microorganisms, including sulfate-reducing bacteria (SRB), are able to form biofilms that adhere to the surfaces of plumbing systems and

processing tanks [7], leading to corrosion of iron and steel due to the production of highly reactive and toxic hydrogen sulfide, with the latter causing blackening and the distinctive "rotten egg" odor in painting products. Furthermore, bacteria embedded in biofilms are more resistant to desiccation and treatment with biocides [8]. In this context, Bosso and Cristinzio [9] studied the tolerance of bacteria and fungi to pentachlorophenol, which is present in various paints as antifouling agent, and were able to observe various strategies such as exclusion from the cell, conversion into a non-toxic compound, or using it as the sole carbon source. These microorganisms comprised (amongst others) well-known paint degraders including aerobic and anaerobic species of the genera *Bacillus*, *Pseudomonas*, and *Desulfitobacterium*, as well as *Penicillium*, *Phanerochaete*, and *Rhizopus* [9]. Additionally, the enforcement of biocides regulation to restrict their use in paints to a necessary minimum causes additional challenges regarding shelf life and premature spoilage, which contradicts the concept of sustainability. Hence, research activities have been intensified to identify major contributors responsible for biodeterioration of paints; however, most studies thus far have focused on culturable organisms using culture-dependent methods. The aim of the present study was thus to (i) combine culture-dependent methods (colony forming units; CFU) and most probable number (MPN) with molecular-biological techniques (colony PCR, sequencing, qPCR), (ii) discuss and compare the contribution of molecular methods in regards with the already proposed approaches and results, and (iii) causally link these data to physical and chemical properties of the investigated paints. Our tested hypothesis was that culture-independent techniques will not only broaden our knowledge on important key players associated with paint spoilage as they can capture yet undetected, non-culturable, and anaerobic microorganisms, but also expand the repertoire of current detection techniques with rapid and reproducible methods, particularly regarding difficult matrices such as paint.

2. Material and Methods

2.1. Physicochemical Analyses

The present study investigated three water-based acrylic paints (paints A to C) from different manufacturers that are frequently used in local painting companies. These paints often show visible microbial contamination (surface growth, malodor) shortly after initial opening (personal communication). The paints were characterized via physical and chemical properties including their water content (Wc), volatile content (Vc), and pigment content (Pc), pH, and aw-value. Wc and Vc were determined by drying the paint specimen at 110 °C to a constant weight and for 60 min (ASTM D2369-20), respectively, while the Pc was evaluated by heating the specimen in a muffle furnace at 450 °C (based on ASTM D3723). The pH was measured using pH indicator stripes (Macherey-Nagel, Allentown, PA, USA). All analyses were performed in triplicate.

2.2. Determination of Colony Forming Units, Colony PCR, and Sequencing

Samples from the three spoiled paints were taken from the surface, serially diluted (10-fold), and plated on nutrient agar (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), then incubated at 30 °C for 24 h prior to enumeration of CFU. Morphologically different colonies were aseptically picked from the Petri dishes and isolated, which then served as a template during PCR amplification using the Red Taq DNA Polymerase Mastermix (2×, VWR, Radnor, PA, USA) and the universal bacterial primer pair 27f and 1492r (Eurofins Genomics, Ebersberg, Germany). PCR conditions included an initial denaturation step at 95 °C for 5 min (the prolonged denaturation step intended to disrupt the cells), followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s, and elongation at 72 °C for 45 s. A final elongation step at 72 °C for 10 min completed the protocol. PCR products were checked on a 1.0% [w/v] agarose gel and purified with the Nucleospin Gel and PCR Clean-up Kit (Macherey Nagel, Düren, Germany) according to the manufacturer's instructions, similar to the study conducted by Bosso et al. [10]. The quantity and purity of the purified PCR products were evaluated via UV-vis spectrophotometry with NanoDrop

2000cTM (PeqLab, Erlangen, Germany), sent to Eurofins Genomics (Germany) for sequencing, and gene sequences compared with existing sequences in available databases using the BLAST software 2.14.0 [11]. The obtained sequences were submitted to GenBank (accession number: SUB13264124).

2.3. Most Probable Number

For selective enumeration of SRB, 180 µL of sterilized sulphite broth (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) were filled into each well of a sterile 96 well microplate. Similar to CFU quantification, 20 µL were withdrawn from the surface of the spoiled paints and added into the first row, followed by a serial dilution of the sample up to 10^{-12} (in the last row). The 96-well plates were placed in an AnaeroPack chamber, activated Microbiologia Anaerocult® A was added to create anaerobic conditions, and the chambers were cultivated at 30 °C for up to three days. The numbers of present SRB were determined according to the MPN Determination from Serial Dilutions by R. Blodgett, FDA [12–14] (please refer to Supplementary Table S1).

2.4. DNA Extraction and qPCR

A sample of 50 mL was taken from the surface of the spoiled paints and filtered through a 0.45 µm membrane (Whatman ®, Kent, UK), washed with PBS, and cut into small pieces with a sterile scalpel prior to extraction. DNA was extracted using the NucleoSpin® Microbial DNA Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The integrity of the DNA was determined on a 1.0% agarose gel and the quantity and purity of the extracted DNA was evaluated via UV-vis spectrophotometry with NanoDrop 2000cTM (PeqLab, Erlangen, Germany).

qPCR was performed on a CFX96 Touch Deep Well Real-Time PCR System (Bio-Rad, Hercules, CA, USA) using the iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA) in 20 µL volumes. Each standard reaction mix contained a final concentration of 1× Supermix reaction premix, 100 nM each primer, 0.04 mg mL^{-1} BSA, and distilled water. For the quantification of the entire bacterial community, the universal primer pairs 338f and 805r were applied [15]. Prior to amplification, the samples were subjected to an initial denaturation step at 95 °C for 5 min. For construction of calibration curves, we used genomic DNA from *Escherichia coli* (DSM 4230) purchased from the German collection of microorganisms and cell cultures (DSMZ). Cycling conditions for quantification of the assays targeting bacterial 16S rRNA gene copies were as follows: 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 90 s (35 cycles). The primers used to specifically target SRB were dsrA_290F and dsrA_660R, and *Desulfovibrio vulgaris* (DSM 644, DSMZ, Braunschweig, Germany) was used as standard [16]. Cycling conditions included an initial denaturation at 95 °C for 3 min, followed by 50 cycles of 40 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C [14]. Each run included negative and non-template controls (UltraPure DNase/RNase-Free Distilled Water, Invitrogen, Carlsbad, CA, USA), and samples were run in duplicate. After quantification, PCR products were checked via melting curve analysis.

2.5. Statistical Analyses

Data analysis was performed using SigmaPlot v15.0 (Systat Software Inc., Chicago, IL, USA). Results are given as mean (n = 3) and error bars correspond to the standard deviation unless stated otherwise.

3. Results

3.1. Physicochemical Properties

All determined physicochemical parameters are summarized in Table 1. All three paint samples exhibited alkaline pH-values ranging from 8.0 to 10.5 and showed similarly high aw-values. The Wc ranged from about 40 to 50% (Table 1). While the Vc was significantly lower in paint B compared with A and C, paint A was characterized by a much lower pigment content (Table 1).

Table 1. Physical and chemical characteristics of the fresh paints. Values are given as means (±SD), $n = 3$. Wc, water content; Vc, volatile content; Pc, pigment content.

Paint	pH	aw-Value	Wc [%]	Vc [%]	Pc [%]
A	8.0	0.98 (0.001)	46.7 (0.6)	67.0 (2.7)	53.2 (2.5)
B	10.5	0.98 (0.002)	50.0 (0.9)	48.8 (0.3)	79.5 (3.5)
C	8.5	0.98 (0.001)	40.4 (0.3)	59.5 (1.6)	84.2 (1.4)

3.2. Microbiological and Molecularbiological Approaches

Culture-based techniques revealed high bacterial counts ranging from 4.2×10^6 CFU mL^{-1} (paint A), 1.8×10^7 CFU mL^{-1} (paint B) to 3.0×10^7 CFU mL^{-1} (paint C). The three different spoiled paint samples revealed the presence of 16 morphologically different species, which were subsequently used to conduct colony PCR with BLAST searches using the rRNA/ITS databases. Among the bacteria, abundant phlya related to Firmicutes (Bacilli) and Proteobacteria were found. At genus level, we were able to identify *Pseudomonas* spp. and *Bacillus* spp. as the dominant bacteria, both being detected in all three investigated paint samples. Besides *Bacillus* and *Pseudomonas* spp., we identified *Staphylococcus* sp., *Flavobacterium* sp., *Microbacterium* sp., *Alcaligenes* sp., *Citrobacter* sp., *Delftia* spp., *Providencia* sp., *Comamonas* sp., *Rhodopseudomonas* sp., and *Rhodococcus* sp. as abundant organisms in spoiled paint (please refer to Table 2).

Table 2. Identified bacteria in the three different paint samples (A to C) using colony PCR and subsequent sequencing.

Organism	Paint	Accession Number	Similarity [%]
Delftia spp.	A	NR_113708.1	>99
	C	NR_113870.1	>99
Flavobacterium sp.	A	NR_104713.1	>99
Microbacterium sp.	A	NR_044936.1	>98
Rhodococcus sp.	A	NR_145886.1	>99
Rhodopseudomonas sp.	A	NR_036771.1	>99
Alcaligenes sp.	B	NR_113606.1	>99
Comamonas sp.	B	NR_114865.1	>97
Providencia sp.	B	NR_115880.1	>98
Citrobacter sp.	C	NR_117752.1	>99
Staphylococcus sp.	C	NR_156818.1	>99
Bacillus spp.	A,B	NR_112636.1	>99
	C	NR_164882.1	>99
Pseudomonas spp.	A,C	NR_114226.1	>99
	B,C	NR_181196.1	>99
	A,C	NR_040802.1	>99

qPCR targeting the 16S rRNA gene was conducted to quantify the entire bacterial community in the surface film of deteriorated paint samples. The results revealed high LOG_{10} 16S rRNA gene abundances ranging from 7.2 ± 0.10 copies mL^{-1} to 8.9 ± 0.002 copies mL^{-1} (Figure 1). Using *dsrA* primers to quantify SRB resulted in positive signals for paint A and C with LOG_{10} abundances of 2.5 ± 0.52 copies mL^{-1} and 3.4 ± 0.32 copies mL^{-1}, respectively. The amplification efficiencies ranged between 94% and 99% with a regression coefficient value (R^2) systematically above 0.99. LOG_{10} abundances of SRB confirmed the results obtained from MPN techniques as a statistical method to estimate the viable numbers of SRB in a sample resulting in 2.50×10^2 and 1.90×10^3 cells mL^{-1} in paint A and C, respectively, as evidenced by a characteristic formation of black iron sulfide precipitates. We were unable to detect SRBs in paint B using both qPCR and MPN technique (Figure 1 and Supplementary Table S1).

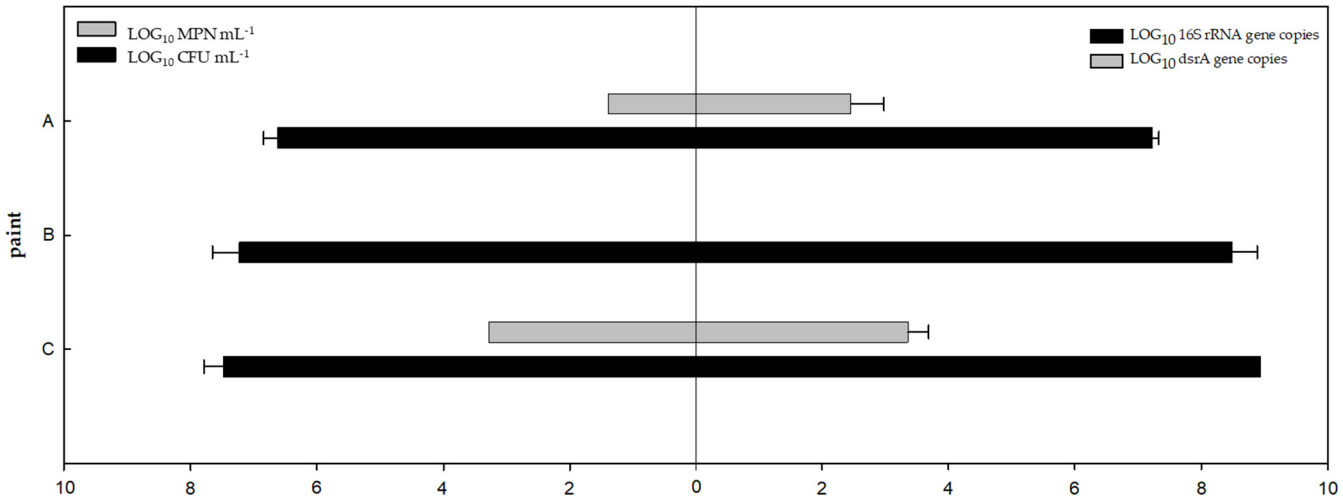

Figure 1. (**Left**): MPN results and colony forming units (both LOG_{10} transformed), (**right**): LOG_{10} gene copy numbers of total bacteria (16S rRNA) and functional genes for SO_4^{2-} reduction (*dsrA*) revealed by qPCR analysis in the three deteriorated paints. Results are given as means (±SD), $n = 3$.

4. Discussion

Water-based paints are highly prone to microbial deterioration as they offer a conducive environment including available nutrient sources, water supply, and suitable pH-values. A variety of products exhibit high water activities (aw > 0.85) that favors microbial growth and accounts for the need of preservatives [17–19]. This available or free water causes (bio)chemical reactions [17–19] that have been shown to have a negative impact on product characteristics including texture, color, and stability and lead to gassing and malodor. Moreover, water-based paints are known to contain recycled water that may be a source of contamination [20]. One of the first signs of deterioration is loss in viscosity and phase separation due to microbes releasing enzymes that primarily attack the cellulose used as thickening agent [21]. Although the paints used in this study are characterized by (slightly) alkaline pH values (Table 1), which lies outside the neutral range preferred by the majority of microorganisms, a high-water availability and pigments serving as carbon and nitrogen sources allowed microbial growth in all tested samples. Etim and Antai [22], investigating the effect of different pH values (and temperatures) on the growth of microorganisms in paints, reported that *Bacillus* and *Pseudomonas* sp. preferred alkaline over acid pH values. Furthermore, Poulsen et al. [23] also reported that pH values up to 11 did not sufficiently remove bacterial contamination from paints entirely, which is consistent with the results in the present investigation.

Using culture-dependent techniques, we detected total bacterial counts in spoiled paints up to 10^7 CFU mL^{-1}. These results are consistent with the findings from Olayide et al. [24], who reported mean bacterial counts in spoiled paints ranging from 2.3×10^6 CFU mL^{-1} to 6.8×10^8 CFU mL^{-1} using nutrient agar. Lower numbers of total viable counts were detected in fresh emulsion and gloss paints, ranging from 1.1×10^3 to 3.6×10^3 CFU mL^{-1} [18]. Subsequent sequencing allowed the identification of common paint degraders including *Pseudomonas* spp., *Bacillus* spp., *Alcaligenes* sp., and *Staphylococcus* sp. in spoiled paints (Table 2). Species of *Bacillus* and *Pseudomonas* were frequently detected in spoiled paints and were found to be associated with alteration of paint characteristics [24–26]. Endospore-forming *Bacilli* are resistant to hostile physical and chemical conditions, such as concentrated biocides, very low pH, and high temperatures, and were thus detected in freshly produced as well as spoiled paint [24]. After germination, they hydroxylate and oxidize the organic matter through oxidation-reduction reactions that cause the pH to cease, which, in turn, results in increasing stability problems [2]. Among bacteria, endospore-forming *Bacilli* and *Pseudomonas* spp., both being present during the initial stages of paint biodeterioration, are able to secret enzymes responsible for

the degradation of pigments as well as cellulosic thickeners [27]. Cellulolytic enzymes predominantly comprise endoglucanases that randomly hydrolyze the β-1,4-linkage of the cellulosic ether chains [27], resulting in respective end products such as cellobiose and glucose being easily accessible and thus facilitating subsequent microbial proliferation. Furthermore, *Pseudomonas* spp. are able to attack cellulosic derivates next to phenolic resins present in binders and stable and difficult to degrade volatile organic compounds (VOCs) such as toluene used as paint thinners [17,25]. Unlike *Bacillus*-related species, *Pseudomonas* spp. are primarily found in spoiled paint, thus highlighting their ability to tolerate both biocides and a high level of heavy metals [24]. Previous investigations also found *Alcaligenes* sp., *Staphylococcus* sp., *Flavobacterium* sp., and *Microbacterium* sp. to be frequently abundant in spoiled paint [3,24,28,29], which agrees with our results. The consistent isolation of the above-mentioned organisms indicates that they constitute characteristic paint degraders; however, we also found less frequently described microorganisms such as *Rhodococcus*, *Providencia*, and *Delftia* spp. (Table 2) that might significantly contribute to the biodeterioration process. Species among these genera are known for their unique metabolic capabilities to break down or transform phenols or (aromatic) hydrocarbons (e.g., toluene) and are resistant and able to degrade heavy metals [30,31]. *Rhodopseudomonas* spp. have gained increasing importance due to their ability to degrade aromatic compounds including benzene, which is a major part of numerous commercial products including paints and ink. In this context, many of the traditional pigments used in paint formulations, as well as bases that provide bulk to the paint and driers, contain heavy metals including cadmium, chromium, cobalt, lead, and zinc [32,33]. Other bacteria isolated from spoiled paints, which were not detected in this study, include (e.g.,) *Arthrobacter* sp., *Azotobacter* sp., *Burkholderia* sp., *Enterobacter* sp., and *Micrococccus* sp. [24].

Almost all studies related to biodeterioration of paints, however, used standard cultivation methods to quantify and biochemical techniques to identify the microbial community. To our knowledge, just one publication by Olayide et al. [24] applied a combination of culture-based techniques and a metagenomic approach to reveal a diverse population of bacteria and archaea in fresh and spoilt water-based paints. Hence, the actual bacterial (and fungal) abundance and diversity is inevitably underestimated. Further limitations include the choice of media and incubation conditions that orientate the microbial groups to be cultivated, while molecular methods allow the examination of microorganisms that cannot be isolated and cultivated with current culture methodologies. They facilitate a rapid and reproducible comparison of different environmental samples, revealing a high specificity and sensitivity [34]. To receive a bigger picture, we combined culture-dependent (CFU), with MPN and culture-independent techniques (colony PCR, qPCR). qPCR targeting the 16S rRNA (universal bacteria) and *dsrA* gene (SRBs) revealed high copy numbers of bacteria in all deteriorated paints, while SRB were only detected in two out of three paints (Figure 1). These results coincided with the results obtained by MPN techniques as a rapid and cheap method to screen paints for the presence of SRB, with positive reactions for paint A and C only (Figure 1). In this context, SRB prefer environments with pH values ranging from 5.0–8.0 [35], with paint B being characterized by higher pH values (Table 1) and thus unfavorable conditions. While increasing the pH in paint samples can be advantageous regarding microbial attack, adjustment during manufacturing to a value typically greater than 8.0 is carried out using neutralizing amines (e.g., 2-amino-2-methyl-1-propanol), with their addition causing instabilities during storage. The negative impacts of contamination with SRB are similar to those resulting from aerobic ones: loss in viscosity, pH shifts, malodor (characteristic rotten eggodor), discoloration, and gassing. Furthermore, SRB are able to form biofilms, making them highly resistant to antimicrobial agents. Using a metagenomic approach, Olayide et al. [24] were also able to detect *Desulfovibrio* sp. in spoiled paint samples, leading to the assumption that SRB are frequently more abundant in spoiled paint than hitherto believed. These results also highlight the importance of techniques beyond traditional cultivation to recover both anaerobic and unculturable organisms associated with paint spoilage. The proposed methods (MPN, qPCR) thus supplement the panel

of culture-dependent techniques and constitute an easy and rapid opportunity to detect microbial contamination in paints; however, further applications such as next-generation sequencing should be addressed to receive a detailed picture of the microbial community in bio-deteriorated paints, indispensable for preventing spoilage.

5. Conclusions

Biodeterioration of painting products, although a well-known "old" challenge in the paint and coating industry, has not received much scientific attention in the past decades. Few studies have thus far been conducted, particularly by researchers in tropical countries, where high temperatures and humidity favor microbial growth and deterioration. However, recently, due to the enforcement of biocides regulation, e.g., the Biocidal Products Regulation (BPR, Regulation (EU) 528/2012), to restrict their use in paints to a necessary minimum, industries have called upon intensive research activities in that respect. Strategies, such as the identification of contamination sources (and their termination) in the production process, the development of novel packaging policies, or the addition of safe bio-additives have been considered to compensate biocide application. However, to "know your enemy", more comprehensive analyses of microbial key players are urgently needed, based on a harmonized approach combining the best of both worlds, culture-dependent techniques and molecular applications. With a detailed knowledge of the microorganisms involved in paint spoilage, more targeted and efficient measures can be taken to avoid contamination without biocides and increase the shelf life of the products. As paint spoilage is still considered a "black box", with essential information about preventing biodeterioration still being missed, future investigations should also focus on other bacterial groups, such as iron bacteria as well as fungi, by using culture-independent approaches such as targeted amplicon sequencing and metagenomics, along with Fourier-transform infrared spectroscopy (FTIR) analyses, which could help to elucidate major structural changes or modifications of contaminated water- and oil-based paints, particularly interesting during storage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/coatings13061055/s1, Table S1. MPN calculation table for 8 tubes according to Blodgett [10–12] and 95% confidence intervals.

Author Contributions: Conceptualization, K.B. and H.S.; methodology, M.M. and A.W.; investigation, M.M. and A.W.; resources, H.S. and K.B.; data curation, M.M. and A.W.; writing—original draft preparation, M.M.; writing—review and editing, A.W., H.S. and K.B.; visualization, M.M.; supervision, H.S. and K.B.; project administration, H.S.; funding acquisition, H.S. and K.B. All authors have read and agreed to the published version of the manuscript.

Funding: This project was funded by The Austrian Research Promotion Agency FFG (InCanPres, FO999898991 and 46998351).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article.

Acknowledgments: The authors would like to thank M.L.G. for providing the paint samples.

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

References

1. Liu, Q.; Gao, D.; Xu, W. Effect of paint process on the performance of modified poplar wood antique. *Coatings* **2021**, *11*, 1174. [CrossRef]
2. Phulpoto, A.H.; Qazi, M.A.; Mangi, S.; Ahmed, S.; Kanhar, N.A. Biodegradation of oil-based paint by Bacillus species monocultures isolated from the paint warehouses. *Int. J. Environ. Sci. Technol.* **2016**, *13*, 125–134. [CrossRef]
3. Ravikumar, H.R.; Rao, S.S.; Karigar, C.S. Biodegradation of paints: A current status. *Indian J. Sci. Technol.* **2012**, *5*, 1977–1987. [CrossRef]

4. Mardones, L.E.; Legnoverde, M.S.; Monzón, J.D.; Bellotti, N.; Basaldella, E.I. Increasing the effectiveness of a liquid biocide component used in antifungal waterborne paints by its encapsulation in mesoporous silicas. *Prog. Org. Coat.* **2019**, *134*, 145–152. [CrossRef]
5. Bellotti, N.; Romagnoli, R.; Quintero, C.; Domínguez-Wong, C.; Ruiz, F.; Deyá, C. Nanoparticles as antifungal additives for indoor water borne paints. *Prog. Org. Coat.* **2015**, *86*, 33–40. [CrossRef]
6. La Rosa, F.R.; Giese, E.C.; Dekker RF, H.; Pelayo, J.S.; de Melo Barbosa, A. Microbiological contamination of water-based paints from an industry in the state of Paraná, Brazil. *Ciências Exatas Ciências Exatas Tecnológicase Terra Londrina* **2008**, *29*, 85–92.
7. Samyn, P.; Bosmans, J.; Cosemans, P. Current Alternatives for In-Can Preservation of Aqueous Paints: A Review. *Mater. Proc.* **2021**, *7*, 18.
8. Abebe, G.M. The role of bacterial biofilm in antibiotic resistance and food contamination. *Int. J. Microbiol.* **2020**, *2020*, 1705814. [CrossRef]
9. Bosso, L.; Cristinzio, G. A comprehensive overview of bacteria and fungi used for pentachlorophenol biodegradation. *Rev. Environ. Sci. Biotechnol.* **2014**, *13*, 387–427. [CrossRef]
10. Bosso, L.; Lacatena, F.; Varlese, R.; Nocerino, S.; Cristinzio, G.; Russo, D. Plant pathogens but not antagonists change in soil fungal communities across a land abandonment gradient in a Mediterranean landscape. *Acta Oecol.* **2017**, *78*, 1–6. [CrossRef]
11. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol* **1990**, *215*, 403–410. [CrossRef] [PubMed]
12. Blodgett, R.J. Serial dilution with a confirmation step. *Food Microbiol.* **2005**, *22*, 547–552. [CrossRef]
13. Blodgett, R.J. Upper and lower bounds for a serial dilution test. *J. AOAC Int.* **2005**, *88*, 1227–1230. [CrossRef]
14. Blodgett, R.J. Measuring improbability of outcomes from a serial dilution test. *Commun. Stat. Theory Methods* **2002**, *31*, 2209–2223. [CrossRef]
15. Yu, Y.; Lee, C.; Kim, J.; Hwang, S. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnol. Bioeng.* **2005**, *89*, 670–679. [CrossRef]
16. Pereyra, L.P.; Hiibel, S.R.; Prieto Riquelme, M.V.; Reardon, K.F.; Pruden, A. Detection and quantification of functional genes of cellulose-degrading, fermentative, and sulfate-reducing bacteria and methanogenic archaea. *Appl. Environ. Microbiol.* **2010**, *76*, 2192–2202. [CrossRef]
17. International Cooperation on Cosmetics Regulation. (n.d.). General and Technical Frequently Asked Questions (FAQs) on Preservatives in Cosmetics. Available online: https://ec.europa.eu/docsroom/documents/15281/attachments/1/translations/en/renditions/native (accessed on 2 June 2023).
18. Decagon Devices (2006). Fundamentals of Water Activity. Available online: https://www.graintec.com.au/media/12856/Fundamentals.pdf (accessed on 2 June 2023).
19. Gatenby, A. Moisture Content and Water Activity—What Are They? [web log post]. 2013. Available online: https://www.cscscientific.com/csc-cientific-blog/moisture-content-and-water-activity-what-are-they (accessed on 2 June 2023).
20. Maduka, C.M.; Igwilo, N.C. Microorganisms Survive in Paints. *Cur. Anal. Biotechnol.* **2019**, *2*, 1–5.
21. Cheroni, S.; Gatti, B.; Margheritis, G.; Formantici, C.; Perrone, L.; Galante, Y.M. Enzyme resistance and biostability of hydroxyalkylated cellulose and galactomannan as thickeners in waterborne paints. *Int. Biodeterior. Biodegr.* **2012**, *69*, 106–112. [CrossRef]
22. Etim, L.B.; Antai, S.P. The effects of temperature and pH bacterial degradation of latex paint in humid environment. *Glob. J. Pure Appl. Sci.* **2014**, *20*, 89–94. [CrossRef]
23. Poulsen, S.E.; Lynge Hansen, S.; Kofoed, M.V.W.; Schjøth-Eskesen, J. *Reducing Biocide Concentrations for Preservation of Water-Based Paints*; Environmental Project No. 2014; The Danish Environmental Protection Agency: Odense, Denmark, 2018.
24. Olayide, O.F.; Ayomikun, K.E.; Temitope, F.O. A first report on the identification of a novel archaea, *Methanospirillum lacunae* from spoilt paints in Lagos, Nigeria using a metagenomic approach. *Sci. Afr.* **2022**, *15*, e01029. [CrossRef]
25. Hasan, S.A.; Jabeen, S. Degradation kinetics and pathway of phenol by *Pseudomonas* and *Bacillus* species. *Biotechnol. Biotechnol. Equip.* **2015**, *29*, 45–53. [CrossRef] [PubMed]
26. Fazenda, J.M.R. *Paints and Varnishes: Science and Technology*; Edgard Blücher: São Paulo, Brazil, 2005.
27. Tothill, I.E.; Seal, K.J. Biodeterioration of waterborne paint cellulose thickeners. *Int. Biodeterior. Biodegrad.* **1993**, *31*, 241–254. [CrossRef]
28. Obidi, O.F.; Aboaba, O.O.; Makanjuola, M.S.; Nwachukwu, S.C.U. Microbial evaluation and deterioration of paints and paint-products. *J. Environ. Biol.* **2009**, *30*, 835. [PubMed]
29. Gorbushina, A.A.; Heyrman, J.; Dornieden, T.; Gonzalez-Delvalle, M.; Krumbein, W.E.; Laiz, L.; Swings, J. Bacterial and fungal diversity and biodeterioration problems in mural painting environments of St. Martins church (Greene–Kreiensen, Germany). *Int. Biodeterior. Biodegr.* **2004**, *53*, 13–24. [CrossRef]
30. Wu, W.; Huang, H.; Ling, Z.; Yu, Z.; Jiang, Y.; Liu, P.; Li, X. Genome sequencing reveals mechanisms for heavy metal resistance and polycyclic aromatic hydrocarbon degradation in Delftia lacustris strain LZ-C. *Ecotoxicology* **2016**, *25*, 234–247. [CrossRef] [PubMed]
31. Bell, B.; Philp, P.; Aw, A.; Christofi, C. The genus rhodococcus. *J. Appl. Microbiol.* **1998**, *85*, 195–210. [CrossRef] [PubMed]
32. Megertu, D.G.; Bayissa, L.D. Heavy metal contents of selected commercially available oil-based house paints intended for residential use in Ethiopia. *Environ. Sci. Pollut. Res.* **2020**, *27*, 17175–17183. [CrossRef]

33. Pandey, P.; Kiran, U.V. Degradation of paints and its microbial effect on health and environment. *J. Crit. Rev* **2020**, *7*, 4879–4884.
34. Pontes, D.S.; Lima-Bittencourt, C.I.; Chartone-Souza, E.; Amaral Nascimento, A.M. Molecular approaches: Advantages and artifacts in assessing bacterial diversity. *J. Ind. Microbiol. Biotechnol.* **2007**, *34*, 463–473. [CrossRef]
35. Koschorreck, M. Microbial sulphate reduction at a low pH. *FEMS Microbiol. Ecol.* **2008**, *64*, 329–342. [CrossRef]