Celebrating the Centenary in Polymer Science: Drawing Inspiration from Nature to Develop Anti-Fouling Coatings

The Development of Biomimetic Polymer Surfaces and their Effect on Bacterial Fouling

Jake McClements[a], Luciana C. Gomes[b], Joshua Spall[c], Fabien Saubade[c], Devine Akhidime[c], Marloes Peeters[a], Filipe J. Mergulhão[b] and Kathryn A. Whitehead[c]*

[a] School of Engineering, Newcastle University, Merz Court, Claremont Road, Newcastle Upon Tyne NE1 7RU, UK;

[b] LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal;

[c] Microbiology at Interfaces, Department of Life Sciences, Chester Street, Manchester Metropolitan University, Manchester, M15GD UK.

*Corresponding Author: K. A. Whitehead, Manchester Metropolitan University, UK.

E-mail address K.A.Whitehead@mmu.ac.uk

Abstract

The development of self-cleaning biomimetic surfaces has the potential to be of great benefit to human health, in addition to reducing the economic burden on industries worldwide. Consequently, this study developed a biomimetic wax surface using a moulding technique which emulated the topography of the self-cleaning *Gladiolus hybridus* (Gladioli) leaf. A comparison of topographies was performed for unmodified wax surfaces (control), biomimetic wax surfaces, and Gladioli leaves using optical profilometry and scanning electron microscopy. The results demonstrated that the biomimetic wax surface and Gladioli leaf had extremely similar surface roughness parameters, but the water contact angle of the Gladioli leaf was significantly higher than the replicated biomimetic surface. The self-cleaning properties of the biomimetic and control surfaces were compared by measuring their propensity to repel *Escherichia coli* and *Listeria monocytogenes* attachment, adhesion, and retention in mono- and co-culture conditions. When the bacterial assays were carried out in monoculture, the biomimetic surfaces retained fewer bacteria than the control surfaces. However, when using co-cultures of the bacterial species, only following the retention assays were the bacterial numbers reduced on the biomimetic surfaces. The results demonstrate that such surfaces may be effective in reducing biofouling if used in the appropriate medical, marine, and industrial scenarios. This study provides valuable insight into the antifouling physical and chemical control mechanisms found in plants, which are particularly appealing for engineering purposes.

Keywords Biomimetic; plant; roughness; superhydrophobic; self-cleaning; anti-fouling**.**

Introduction

There has been significant interest directed towards producing biomimetic surfaces with controlled surface wetting properties [1]. The surface properties of plant leaves are promising models to mimic [2], [3], and consequently, different approaches have been taken to replicate them [4]–[7]. Much of this work has focused on altering surface topography and chemistry to produce superhydrophobic surfaces. It is generally considered that the topography of plant surfaces is the main factor influencing their hydrophobicity [2], [8]. In particular, hierarchical structures at both the micro ($\geq$ 0.5 µm) and macro levels ($\geq$ 10 µm), called the Lotus effect, are associated with superhydrophobicity of leaf surfaces [9]. These surfaces are also self-cleaning, meaning that the rolling action of water droplets alone is enough to remove contaminants and microorganisms from the leaf surfaces. Numerous biomimetic surfaces which exhibit anti-contamination, self-cleaning, and water repellent properties have been developed based on the topography of superhydrophobic leaves [4], [5], [10], [11]. These properties are extremely desirable for many applications where regular cleaning of surfaces is required to reduce surface biofouling, which can be time-consuming and have a significant economic burden [12], [13].

Despite intensive efforts to replicate such topographical features on surfaces to produce an anti-adhesive effect, many biomimetic surfaces produced with topographical features still need to be chemically modified to make them self-cleaning [5]. It has been observed that numerous plant surfaces are hydrophobic (water contact angles > 110°), or even superhydrophobic (water contact angles > 150°) [14]. However, the exact interplay between the surface chemistry, topography, and their influence on surface physiochemistry is still not completely understood, and there remains a great difficulty in producing biomimetic surfaces with effective self-cleaning properties. Thus, an understanding of the interactions at the

surface-microorganism interface is essential to further the development of synthetically made anti-adhesive surfaces which could provide a major benefit to industry.

Two important pathogens that occur in the food industry are *Listeria monocytogenes*, which is an opportunistic food-borne pathogen and the causative agent of listeriosis, and *Escherichia coli*, which is a bacterial pathogen found in water and food [15], [16]. Bacterial attachment, adhesion, and retention are a prerequisite for biofilm formation, and such issues can lead to poor hygienic conditions in food processing environments [17]. Most surfaces, when tested for their anti-adhesive properties, use single species of bacteria [18]. However, within the environment, bacteria are more usually found living in multicultural, symbiotic relationships, which can alter the pathogenicity and metabolism of the individual species, increasing surface attachment and cell density [15], [19], [20]. Despite this, little is known regarding the influence that biomimetic surfaces have on bacteria in co-culture, which is more relevant to the environments found in the food industry.

The aim of this work was to replicate the self-cleaning surface of the *Gladiolus hybridus* (Gladioli) leaf using silicone material. A negative mould of the leaf surface was produced and dental wax was utilised to create a biomimetic surface. The water contact angles of the prepared biomimetic surfaces were compared to those of the Gladioli leaf, as well as an unmodified (smooth with no impression) wax surface which acted as a control. Furthermore, the self-cleaning properties of the biomimetic wax surface were also investigated to examine how effectively it could repel bacteria in mono- and co-cultures. Wax surfaces were used as a model system due to their similar properties to polymers and their ease of moulding. These results provide valuable insight into how emulating the topography of a self-cleaning leaf can affect the anti-fouling properties of a replicated biomimetic surface.

Materials and Methods

Leaf collection

The Gladioli leaf was selected based on its propensity to repel water droplets from its leaves in an immediate rolling fashion following spraying with water for 1 min. The Gladioli leaves were collected (Westhoughton, Greater Manchester, UK) between September and November 2017. Clean leaf samples that had fully developed and were four weeks old were collected at the base using a clean knife. The leaf samples were wrapped in aluminium foil so that they remained flat and transported to Manchester Metropolitan University via car. The leaf samples were either used within 2 - 4 h of picking or stored at 4 °C ($\leq$ 24 h) before use. A number of separate batches of mature leaves were collected on different days over the two-month experimental period ($n = 10$).

Determination of the water contact angles

The water contact angles of each surface were determined using contact angle goniometry with a KRÜSS sessile drop goniometer (GH11MODEL KRÜSS, France) and PC-based data analysis system. The water droplet volume was 5 µL and was dispensed using a micro-syringe ($n = 10$) [21].

Characterisation of surface topography

Optical profilometry images (magnification 20x) of the surface topographies were taken as previously described [22] using a MicroXAM (phase shift) surface mapping microscope (ADE corporation, XYZ model 4400 mL system) with an AD phase shift controller (Omniscan, UK). Each analysis was carried out using extended range vertical scanning interferometry, and the image analysis system MAPVIEW AE 2.17 (Omniscan, UK) was used to obtain the average roughness (S_a) and average peak-to-valley roughness (S_{pv}) ($n = 9$). The

surfaces were prepared for scanning electron microscopy (SEM) using an adapted protocol [23]. The surfaces were soaked in 4 % v/v glutaraldehyde (Agar Scientific, UK) for 24 h at 4 °C, before being washed with sterile distilled water, dried overnight, and cut into ca. 6 mm^2 coupons. Samples were then dehydrated in a series of absolute ethanol/water solutions with increasing concentrations of ethanol (30, 50, 70, 90, and 100% v/v) for 10 min at each concentration. After drying, the samples were fixed to SEM stubs (Agar Scientific, UK) using carbon tabs (Agar Scientific, UK) and stored in a desiccator until visualisation.

The coupons were then fixed (adaxial side up) to SEM stubs using a conductive double-sided adhesive pad (Agar Scientific, UK), before sputter coating with gold using a SEM coating system (Polaron, UK). The sputter coating conditions were as follows: 5 mA (plasma current), pressure < 0.1 mbar, 800 V, argon gas for 30 s. The secondary electron detector (SE2) of a Supra 40VP SEM (Carl Zeiss Ltd., UK) was used to obtain the images at an accelerating voltage of 2 kV.

Production of the surfaces

Biomimetic replicate coupons emulating the Gladioli leaf were fabricated as follows; The leaf was attached to a surface using double-sided tape with the adaxial surface uppermost. A silicone batch that uses a dual-component system (Duosil silicone Shera, Germany) was produced according to the manufacturer's instructions by mixing the components in a 1:1 ratio, and poured at room temperature onto the leaf surface, in order to produce a negative mould. The silicone mold was then removed gently after curing and the leaves were discarded. The negative silicone mould was adhered to a mould base with double-sided tape (3M, UK). A dental modelling wax (Kemdent Eco dental wax, UK) was poured onto the negative mould creating a positive wax surface of each leaf. A 15 mm diameter steel hole punch (Trimming shop, UK) was used to create equally sized individual coupons. The control surface was made

in the same manner as above but without the leaf being used to make an impression in the silicone mold.

Bacterial preparation

Escherichia coli NCIB 9484 [24], a common laboratory *E. coli* strain, or *Listeria monocytogenes* Scott A, an isolate from a food-borne listeriosis outbreak [25], were prepared from tryptone soya agar (TSA) (Oxoid, US) at 4 °C (Liebherr; Switzerland), were placed into 10 mL of tryptic soy broth (Oxoid, US) and incubated for 18 h at 37 °C on an orbital shaker set at 150 rpm (New Brunswick Scientific, US). Cultures were then washed three times by centrifugation (Rotina 380, Hettich, Germany) at 1721 RPM rinsing with sterile distilled water in between. Cultures were diluted to an absorbance of 0.5 (+/- 0.05) at 540 nm on a spectrophotometer (Jenway, UK) equating to 3.4 x 10^8 *E. coli* colony forming units (CFU)/mL and 7.60 x 10^8 *L. monocytogenes* CFU/mL. The coupons were analysed for attachment, adhesion, and retention assays with either a monoculture or a coculture of the selected microorganisms. For the coculture, an equal volume of both monocultures were mixed just before use.

Attachment and adhesion assays

In order to determine the attachment (spray with wash assay) or adhesion (spray assay) of the bacteria to the biomimetic and control surfaces, eight replicates of each surface were attached to a stainless steel tray, which was angled at approximately 45°, using double-sided tape. A monoculture or coculture suspension was sprayed onto the coupons using a compressed gas paint sprayer (Spraycraft Universal Air Propellant, Shesto, UK) for 5 s at a distance of 10 cm inside a class II laminar flow cabinet (Faster, Italy). Immediately after spraying, the surfaces were divided into two sets, one was laid horizontally and left to dry

(spray assay, adhesion), whilst the other was sprayed with sterile distilled water and dried (spray with wash assay, attachment). The surfaces were then prepared for CFU enumeration ($n = 3$) and SEM imaging ($n = 1$). The coupons from the spray assay were swabbed with 70% ethanol on their sides to remove non-attached bacteria. Each coupon was added to 2 mL of phosphate-buffered saline (PBS, Oxoid, US) and vortexed for 1 min. Coupons were extracted and placed into a separate 2 mL of PBS and vortexed again to ensure the removal of most adhered cells (95-99%), which was confirmed by SEM. The contents of each of the two universals of PBS were mixed, creating the suspension to be used for serial dilutions. Following this, three replicates of the bacterial suspension, with a volume of 10 μL from each dilution were plated out onto agar (Oxoid, UK). The agar plates were incubated for 18 h at 37 °C. A colony enumeration was then performed in three independent experiments ($n = 9$). In the case of monoculture assays, the bacteria were grown on TSA. However, for bacterial enumeration following the co-culture assays, *E. coli* was grown on MacConkey agar (Oxoid, UK) and *L. monocytogenes* was grown on Oxford agar (Sigma, UK).

Retention assays

Bacteria were prepared using the bacterial preparation method. Each biomimetic coupon was submerged in 25 mL of cell suspension for 1 h at 37 °C. Once incubated, the cell suspension was poured off and 25 mL of sterile distilled water was used to rinse the coupons. Each coupon was swabbed with 70% ethanol on the abaxial plane and its sides before being added to 2 mL of PBS and vortexed for 1 min. Following this, the coupons were placed into a separate 2 mL of PBS and vortexed again to ensure the removal of most adhered cells (95-99%), which was confirmed by SEM. Each of the two universals of PBS was then mixed, creating the suspension to be used for serial dilutions.

Bacterial co-culture assays

The bacterial co-culture assays were prepared using the same procedure as the monoculture assays, except that the bacteria were enumerated and mixed in equal quantities in order to obtain a final cell concentration of $\sim 5.0 \times 10^8$ CFU/mL.

Statistical analysis

Statistical analysis of the data was carried out using Prism 8, using unpaired, non-parametric Mann-Whitney testing. The error bars shown in the graphs correspond to the standard error of the mean. Significant differences were determined at $p < 0.05$. Asterisks denote significance where $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, and $****p \leq 0.0001$.

Results

Physicochemical properties and topography

The images of the biomimetic surface showed that the macro and micro topography was very similar to the original Gladioli leaf (Figure 1d), and demonstrated features of macro topography and also the stomas of the leaf surface (Figure 1g). It was also observed that the size of the surface features was within the same range as the Gladioli leaf, which showed a fairly homogeneous distribution of raised nodules on the surface with consistent heights and diameters of approximately 4 and 5 μm, respectively (Figure 1h). At the nano level, it was observed that the topography of the Gladioli surface, characterised by a dense distribution of wax nanocrystals (Figure 1i), was not replicated on the biomimetic surface (Figure 1f).
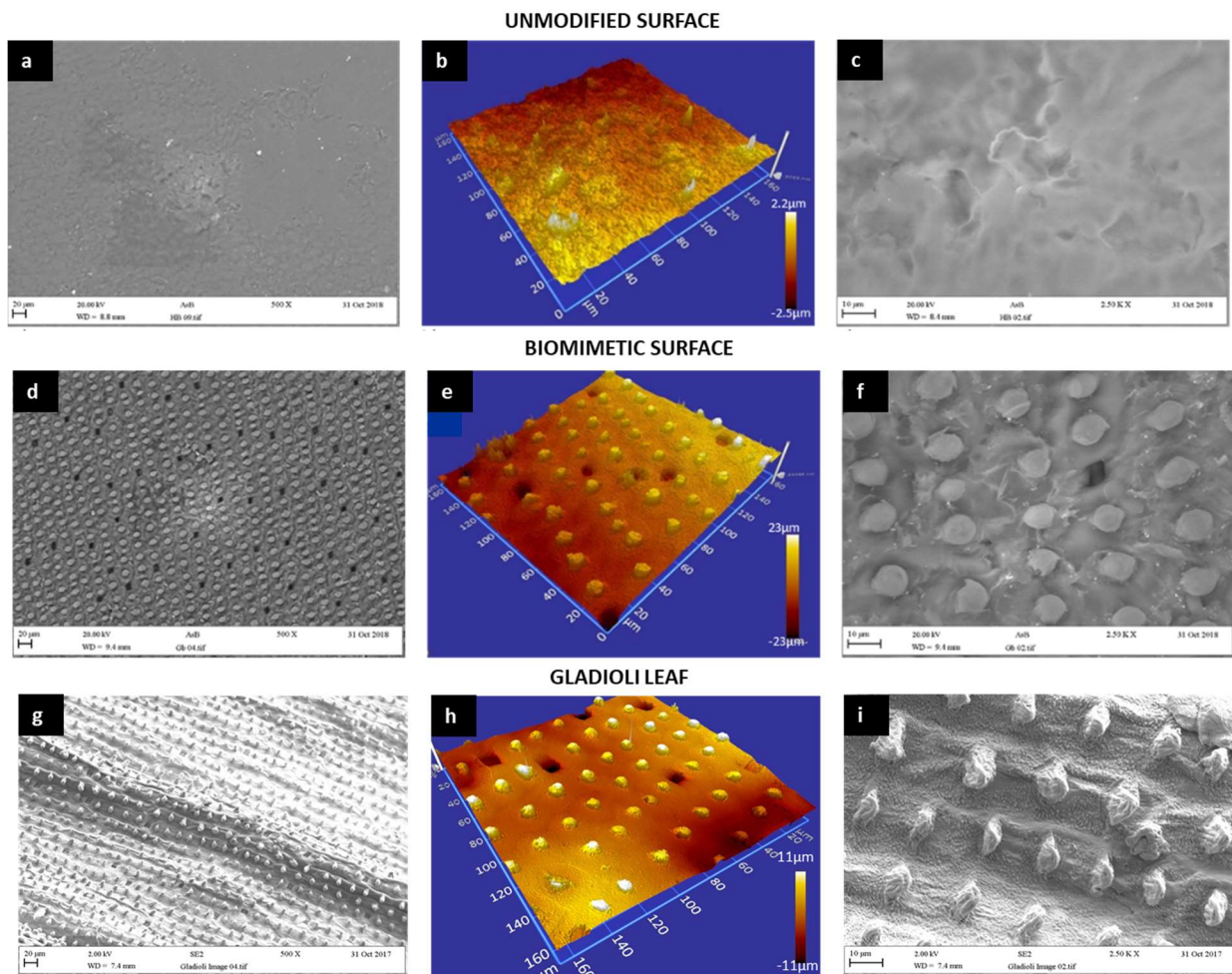
Figure 1. SEM and optical profilometry images of the a-c) unmodified wax surface, d-f) biomimetic wax surface, and g-i) original Gladioli leaf.

The surface roughness of the biomimetic surface and Gladioli leaf were determined and it was found that there was no significant difference in the S_a (4685 and 3088 nm, respectively) or S_{pv} values (63821 and 75191 nm, respectively).
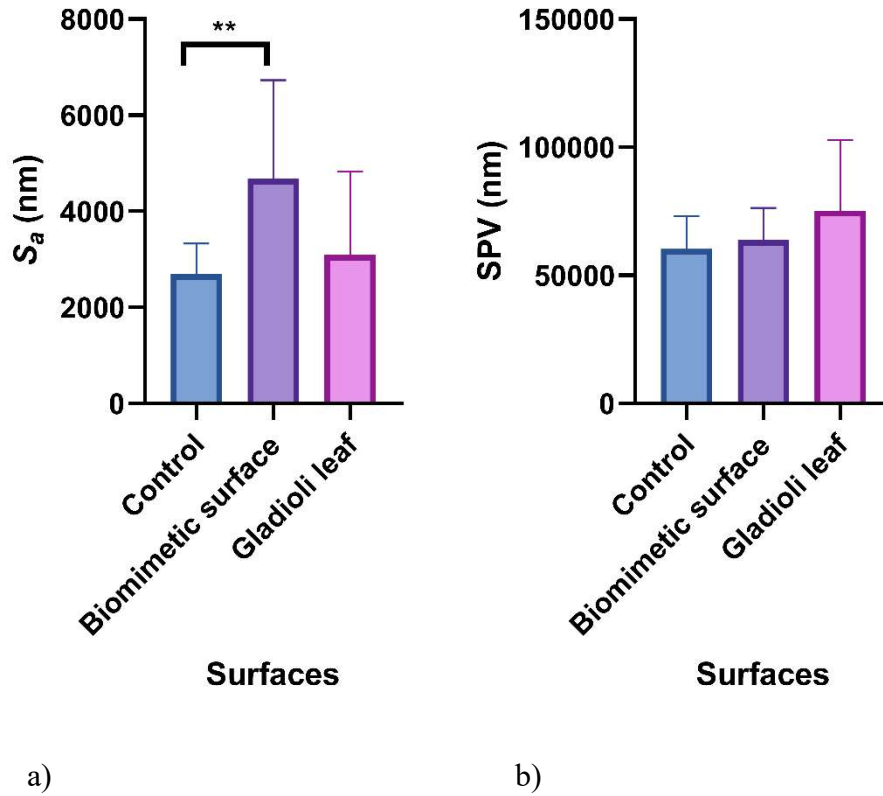
Figure 2. Surface roughness parameters (S_a (a) and S_{pv} (b)) of the unmodified wax surface, biomimetic wax surface, and original Gladioli leaf obtained from the optical profilometry images. The means + standard deviations are presented and asterisks denote significance (**$p \leq 0.01$).

The water contact angles of each surface were taken to determine their wettabilities (Figure 3). The results demonstrated that the unmodified wax surface was the least hydrophobic (99.9°). The replicated biomimetic was surface demonstrated a further increase in contact angle (115.4°), whilst the Gladioli leaf surface was the most hydrophobic (149.7°). There was a significant difference between the water contact angle values for all the surfaces ($p < 0.001$).
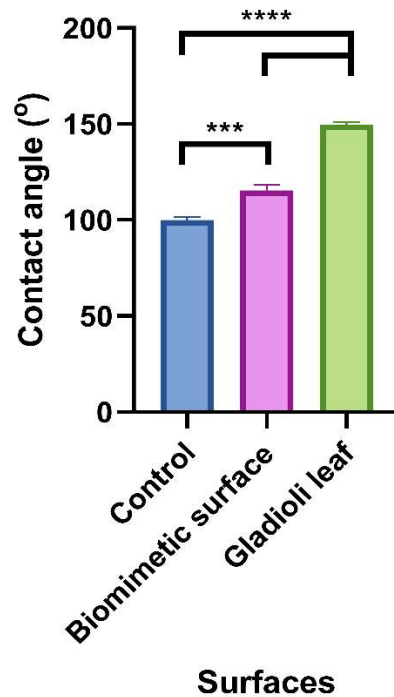
Figure 3. Water contact angles of the unmodified wax surface, biomimetic wax surface, and original Gladioli leaf. The means + SDs are presented and asterisks denote significance (***p ≤ 0.001 and ****p ≤ 0.0001).

Monoculture assays

The unmodified and biomimetic wax surfaces were analysed with attachment, adhesion, and retention assays to determine the effect that varying topography had on the microbial binding. It is important to investigate each of these bacterial binding mechanisms individually to achieve a more complete understanding of the anti-fouling properties of the surfaces. The bacteria were tested in mono- and co-culture to determine the differences that the inclusion of the two different species had on bacterial binding to the surfaces.

The results demonstrated that on all the surfaces, when tested using bacteria in monoculture, greater numbers of *E. coli* and *L. monocytogenes* were determined on the control surfaces when compared to the biomimetic surfaces following all the assays (control surface: *E. coli* attachment - 2.37 x 10^6 CFU/cm^2, adhesion - 1.37 x 10^6 CFU/cm^2, retention - 8.99 x

10^5 CFU/cm^2, *L. monocytogenes* attachment - 4.53 x 10^6 CFU/cm^2, adhesion - 2.89 x 10^6 CFU/cm^2, retention 3.83 x 10^3 CFU/cm^2; biomimetic surface: *E. coli* attachment - 2.47 x 10^4 CFU/cm^2, adhesion - 1.31 x 10^4 CFU/cm^2, retention - 4.31 x 10^2 CFU/cm^2, *L. monocytogenes* attachment - 1.04 x 10^4 CFU/cm^2, adhesion - 1.33 x 10^4 CFU/cm^2, retention - 6.94 x 10^1 CFU/cm^2) (Figure 4). For both the *E. coli* and *L. monocytogenes,* there were significant differences between the numbers of bacteria determined on the control surface compared to the biomimetic surface ($p \leq 0.01$).
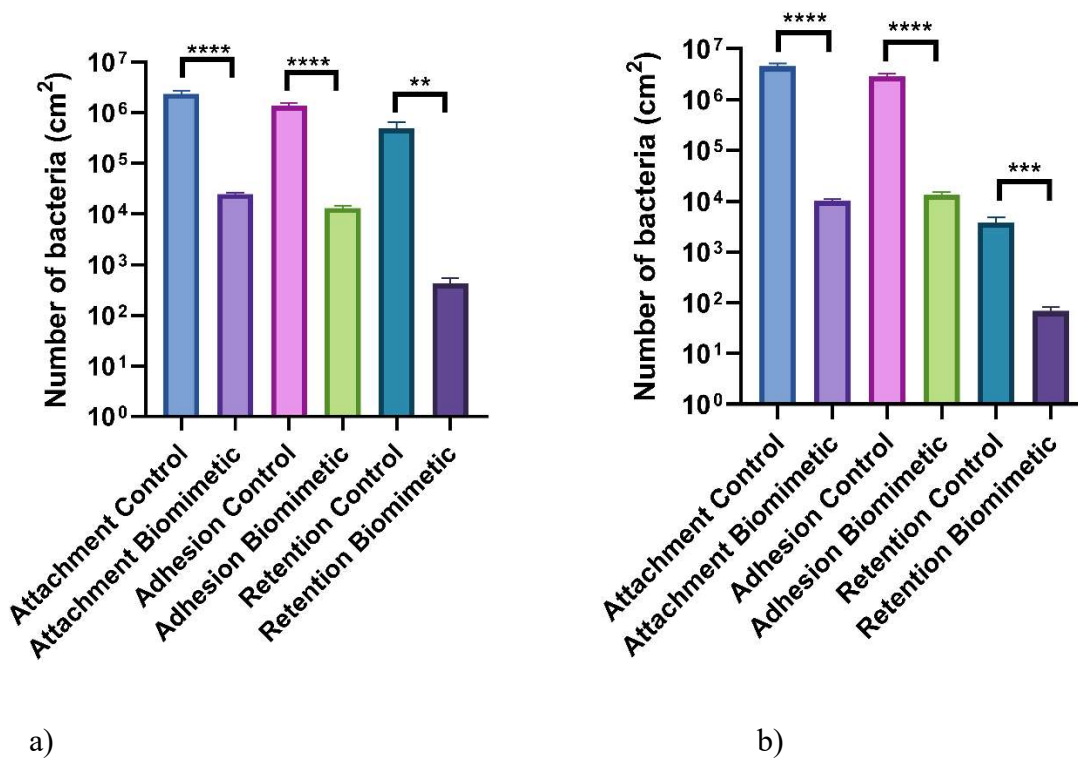

a)   b)

Figure 4. Number of a) *E. coli* and b) *L. monocytogenes* culturable cells bound to the biomimetic and unmodified wax surfaces (control) following attachment, adhesion, and retention monoculture assays. The means + SDs for three independent experiments are presented. Asterisks denote significance (**$p \leq 0.01$, ***$p \leq 0.001$, and ****$p \leq 0.0001$).

Bacterial co-culture assays

The testing of the surfaces in bacterial co-cultures demonstrated that there was a different trend in the numbers of bacteria bound to the surfaces compared to the assays using monoculture bacteria (Figure 5). When applied in co-culture, the differences in the numbers of the bacteria attached to the different surfaces were less pronounced. In addition, following the attachment and adhesion assays for both the *E. coli* and *L. monocytogenes* strains, more bacteria were retained on the biomimetic surfaces (control surface: *E. coli* attachment - 1.29 x 10^4 CFU/cm^2, adhesion - 6.77 x 10^3 CFU/cm^2, *L. monocytogenes* attachment - 2.71 x 10^4 CFU/cm^2, adhesion - 1.28 x 10^4 CFU/cm^2; biomimetic surface: *E. coli* attachment - 5.69 x 10^4 CFU/cm^2, adhesion - 1.98 x 10^4 CFU/cm^2, *L. monocytogenes* attachment - 4.47 x 10^4 CFU/cm^2, adhesion - 3.92 x 10^4 CFU/cm^2). However, significantly less *E. coli* (89%) and *L. monocytogenes* (63%) were retained on the biomimetic surfaces after the retention assays. Additionally, there were significant differences in the number of cells retained following all the assays for the *E. coli* ($p \leq 0.05$), while only one significant difference was demonstrated for *L. monocytogenes* adhesion assay ($p \leq 0.05$).
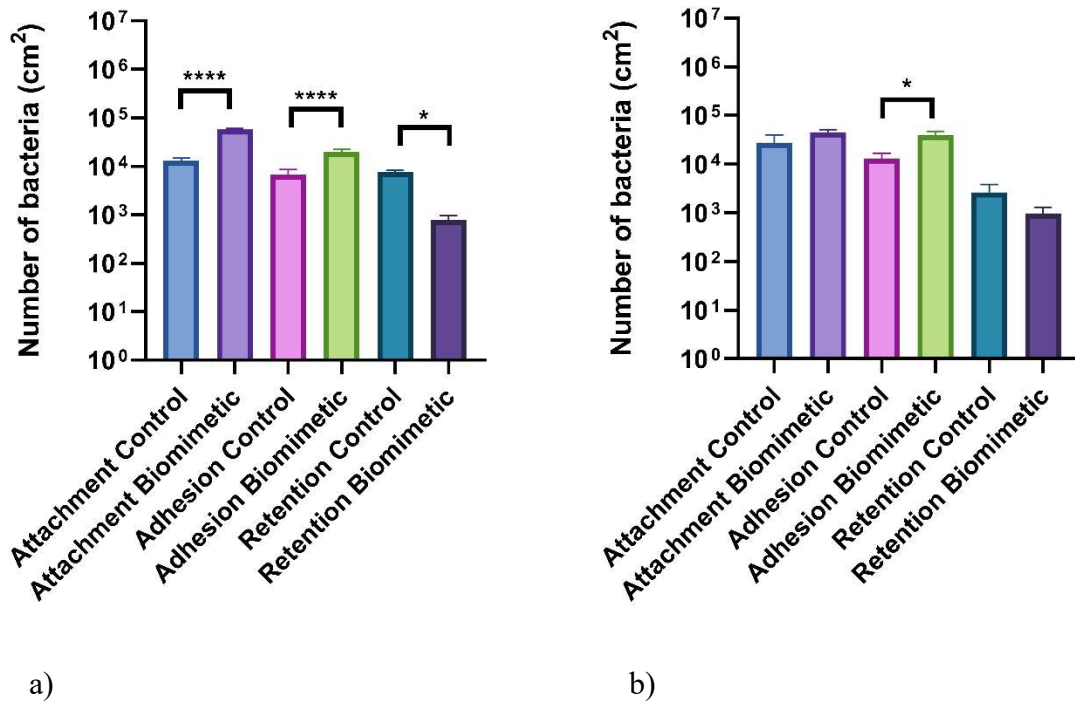
Figure 5. Number of a) *E. coli* and b) *L. monocytogenes* culturable cells bound to the biomimetic and unmodified wax surfaces (control) following attachment, adhesion, and retention co-culture assays. The means + SDs for three independent experiments are presented. Asterisks denote significance (*$p \leq 0.05$ and ****$p \leq 0.0001$).

Following microbial binding on the biomimetic surfaces, it was demonstrated that the bacteria bound on the surface were not influenced by the surface features (Figure 6). When in co-culture, it was not possible to differentiate between the two different bacterial cell types on the surfaces (Figure 6c).
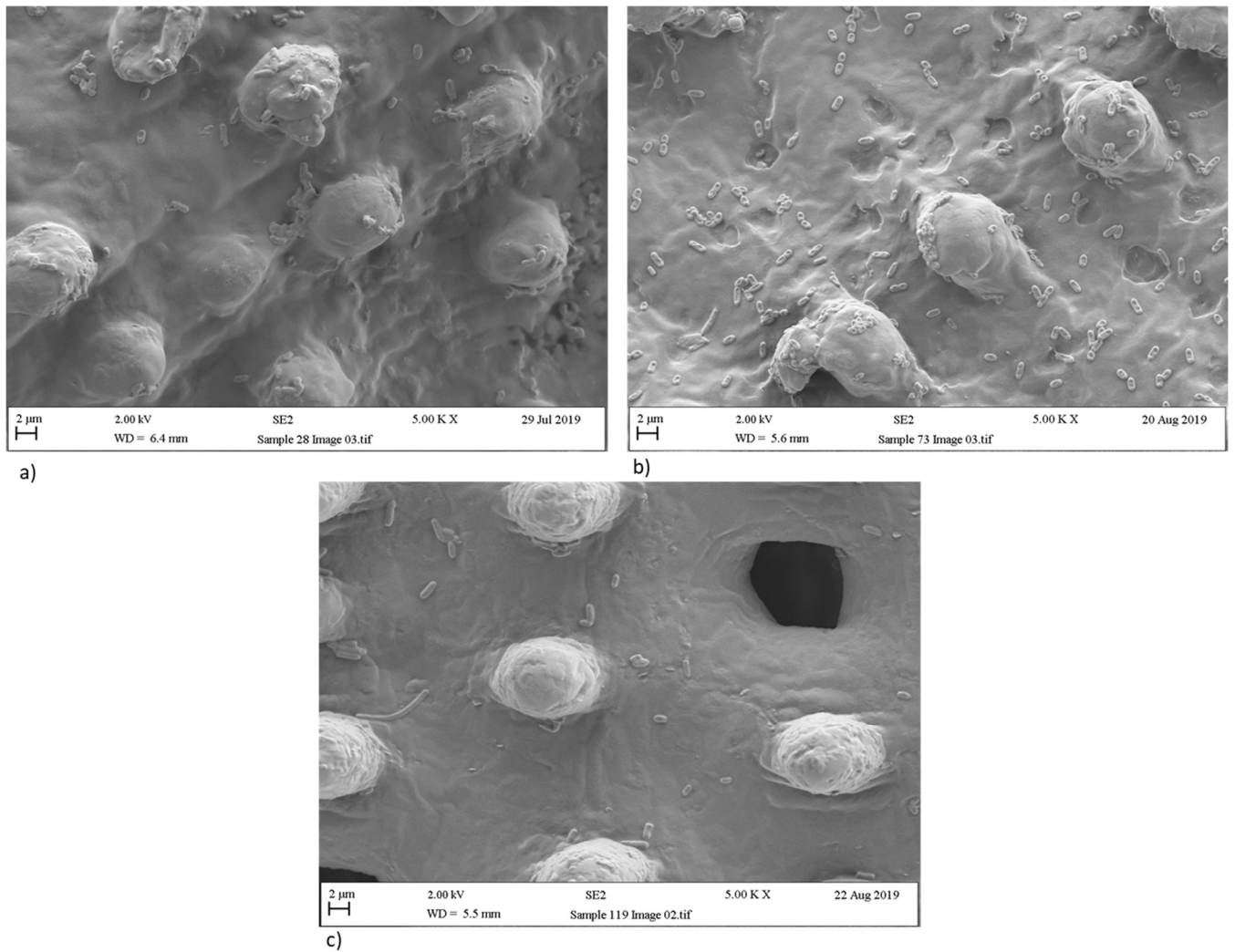
Figure 6. Scanning electron micrographs of bacteria retained on biomimetic surfaces: a) *E. coli* in monoculture, b) *L. monocytogenes* in monoculture, and c) *E. coli* and *L. monocytogenes* in co-culture.

Discussion

The production of biomimetic surfaces has been suggested to be an important development to enhance surface hygiene [4,5]. In this work, replica biomimetic surfaces of Gladioli leaves were produced and tested against two important food pathogens, *E. coli* and *L. monocytogenes*, in mono- and co-culture to determine if the biomimetic surfaces reduced bacterial attachment, adhesion, and retention. The Gladioli leaf was selected since in previous

work, the authors had demonstrated that from the selected leaves of twelve plant species, the Gladioli leaf demonstrated higher water repellency characteristics [26].

Imaging of the biomimetic surface and Gladioli leaf demonstrated that they had very similar micro and macro topographies characterised by evenly distributed raised nodules. However, the Gladioli leaf showed a high density of wax nanocrystals, which is characteristic of a hydrophobic, self-cleaning leaf [2]. The average surface roughness parameters of the Gladioli and biomimetic surfaces were not significantly different. There was a difference in the water contact angles between the Gladioli leaf and the biomimetic surface, resulting in an increased wettability of the biomimetic surface. However, generally, the results demonstrated that the presented moulding method could be utilised to emulate the surface topography and hydrophobicity of a self-cleaning leaf with a high degree of accuracy.

It is known that bacteria act differently when tested in monoculture compared to co-culture conditions [17,20], yet many microbial assays still test the antimicrobial and anti-adhesiveness of surfaces using single bacterial species. Three tests were carried out (attachment, adhesion, and retention assays) which enabled to assess the propensity of bacteria to bind to the surfaces.

Following surface characterisation, the unmodified and biomimetic wax surfaces were analysed using three different approaches (attachment, adhesion, and retention) to examine their anti-fouling properties in mono- and co-cultures. It has been suggested that microbial interactions in complex systems may be neutral, positive, or negative [27]. For the mono-cultured assays, lower numbers of bacteria were bound to the biomimetic surfaces following all the assays. However, using the co-cultured bacteria, the numbers retained were higher on the biomimetic surfaces. These results clearly demonstrated that the effect of surface properties on the mono- and co-cultures of bacteria was different.

With the exception of *E. coli* in co-culture on the control surface, the numbers of bacteria retained on the surfaces following the retention assays were the lowest. The retention assays use surfaces that are submerged in the bacterial suspension for 1 h, which are then washed to remove any unbound bacteria. This suggests that such surfaces may reduce bacterial binding when used in similar conditions. These results further highlight the need for assay selection that represents the environmental or industrial applications.

Plant leaves are multifaceted biological systems and their anti-fouling characteristics are influenced by a complex interplay between their topography, composition, and physicochemical properties. Our results suggest that these interactions with bacteria are even more complex since the surfaces interacted differently with bacteria in mono-culture compared to co-culture. Much of the work carried out on the effects of co-culturing bacteria has investigated the effects of culture conditions on antimicrobial activity. For example, when *Bacillus amyloliquefaciens* was grown with *Escherichia coli*, the antimicrobial activity of the *B. amyloliquefaciens* was increased [28]. In a biofilm work using a range of mixed bacterial species, it was demonstrated that when compared to bacteria grown in monoculture, in multispecies consortia around 20% of biofilm formation was enhanced [17]. In contrast, it has been shown that when growing *L. monocytogenes* with either *Pseudomonas fluorescens, Shewanella baltica*, or *Serratia proteamaculans,* the amount of *L. monocytogenes* was reduced [29]. However, when developing a mixed biofilm of *L. monocytogenes* and *Salmonella enterica* on stainless steel coupons, after 144 h there were similar numbers of both bacterial species, which indicated that there were no negative interactions between these bacteria [30]. Despite this, little work has been carried out on how bacteria affect each other in co-culture when determining the prerequisites of bacterial binding to a surface before biofilm formation occurs. Work by Klayman et al., [15] demonstrated that when introduced alone, planktonic *E. coli* were unable to attach to a glass surface. However, when the *E. coli* strain was introduced

simultaneously with *P. aeruginosa*, both bacteria co-adhered to the surface. In addition, when *E. coli* were introduced into a flow cell pre-colonized with a *P. aeruginosa* biofilm, they found that 10-fold more bacteria were retained than when the bacteria had been applied using co-inoculation [15]. Thus, the effect of surface properties on bacterial co-culture binding requires further investigation. However, work using biofilms of *E. coli* and *P. aeruginosa* determined that the patterned topography of a surface promoted the growth of *E. coli* rather than *P. aeruginosa* [31]. This was not observed in our work, which could be due to the differences in the bacterial species used.

Wax acted as a model surface due to its low cost, ease of moulding, and similar physiochemical properties to several hydrophobic leaves [32]. Furthermore, waxes have similar properties to some polymers, particularly those with low molecular weights [33], [34]. Polymers are a vital part of the coatings industry. For example, polymer coatings are common on ship's hulls as marine fouling has been shown to reduce ships' efficiency by up to 86%, which has huge financial and environmental implications [35], [36]. Furthermore, the value of anti-microbial peptides for use in coatings within the medical industry was estimated at US $1.06 billion in 2015 [37], [38]. The present study is significant as it characterises leaves for the rational design of polymer surfaces for anti-fouling applications. This allows us to gain a greater understanding of the self-cleaning properties of leaves and how they can be reproduced in simple and cost-effective biomimetic surfaces using polymers.

Conclusions

A moulding technique was utilised to fabricate a biomimetic wax surface based on the self-cleaning Gladioli leaf. Imaging demonstrated that the biomimetic surface had a very similar topography and surface roughness to the Gladioli leaf. When using bacteria in monocultures, the biomimetic surfaces retained fewer bacteria than the control surfaces.

However, when using co-cultures of the bacterial species, the bacterial cell numbers were greater on the biomimetic surfaces. This study provides valuable insight into the nature of self-cleaning surfaces which will be applicable to a range of industries.

References

[1]   S.-H. Hsu, K. Woan, and W. Sigmund, "Biologically inspired hairy structures for superhydrophobicity," *Mater. Sci. Eng. R Reports*, vol. 72, pp. 189–201, 2011, doi: 10.1016/j.mser.2011.05.001.

[2]   W. Barthlott, M. Mail, and C. Neinhuis, "Superhydrophobic hierarchically structured surfaces in biology: evolution, structural principles and biomimetic applications," *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.*, vol. 374, p. 20160191, 2016, doi: 10.1098/rsta.2016.0191.

[3]   W. Barthlott, M. Mail, B. Bhushan, and K. Koch, "Plant surfaces: Structures and functions for biomimetic innovations," *Nano-Micro Lett.*, vol. 9, p. 23, 2017, doi: 10.1007/s40820-016-0125-1.

[4]   F. H. Rajab, C. M. Liauw, P. S. Benson, L. Li, and K. A. Whitehead, "Production of hybrid macro/micro/nano surface structures on Ti6Al4V surfaces by picosecond laser surface texturing and their antifouling characteristics," *Colloids Surfaces B Biointerfaces*, vol. 160, pp. 688–696, 2017, doi: 10.1016/j.colsurfb.2017.10.008.

[5]   F. H. Rajab, C. M. Liauw, P. S. Benson, L. Li, and K. A. Whitehead, "Picosecond laser treatment production of hierarchical structured stainless steel to reduce bacterial fouling," *Food Bioprod. Process.*, vol. 109, pp. 29–40, 2018, doi: 10.1016/j.fbp.2018.02.009.

[6]   A. I. K. S. Rupp and P. Gruber, "Biomimetic groundwork for thermal exchange structures inspired by plant leaf design," *Biomimetics*, vol. 4, p. 75, 2019, doi: 10.3390/biomimetics4040075.

[7]   J. Li, Y. Zhou, W. Wang, F. Du, and L. Ren, "A bio-inspired superhydrophobic surface for fog collection and directional water transport," *J. Alloys Compd.*, vol. 819, p. 152968, 2020, doi: 10.1016/j.jallcom.2019.152968.

[8]   G. Wang, Z. Guo, and W. Liu, "Interfacial effects of superhydrophobic plant surfaces: A Review," *J. Bionic Eng.*, vol. 11, pp. 325–345, 2014, doi: 10.1016/S1672-6529(14)60047-0.

[9]   W. Barthlott and C. Neinhuis, "Purity of the sacred lotus, or escape from contamination in biological surfaces," *Planta*, vol. 202, pp. 1–8, 1997, doi: 10.1007/s004250050096.

[10]   A. Peter, A. H. A. Lutey, S. Faas, L. Romoli, V. Onuseit, and T. Graf, "Direct laser interference patterning of stainless steel by ultrashort pulses for antibacterial surfaces," *Opt. Laser Technol.*, vol. 123, p. 105954, 2020, doi: 10.1016/j.optlastec.2019.105954.

[11]   A. H. A. Lutey *et al.*, "Towards laser-textured antibacterial surfaces," *Sci. Rep.*, vol. 8, p. 10112, 2018, doi: 10.1038/s41598-018-28454-2.

[12]   F. Geyer *et al.*, "When and how self-cleaning of superhydrophobic surfaces works," *Sci. Adv.*, vol. 6, p. eaaw9727, 2020, doi: 10.1126/sciadv.aaw9727.

[13]   Q. Xu, W. Zhang, C. Dong, T. S. Sreeprasad, and Z. Xia, "Biomimetic self-cleaning surfaces: synthesis , mechanism and applications," *J. R. Soc. interface*, vol. 13, p.

20160300, 2016, doi: 10.1098/rsif.2016.0300.

[14]   B. Aryal and G. Neuner, "Leaf wettability decreases along an extreme altitudinal gradient," *Oecologia*, vol. 162, pp. 1–9, 2010, doi: 10.1007/s00442-009-1437-3.

[15]   B. J. Klayman, P. A. Volden, P. S. Stewart, and A. K. Camper, "*Escherichia coli* 0157:H7 requires colonizing partner to adhere and persist in a capillary flow cell," *Environ. Sci. Technol.*, vol. 43, pp. 2105–2111, 2009, doi: 10.1021/es802218q.

[16]   A. Z. de Grandi, U. M. Pinto, and M. T. Destro, "Dual-species biofilm of *Listeria monocytogenes* and *Escherichia coli* on stainless steel surface," *World J. Microbiol. Biotechnol.*, vol. 34, p. 61, 2018, doi: 10.1007/s11274-018-2445-4.

[17]   H. L. Røder *et al.*, "Interspecies interactions result in enhanced biofilm formation by co-cultures of bacteria isolated from a food processing environment," *Food Microbiol.*, vol. 51, pp. 18–24, 2015, doi: 10.1016/j.fm.2015.04.008.

[18]   J. M. R. Moreira, L. C. Gomes, K. A. Whitehead, S. Lynch, L. A. Tetlow, and F. J. Mergulhão, "Effect of surface conditioning with cellular extracts on *Escherichia coli* adhesion and initial biofilm formation," *Food Bioprod. Process.*, vol. 104, pp. 1–12, 2017, doi: 10.1016/j.fbp.2017.03.008.

[19]   S. K. Filoche, S. A. Anderson, and C. H. Sissons, "Biofilm growth of *Lactobacillus* species is promoted by *Actinomyces* species and *Streptococcus mutans*," *Oral Microbiol. Immunol.*, vol. 19, pp. 322–326, 2004, doi: 10.1111/j.1399-302x.2004.00164.x.

[20]   L. C. Gomes, J.-C. Piard, R. Briandet, and F. J. Mergulhão, "*Pseudomonas grimontii* biofilm protects food contact surfaces from *Escherichia coli* colonization," *LWT - Food Sci. Technol.*, vol. 85, pp. 309–315, 2017, doi: 10.1016/j.lwt.2017.03.005.

[21]   K. A. Whitehead *et al.*, "The effect of the surface properties of poly(methyl methacrylate) on the attachment, adhesion and retention of fungal conidia," *AIMS*

Bioeng., vol. 7, pp. 165–178, 2020, doi: 10.3934/bioeng.2020015.

[22]   A. Skovager, K. Whitehead, D. Wickens, J. Verran, H. Ingmer, and N. Arneborg, "A comparative study of fine polished stainless steel, TiN and TiN/Ag surfaces: Adhesion and attachment strength of *Listeria monocytogenes* as well as anti-listerial effect," *Colloids Surfaces B Biointerfaces*, vol. 109, pp. 190–196, 2013, doi: 10.1016/j.colsurfb.2013.03.044.

[23]   K. A. Whitehead, L. A. Smith, and J. Verran, "The detection and influence of food soils on microorganisms on stainless steel using scanning electron microscopy and epifluorescence microscopy," *Int. J. Food Microbiol.*, vol. 141, pp. S125–S133, 2010, doi: 10.1016/j.ijfoodmicro.2010.01.012.

[24]   C. O. Gill and N. Penney, "Penetration of bacteria into meat," *Appl. Environ. Microbiol.*, vol. 33, pp. 1284–1285, 1977, doi: 10.1128/aem.33.6.1284-1286.1977.

[25]   Y. Briers, J. Klumpp, M. Schuppler, and M. J. Loessner, "Genome sequence of *Listeria monocytogenes* Scott A, a clinical isolate from a food-borne listeriosis outbreak," *J. Bacteriol.*, vol. 193, pp. 4284–4285, 2011, doi: 10.1128/JB.05328-11.

[26]   F. Saubade, L. I. Pilkington, C. M. Liauw, L. C. Gomes, J. McClements, M. Peeters, M. El Mohtadi, F. Mergulhão and K. A. Whitehead, "Principal component analysis to determine the surface properties that influence the self-cleaning action of hydrophobic plant leaves," *Langmuir,* Accepted.

[27]   K. Faust and J. Raes, "Microbial interactions: From networks to models," *Nat. Rev. Microbiol.*, vol. 10, pp. 538–550, 2012, doi: 10.1038/nrmicro2832.

[28]   L. Benitez, A. Correa, D. Daroit, and A. Brandelli, "Antimicrobial activity of Bacillus amyloliquefaciens LBM 5006 is enhanced in the presence of *Escherichia coli*," *Curr. Microbiol.*, vol. 62, pp. 1017–1022, 2011, doi: 10.1007/s00284-010-9814-z.

[29]   H. E. Daneshvar Alavi and L. Truelstrup Hansen, "Kinetics of biofilm formation and

desiccation survival of *Listeria monocytogenes* in single and dual species biofilms with P*seudomonas fluorescens, Serratia proteamaculans* or *Shewanella baltica* on food-grade stainless steel surfaces," *Biofouling*, vol. 29, pp. 1253–1268, 2013, doi: 10.1080/08927014.2013.835805.

[30]   M. Kostaki, N. Chorianopoulos, E. Braxou, G.-J. Nychas, and E. Giaouris, "Differential biofilm formation and chemical disinfection resistance of sessile cells of *Listeria monocytogenes* strains under monospecies and dual-species (with *Salmonella enterica*) conditions," *Appl. Environ. Microbiol.*, vol. 78, pp. 2586–2595, 2012, doi: 10.1128/AEM.07099-11.

[31]   A. Bhattacharjee, M. Khan, M. Kleiman, and A. I. Hochbaum, "Effects of growth surface topography on bacterial signaling in coculture biofilms," *ACS Appl. Mater. Interfaces*, vol. 9, pp. 18531–18539, 2017, doi: 10.1021/acsami.7b04223.

[32]   V. S. Saji, "Wax-based artificial superhydrophobic surfaces and coatings," *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 602, p. 125132, 2020, doi: 10.1016/j.colsurfa.2020.125132.

[33]   M. A. AlMaadeed, S. Labidi, I. Krupa, and M. Ouederni, "Effect of waste wax and chain structure on the mechanical and physical properties of polyethylene," *Arab. J. Chem.*, vol. 8, pp. 388–399, 2015, doi: 10.1016/j.arabjc.2014.01.006.

[34]   L. Shen, J. Severn, and C. W. M. Bastiaansen, "Drawing behavior and mechanical properties of ultra-high molecular weight polyethylene blends with a linear polyethylene wax," *Polymer*, vol. 153, pp. 354–361, 2018, doi: 10.1016/j.polymer.2018.01.083.

[35]   C. I. Idumah, C. M. Obele, E. O. Emmanuel, and A. Hassan, "Recently emerging nanotechnological advancements in polymer nanocomposite coatings for anti-corrosion, anti-fouling and self-healing," *Surfaces and Interfaces*, vol. 21, p. 100734,

2020, doi: 10.1016/j.surfin.2020.100734.

[36]   W. H. Wang, Z. Yang, L. Yue, H. Sun, H. Wang, Z. Guo, F. Cheng and S. Chen, "pH responsive antifouling and antibacterial multilayer films with self-healing performance," *Chem. Eng. J.*, vol. 356, pp. 130–141, 2019, doi: 10.1016/j.cej.2018.08.181.

[37]   G.M. Intelligence, "Global anti microbial peptides market report 2030: based on peptides type, based on products, based on application & by region with COVID-19 impact | Forecast Period 2017-2030," 2020. [Online]. Available: https://www.goldsteinresearch.com/report/anti-microbial-peptides-market-outlook-2024-global-opportunity-and-demand-analysis-market-forecast-2016-2024.

[38]   M. Kazemzadeh-Narbat *et al.*, "Strategies for antimicrobial peptide coatings on medical devices: a review and regulatory science perspective," *Crit. Rev. Biotechnol.*, vol. 41, pp. 94–120, 2021, doi: 10.1080/07388551.2020.1828810.