CHAPTER 6

Application of atomic force microscopy in food microorganism research

Lin Zhao[1,2] **and Hongshun Yang**[1,2]
[1]Department of Food Science and Technology, National University of Singapore, Singapore, Singapore
[2]National University of Singapore (Suzhou) Research Institute, Suzhou Industrial Park, Suzhou, Jiangsu, P. R. China

6.1 Introduction

Foodborne disease has become a major public health challenge worldwide, causing suffering to patients and bringing considerable financial burden to society. Many foods are vulnerable to be spoiled or contaminated by disease-causing microbes or pathogens, due to environmental contamination or improper handling occurring at any point in food production, processing, delivery, and consumption (Heaton & Jones, 2008). According to the 2017 annual report released by Centre for Disease Control and Prevention (CDC), 841 cases of foodborne disease outbreaks were reported in the US, most of which could be attributed to norovirus infection, followed by *Salmonella*, Shiga toxin-producing *Escherichia coli*, and *Clostridium perfringens* successively ("Surveillance for Foodborne Disease Outbreaks, 2019"). Therefore, understanding the characteristics of microorganisms in different species, states, and environmental conditions is of great significance for the prevention of foodborne disease outbreaks, among which the study of microbial surface properties could be one crucial part.

While microorganisms can alter their surface structure or cell profile as a survival mechanism under adverse environments, little is known about their cell surface dynamics with respect to the cellular interactions or the interactions with environment at the nanoscale, due to their small sizes (Araújo, Viana, Gómez, Pontes, & Frases, 2019). However, atomic force microscopy (AFM) can be used as an examination tool to monitor real-time microbiological systems, by providing high nanoscale resolution nondestructively in both air and fluid environments, which cannot be achieved by light microscopy (LM) or transmission/scanning electron microscopy (TEM/SEM) (Yang

Fundamentals and Application of Atomic Force Microscopy for Food Research
DOI: https://doi.org/10.1016/B978-0-12-823985-8.00007-4

et al., 2007). The disadvantages of LM, TEM, and SEM in the study of microbial systems (e.g., pretreatment needed, only 2D images obtained, samples in nonnative status, etc.) can be overcome by AFM, as the latter can provide different views of the sample and unique insights into the microbial surface structure by atomic-scale imaging after minimal sample preparation (Braga & Ricci, 2004). The advantages and disadvantages of AFM, LM, TEM, SEM, and CLSM are compared and summarized in Table 6.1. Moreover, AFM is not only a surface imaging tool, considering its force measurement capability offering further details on the interactions and mechanical properties of macromolecules at microbial surfaces, which can extend the AFM application in the characterization of microbial surface function.

Besides studying "bad" bacteria that can cause serious diseases and food poisoning, "good" bacteria with benefits to human body are also worth studying, and their surface protein layers are thought to play key roles in cell–host interactions (De Sa Peixoto et al., 2015). This chapter summarizes recent studies on the applications of AFM in food microorganisms at different scales: from single cells to the biofilms, from foodborne pathogens to beneficial bacteria, from prokaryotic microorganisms to eukaryotic microorganisms and viruses, characterizing their surface properties and illustrating the molecular interactions in relation to the microbial adhesion or aggregation by probing their surface structures and other physical properties. The current trend of combining AFM and other complementary techniques is also introduced briefly, exploring more possibilities of AFM application in the field of food microorganism.

6.2 Single microbial cell studies

6.2.1 Morphological change evaluation

Atomic force microscopy provides new opportunities to analyze single microorganism cells qualitatively and quantitatively after antimicrobial treatments, which was almost impossible before as conventional methods mainly focused on large numbers of cells. Some researchers used it to compare the nanostructures of *E. coli* and *Listeria innocua* to investigate the cell morphological and dimensional changes after acidic electrolysed water (AEW) and levulinic acid (LA) treatments, respectively (Fig. 6.1) (Zhao, Zhao, Phey, & Yang, 2019). AFM images indicated that the surfaces of both bacteria were wrinkled and became irregular after being treated by LA alone and LA combined with AEW, accompanied by the leakage of intracellular contents as some small substances were observed around the cells, which could be the aggregation of the cytoplasmic components due

Table 6.1 Comparison of the advantages and disadvantages of different microscopy techniques.

Characteristics	Microscopy[a] AFM	LM	SEM	TEM	CLSM
Advantages	High resolution; nanoscale; minimal sample preparation; near-native status; 2D and 3D; in air/liquid; in-situ, continuous process; can be manipulated	Large scan area; fast scan speed; economical	High resolution; nanoscale; fast scan speed	High resolution; nanoscale; fast scan speed	Study dynamic process; fast scan speed; 2D and 3D; in situ
Disadvantages	Small scan size; slower scan speed; difficult for soft material	Only 2D; needs pretreatment; low resolution and magnification	Only 2D; needs pretreatment; nonnative status	Only 2D; needs pretreatment; nonnative status	Complicated operation; needs pretreatment

[a] *AFM*, atomic force microscopy; *LM*, light microscopy; *SEM*, scanning electron microscopy; *TEM*, transmission electron microscopy; *CLSM*, confocal light scanning microscopy; *2D*, two dimensional; *3D*, three dimensional.

Source: Reprinted with permission from John Wiley and Sons, Inc. (Yang, H., Wang, Y., Lai, S., An, H., Li, Y., & Chen, F. (2007). Application of atomic force microscopy as a nanotechnology tool in food science. *Journal of Food Science, 72*(4), R65–R75).

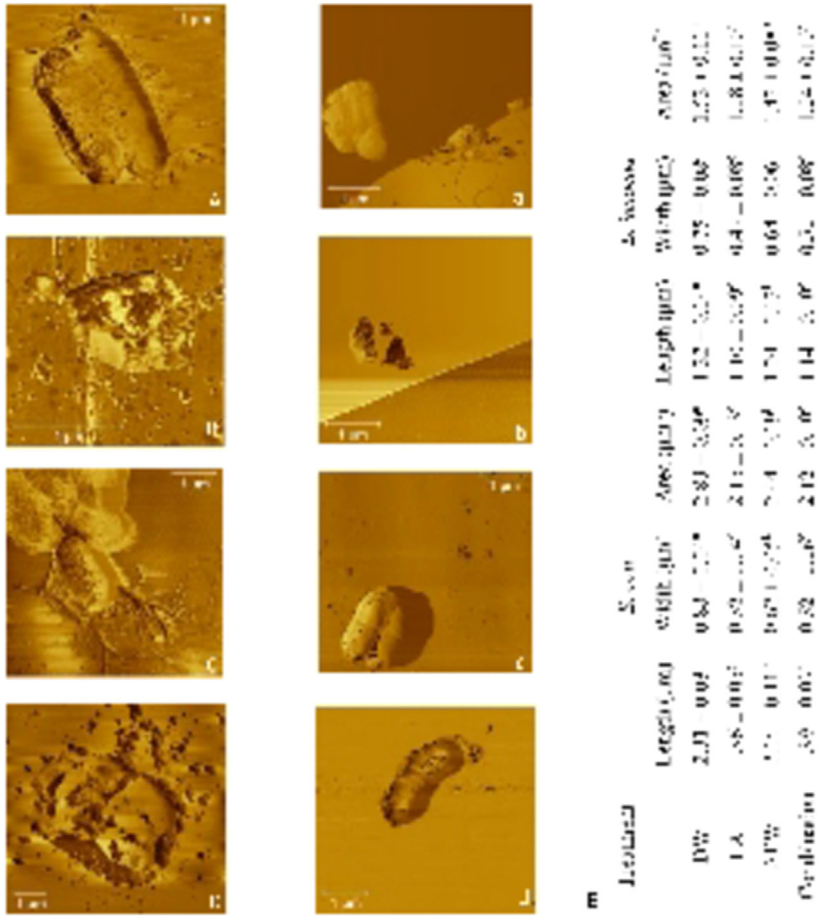

Figure 6.1 Atomic force microscopy (AFM) images of *Escherichia coli* (A–D) and *Listeria innocua* (a–d) cells after DW (A, a), LA (B, b), AEW (C, c) and combination (D, d) treatments. Effects of different treatments on the quantification of their dimension (E). Note: *DW*, deionized water; *LA*, levulinic acid; *AEW*, acidic electrolysed water; Combination: AEW + LA. Different letters represent significant differences between different treatments with the same parameter ($P < .05$). *All subgraphs are reproduced with permission from Elsevier (From Zhao, L., Zhao, M. Y., Phey, C. P., & Yang, H. (2019). Efficacy of low concentration acidic electrolysed water and levulinic acid combination on fresh organic lettuce (*Lactuca sativa *Var. Crispa L.) and its antimicrobial mechanism.* Food Control, 101, *241–250. https://doi.org/10.1016/j.foodcont.2019.02.039).*

to the change of cell membrane permeability. Besides, the quantitative changes, including changes in length, width, and area of the cells obtained from hundreds of AFM parallel images, also supported the above analysis as the leakage of cytoplasm could cause smaller cellular sizes (Fig. 6.1E). Moreover, AFM could also be used to investigate the morphological

changes of *L. monocytogenes* after being treated by nisin and grape seed extract (Zhao, Chen, Zhao, He, & Yang, 2020). Coarse and irregular cell surface and deformed cell structure were observed in the samples under this combined treatment, following decreased width and height and increased surface roughness compared to the untreated cells. Another study revealed the morphological changes of pathogenic and nonpathogenic *E. coli* after mild heat and lactic acid treatment, respectively (Chen et al., 2022). Under AFM examination, although both types of *E. coli* became inflated and had smoother surface, pathogenic *E. coli* had higher cell surface roughness after being treated with lactic acid alone or combined with mild heat treatments compared to its counterpart, demonstrating less extent of membrane disruption of pathogenic strain.

6.2.2 Antimicrobial mechanism evaluation

Due to its high resolution and sensitivity, AFM is a promising approach to help unravel the bactericidal mechanisms of different antimicrobial agents on different microbial species. One study demonstrated that (-)-epigallocatechin-3-gallate (EGCG), a main component of tea catechins, inhibited the growth of Gram-positive and -negative bacteria in different modes: EGCG damaged the cell wall of Gram-positive cells by binding to their peptidoglycan layers, leading to the shrinkage of cell envelope and the rupture of cells. However, the profile changes of Gram-negative bacterial cell walls could be attributed to the H_2O_2 generated by EGCG, which caused membrane degradation as pore-like lesions were observed in AFM images (Cui et al., 2012). Another study used *E. coli* (Gram-negative bacteria representative), *Staphylococcus xylosus* (Gram-positive bacteria representative), and *Zygosaccharomyces bailii* (food-spoilage yeast representative) as models to investigate the interactions between monocaprylate and cell membranes. The differences of membrane fluidity and phospholipid composition among different species of microorganisms played an important role in their susceptibility to monocaprylate, which only integrated into the liquid disordered lipid phase of the membrane as visualized by AFM imaging (Hyldgaard, Sutherland, Sundh, Mygind, & Meyer, 2012). Moreover, some researchers found that the *E. coli* surface was significantly remodeled after 2,4-dichlorophenoxyacetic acid (2,4-D) treatment under AFM examination, with increased surface roughness and altered envelope integrity, indicating 2,4-D cannot only enter the cell leading to oxidative damage but also interact with surface macromolecules directly (Bhat et al., 2015). The studies mentioned above and others related to the surface structure imaging are summarized in Table 6.2.

Table 6.2 Atomic force microscopy imaging for different food microorganisms.

Food microorganism	Operation mode	Sample carrier and imaging environment	Treatment on cells	Observations	References
Candida albicans	Tapping	Glass substrate, air	Lemon grass oil and its vapor	Variable roughness and height; 3D morphological changes	Tyagi and Malik (2010)
Escherichia coli; Staphylococcus aureus	Tapping	Glass coverslip, air	EGCG and H_2O_2	Perforations or grooves in the cell envelopes; cell lysis; damage to the cell walls	Cui et al. (2012)
E. coli; Staphylococcus xylosus; Zygosaccharomyces bailii	Intermittent contact	Cell-TAK-coated coverslip, air	Monocaprylate	Indentations and membrane disruption; rougher surface	Hyldgaard et al. (2012)
E. coli	Contact	Fixation coverslip, air	2,4-dichlorophenoxyacetic acid	Increase in surface roughness and negative charge; loss of envelope integrity	Bhat et al. (2015)
Aspergillus oryzae	Contact	Isopore polycarbonate membrane, liquid	–	Different surface morphology and macromolecular interactions during spore germination	Van der Aa, Asther, and Dufrêne (2002)

E. coli; Pichia pastoris; Aureobasidium pullulans	Tapping	Stainless steel coupon, air	Low concentration neutralized electrolysed water and ultrasound combination	Disordered cellular structure and irregular surface with breaches in the wall	Zhao, Zhang, and Yang (2017)
Lactobacillus crispatus	Contact	Poly-L-lysine covered glass slide, liquid	–	Variety in surface properties among different strains; elasticity and force maps	Schaer-Zammaretti and Ubbink (2003)
E. coli; Salmonella typhimurium	Noncontact	Mica sheet, air	Low concentration electrolysed water combined with short-time heat	Cell shrinkage; breakage of flagella and some indentations; visible lesions and impaired membrane structure	Liu, Jin, Feng, Yang, and Fu (2019)
E. coli	Tapping	Stainless steel coupon, air	Electrolysed water, H_2O_2 and citric acid	Shrunk and fracture; decreased length, width and height	Zhang and Yang (2017)
Rhodococcus ruber; Rhodococcus opacus; Micrococcus luteus; E. coli; Pseudomonas fluorescence	Tapping	Glass coverslip, air	*n*-decane, cyclohexane, toluene, butanol and acetonitrile	Changes in size, surface/volume ratio and surface roughness	Kuyukina, Ivshina, Korshunova, and Rubtsova (2014)

(*Continued*)

Table 6.2 (Continued)

Food microorganism	Operation mode	Sample carrier and imaging environment	Treatment on cells	Observations	References
Listera innocua	Tapping	Mica sheet, air	Sodium hypochlorite and lactic acid combination	Irregular cell body; lower cell width and height; higher root-mean-square (RMS) roughness	Chen et al. (2019)
E. coli	Tapping	Mica sheet, air	Lactic acid	Increased cell width; decreased height and surface roughness; the morphology of pathogenic strain maintained better than nonpathogenic strain	Chen et al. (2020)
Listera innocua	Tapping	Mica sheet, air	Electrolysed water generated by adding $NaHCO_3$ before/after electrolysis	Irregular and shrunken cell surface; decreased width and height	He, Zhao, Chen, Zhao, and Yang (2021)

In addition to the big contribution AFM makes to dynamic topographical characterization of microbial surfaces after different treatments, AFM investigation of microbial biofilms is also a vital part in the study of microbial systems, helping to identify the structural details at different stages of biofilm formation and determine the external effects on biofilm adhesiveness (Wright, Shah, Powell, & Armstrong, 2010), which is introduced in the following section.

6.3 Microbial biofilm studies

6.3.1 Biofilm morphological imaging

AFM is a useful tool to study the formation and adhesion of biofilms. Studies show that microorganisms can form biofilms on different biotic surfaces (food surfaces) as well as abiotic ones (food contact surfaces) as their survival strategy during food processing, and remain in adhesion despite short exposure time, posing a threat to food safety and human health (Papaioannou, Giaouris, Berillis, & Boziaris, 2018). Biofilm can be defined as a community of microbial cells embedded within extracellular polymeric substance (EPS) composed of a mixture of compounds like nucleic acids, polysaccharides, proteins, and lipids (Park et al., 2012). Once the bacteria are attached to the surface and form biofilms, they will exhibit far more tolerance and persistence than their planktonic counterparts against sanitizers, partly due to the complex structure of EPS. Therefore, recognizing the roles EPS materials play in the biofilm structural differentiation is of great importance to facilitate development of appropriate control strategies to biofilms existed on the food or food contact surfaces.

One advantage of AFM in biofilm study is that it can characterize EPS structure and components in the biofilm's natural status without affecting its original formation process. Some researchers used AFM to demonstrate the effect of flow rate on the architecture of *E. coli* O157:H7 biofilms formed on a microfluidic platform under laminar flows, with the aim of better understanding how biofilms respond and adapt to the environmental stress such as flow conditions during their culture period (Lim et al., 2008). More biofilms were produced under flow culture at a rate of 5 μL/min in 5 days when compared to those under a static culture condition, with increased surface coverage rate observed in fluidic condition. Moreover, the bacteria suffered more stress under fluidic condition, with bumpy surface and increased cell surface roughness due to more EPS produced to adapt the environmental stress. The changes in cellular size and

biofilm height also indicated the stress imposed by the flow conditions, as a smaller cell size and lower biofilm depth were noticed under laminar flow. The study showed direct evidence that bacteria could alter their morphology of biofilms in the face of an environmental stress, and AFM could provide a 3D high-resolution model to help us better understand the dynamic process within biofilms in nanoscale.

6.3.2 Biofilm adhesive property study

In addition to the imaging of microbial biofilms, AFM could also be applied to study the elasticity and adhesive properties of biofilms by force measurement. Some researchers pressed the tip into the cells to determine the adhesive forces between the tip and the cell surface biomolecules and obtain the cellular spring constant through a series of force cycles (Volle, Ferguson, Aidala, Spain, & Núñez, 2008b). Based on the AFM images, the different fibrous structures (pili, fimbriae, and flagella) on the cell surfaces of five different bacterial strains (two Gram-positive, three Gram-negative) might adhere to the tip and cause different force curve cycles, which included both the extension and the retraction curves with different force and distance components in different cell types (Fig. 6.2). It was noticed that Gram-positive cells had higher spring constants than -negative strains, indicating they are stiffer when probed, which could be attributed to their thick cell walls of peptidoglycan as well as high cellular turgor pressures. Moreover, the extension and retraction curves showed us that the outside surfaces of all biofilm-colonising cells were coated by a soft layer of EPS, which contributed to different patterns of adhesion among different strains of cells due to its different thickness and stickiness. Larger forces and shorter distances were noted in the Gram-positive cells during adhesion events, whereas the Gram-negative cells were characterized by smaller forces and longer distances, reflecting different strains' different biofilm forming abilities.

Currently, many AFM studies were focused on imaging and force measurements together, providing a more in depth understanding of the adhesion mechanisms of biofilms on the surface and leading to new strategies on how to avoid biofilm formation during food processing. Rodriguez et al. used AFM to study the effects of various probe parameters (e.g., probe contact time, probe loading force, probe loading pressure, and probe material) and the relative humidity on the adhesiveness of *Listeria* biofilms at a cellular level, hoping to gain a deeper insight into the mechanism of bacterial transfer from food equipment surfaces to foods, which often happens in food industry (Rodriguez, Autio, & McLandsborough, 2008). They found that *Listeria*

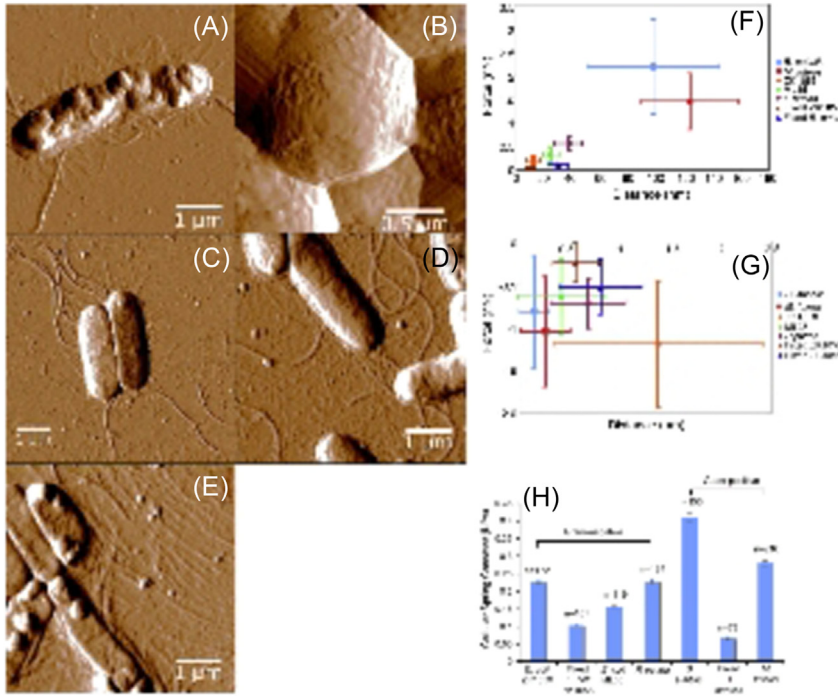

Figure 6.2 Atomic force microscopy (AFM) imaging of biofilms: (A) *Bacillus subtilis*; (B) *Micrococcus luteus*; (C) *Escherichia coli* ZK 1056; (D) *Pseudomonas putida*; and (E) *E. coli* ML 35. AFM force measurement: the distance and force components of the extension curve (F) and retraction curve (G) and cellular spring constants (H) for biofilms and fixed planktonic cells. *All subgraphs are reproduced with permission from Elsevier (From Volle, C. B., Ferguson, M. A., Aidala, K. E., Spain, E. M., & Núñez, M. E. (2008a). Spring constants and adhesive properties of native bacterial biofilm cells measured by atomic force microscopy.* Colloids and Surfaces B: Biointerfaces, *67(1), 32–40. https://doi.org/10.1016/j.colsurfb.2008.07.021).*

grew as single cells or in the form of microcolonies on the surface of stainless steel coupons, instead of colonizing the entire surface. After imaging, the force–distance curves were portrayed, which revealed that the biofilms had a more tendency to adhere to hydrophobic surfaces than hydrophilic surfaces based on the pull-off force measurement by using two different colloidal probes, whereas other parameters mentioned above had little effect on biofilm adhesiveness.

6.3.3 Biofilm dynamic process study

In addition, the dynamic cellular processes within biofilms can also be imaged and monitored by using AFM. Some researchers investigated the

morphological changes of *E. coli* cells during biofilm formation in dilute and rich media, respectively, showing the dynamic process of cell adherence, cell division and reproduction, and EPS excretion under AFM scanning (Núñez, Martin, Chan, & Spain, 2005). Moreover, the researchers also used *Bdellovibrio*, a predatory bacterium that can invade and attack Gram-negative bacteria, as a tool for eliminating *E. coli* biofilms in this study. AFM results demonstrated the dynamic interactions between the predator and prey, such as the morphological changes of *Bdellovibrio* including the structures of flagella and pili involved in their motility and adhesion and the structural changes of *E. coli* including the transformation of shape (Fig. 6.3). Other researchers further investigated the changes of

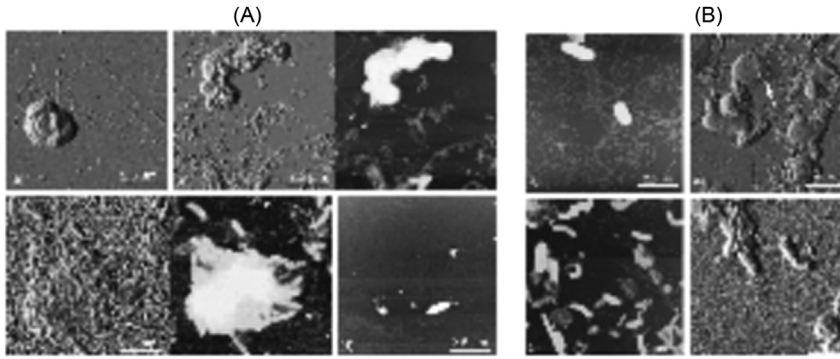

Figure 6.3 The dynamic processes of *Bdellovibrio*s added to *Escherichia coli* biofilm cultured in a dilute medium (A) and rich medium (B), respectively. A: (a) Bdelloplasts, prey cells with predatory *Bdellovibrios* growing inside (image taken after biofilm growing and infected by *Bdellovibrio* for 24 h); (b) Bdelloplasts and *E. coli* cell debris. Left side is a deflection image and right side is a height image (dark to white means low to high) (image taken after biofilm growing and infected by *Bdellovibrio* for 48 and 24 h, respectively); (c) A frantic feeding in progress. Deflection and height images both show the centered Bdelloplasts were surrounded by the well-fed *Bdellovibrio*s (imaging time same as (b)); (d) Within 24–48 h, almost all *E. coli* prey cells were infected. Only debris and *Bdellovibrios* can be found (imaging time the same as (a)). B: (a) A lot of abandoned flagella and a few *E. coli* cells after 24 h biofilm initiation; (b) Bdelloplasts containing *Bdellovibrio*s inside (arrow) after 48 h biofilm initiation; (c) *E. coli* cells were attacked by *Bdellovibrio* predators, most of which were well-fed and healthy, while some of which were narrow and unhealthy after 48 h biofilm initiation; (d) clusters of *E. coli* cells still existed despite not in large communities after 5 days of *Bdellovibrio* attacks. *All subgraphs are reproduced with permission from Elsevier (From Núñez, M. E., Martin, M. O., Chan, P. H., & Spain, E. M. (2005). Predation, death, and survival in a biofilm: Bdellovibrio investigated by atomic force microscopy*. Colloids and Surfaces B: Biointerfaces, 42*(3–4), 263–271. https://doi.org/10.1016/j.colsurfb.2005.03.003).*

elasticity and adhesive properties of *E. coli* biofilms after being invaded by *Bdellovibrio*, finding a series of chemical and physical changes, which can be probed by AFM (Volle et al., 2008a). The invaded prey cells were shorter, rounder, and softer than normal cells, with lower spring constants and larger pull-off forces, indicating a decrease in *E. coli* biofilm's elasticity and an increase in adhesiveness after the invasion by *Bdellovibrio* predator. Overall, these studies open up an opportunity of imaging and measuring dynamic events happened within biofilms.

6.4 Microbial macromolecule studies

In addition to above studies at the cellular level of whole cells, AFM can also be applied to characterize the biomolecules on the surface of microorganisms at the macromolecular level, unraveling key microbial problems by examining the interactions between AFM tips and single molecules. In the force spectroscopy mode, the nanomechanical properties of surface macromolecules such as proteins, nucleic acids, and polysaccharides can be probed and characterized, and when the tip is coated or immobilized with specific molecules, some specific interactions could be measured due to the molecular recognition (Alsteens et al., 2017).

6.4.1 Surface layer protein study

Surface layer (S layer) is a part of the bacterial cell wall and encloses the whole cell surface, which, as the outermost interaction zone, keeps cell safe from the external environment. It consists of monomolecular arrays or double layers of strain-dependent surface layer proteins (Slps) built via self-assembly in vivo, which also possess the natural capability to reassemble into stable crystalline structures in vitro, such as solid surface and the air–water interface, providing a suitable condition for AFM analysis. Some researchers used AFM to explore the structural changes of surface layer protein A (SlpA), which was isolated from *Propionibacterium freudenreichii* and deposited on mica sheet for recrystallization, after being exposed to different environmental conditions. SlpA was found to form a paracrystalline hexagonal lattice on a mica sheet, which was maintained but became softer upon mild heating (Fig. 6.4A). Although its lattice assembly was not modified with increase in temperature, the increasing mobilities of this structure could affect the cell–cell or cell–environment interactions, due to a high proportion of disordered and flexible regions existing in SlpA, which could serve as a brush at the bulk/protein interface. On

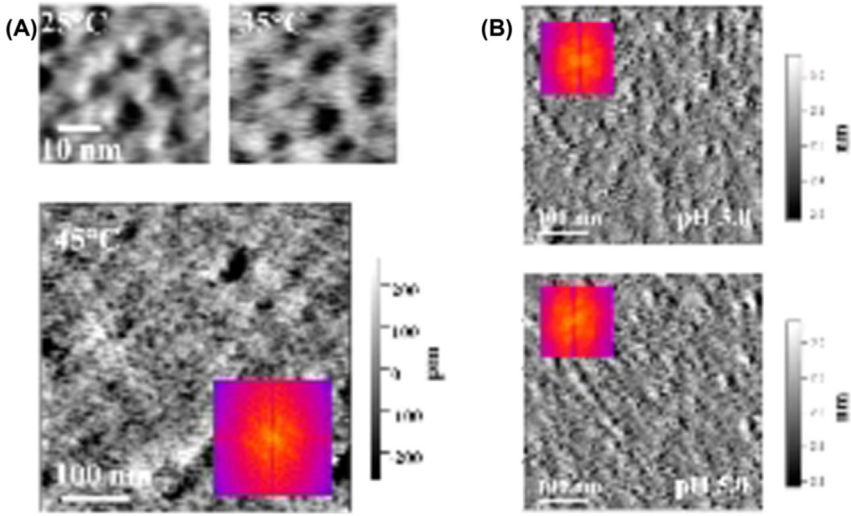

Figure 6.4 Atomic force microscopy images of the recrystallized SlpA layer from *Propionibacterium freudenreichii* PFCIRM 118 strain at pH 6.7 under different temperatures (A) and at 25°C under different pH (B). The red figures inserted are the Fourier-transformed images. *All subgraphs are reproduced with permission from American Chemical Society (From De Sa Peixoto, P., Roiland, C., Thomas, D., Briard-Bion, V., Le Guellec, R., Parayre, S., Deutsch, S. M., Jan, G., & Guyomarc'h, F. (2015). Recrystallized S-layer protein of a probiotic propionibacterium: Structural and nanomechanical changes upon temperature or pH shifts probed by solid-state NMR and AFM.* Langmuir, 31*(1), 199–208. https://doi.org/10.1021/la503735z).*

the other hand, when the environmental pH decreased below the isoelectric point (pI) of the SlpA (∼4.71), the protein film experienced significant changes of protein–water internal bonds (Fig. 6.4B), which was also reported in another study (Toca-Herrera, Moreno-Flores, Friedmann, Pum, & Sleytr, 2004), in which researchers used AFM as well and found visible degradation of the protein film when the pH decreased to 3.0. These results again proved that the environmental stresses can result in structural changes of protein regions existed in S layer films, accompanied by elasticity variations quantified by AFM technique. In another study, wild-type S layer glycoprotein isolated from *Geobacillus stearothermophilu* and its nonglycosylated counterpart on gold-covered sensor surfaces was also examined by AFM, comparing the differences of their recrystallization process and the surface properties in the presence or absence of the glycan residues (Schuster & Sleytr, 2015). They found that the water had a tendency to bind or couple to the outermost glycan residues of the wild-type

glycoprotein, causing higher surface nano-roughness due to the water-exposed glycans.

6.4.2 Surface molecular interaction study

In addition to probing surface layer proteins, other cell surface macromolecules that can generate intercellular interactions and mediate cell adhesion and aggregation behaviors can also be measured by AFM. Some researchers used biofunctionalized probes to investigate the lectin–carbohydrate interactions happening during the flocculation process of yeast cells, which is an important step in food fermentation industry (Touhami, Hoffmann, Vasella, Denis, & Dufrêne, 2003). The adhesion forces between oligoglucose- or lectin-terminated probes and yeast cells under flocculating and nonflocculating conditions were measured, respectively, showing quite different modes of specific interactions between lectins and glucose residues, with around 120 pN adhesion forces recorded under a flocculating condition by using both two functionalized probes, whereas no interaction was observed under a nonflocculating condition. These results provided new insight into the flocculation mechanisms of yeast cells at the molecular level.

Besides lectin and carbohydrate interactions, other molecular recognition, such as enzyme and substrate, ligand and receptor, complementary strands of DNA, antibody and antigen, etc., can also be measured by using biologically modified AFM tip, which plays an important role in detecting foodborne pathogens as part of nanotechnology application in food safety (Hinterdorfer & Dufrêne, 2006). AFM-based methods allow imaging of cells' molecular recognition sites on biointerfaces, followed by mapping a series of kinetic and mechanical properties of binding sites. More importantly, when combined with biofunctionalized probes, AFM can provide more specific and sensitive binding to achieve single molecular interaction detection, which is hard to be realized by conventional tips (Wong, Joselevich, Woolley, Cheung, & Lieber, 1998).

Among many AFM applications based on microbial molecules, the development of specific and sensitive biosensors for microorganism identification is one of the fundamental and critical prospects in food industry, and the antibody–antigen adsorption could be one advanced strategy. Some researchers used AFM to evaluate the surface morphology of the protein A and the antibody attachment to protein A, which is a cell wall protein and has natural affinity towards immunoglobulin molecules for

analyte binding (Lee, Pillai, Singh, & Willing, 2008). On combining the tapping and contact modes, the alterations of average step height between the solid gold surface and the protein A layer were increased from 3.0 ± 1.0 nm to 6.0 ± 1.0 nm, an interesting phenomenon observed when the protein A was attached to antibody. These results indicated that the antibodies grew in an "island model" and adsorbed randomly across the protein A layer, providing partial surface coverage and forming single monolayer on the gold quartz crystals. This study could contribute significantly to the development of more sensitive and specific biosensors to detect microbial contamination in food safety control.

6.5 Representatively reported atomic force microscopy studies about different types of microorganisms

6.5.1 Prokaryotic microorganisms

Prokaryotic cells include bacteria and archaea (Koonin & Wolf, 2008). Although, as mentioned above, a number of bacteria are pathogenic and can cause food poisoning and infectious diseases in humans, most bacteria are harmless, and many are helpful. By characterizing microbial surface properties, AFM can not only provide us with clues for controlling microbial contamination in food industry, but also provide us with the scope for probiotics' characterization, unraveling probiotic properties at the nanometer level, and thus providing more guidance for the application of probiotics in the host's gastro-intestinal tract or in food industrial process.

Lactobacillus species are well-known probiotics normally residing in human's digestive, urinary, and genital systems, helping to break down food and relieve general digestion problems (Heeney, Gareau, & Marco, 2018). Surface properties of lactobacilli explored by AFM could provide us detailed information of different microbial adhesion and aggregation abilities among different strains under native conditions. For example, some researchers used AFM to compare *L. crispatus*, *L. helveticus*, and *L. johnsonii*'s surface topography and molecular interactions, and found the surface of the first two species were covered completely by the S layer, leading to the absence of adhesion peaks on force volume images (Schaer-Zammaretti & Ubbink, 2003), whereas *L. johnsonii* strains showed high adhesion forces and soft gel-like behaviors that might be associated with surfaces rich in polysaccharides or peptidoglycans. This study might

be one of the first attempts to use AFM for investigating the surface properties of different lactic acid bacteria around 20 years ago.

On the other hand, it is well believed that pili play an important role in probiotic-host interactions; however, up to now, a limited number of studies related to the characterization of the pili structure and function in a native state have been conducted, as purifying pili is a challenging and costly task (Spacova, O'Neill, & Lebeer, 2020). Fortunately, some researchers used AFM to investigate the pili structure of *Lacticaseibacillus rhamnosus* GG, which is also one probiotic strain with antipathogenic and immunomodulatory properties (Dos Santos Morais et al., 2020). By dripping bacterial suspension directly on mica substrates, the pili structure could be imaged and their length, height, and thickness could all be measured under AFM examination. Moreover, AFM could even recognize different pilin types based on their molecular size, establishing new mechanistic insights into adhesion capacity of probiotic strains (Kant, Palva, von Ossowski, & Krishnan, 2020). As most fermented foods on the market are probiotic foods, imaging the structural and physicochemical properties of probiotics' surfaces with AFM can have wide-ranging implications for selecting optimal probiotics in food processing.

6.5.2 Eukaryotic microorganisms

Eukaryotes are an extraordinarily diverse group with more complex external and internal structures than prokaryotes (Medinger et al., 2010). Algae, protozoa, fungi, and helminths are all examples of eukaryotic microorganisms, and since their sizes are generally larger than the bacterial sizes, imaging them via AFM becomes more difficult and time-consuming due to an increased height and broader scanned area. Fortunately, by immobilizing them on silicon surface coated with glutaraldehyde, eukaryotic microorganisms such as *Pichia pastoris* (yeast representative) and *Chlorella vulgaris* (algae representative) could be imaged repeatedly and stably by AFM under physiological conditions, without being removed by the AFM probe tip during repeated and long-time scanning (Günther, Suhr, Raff, & Pollmann, 2014).

In addition, considering many fungal pathogens can bind to abiotic surfaces to form biofilms, using AFM to decipher the underlying molecular interactions in fungal adhesion could provide us more strategies for combating fungal infections. For example, some researchers investigated

the role of cell adhesion proteins of *Candida glabrata* by means of AFM (El-Kirat-Chatel et al., 2015). According to the data of hydrophobic force and interaction force obtained from chemical force microscopy (CFM) and single-cell force spectroscopy (SCFS), respectively, Epa6, a principal adhesin protein belonging to Epa family at the cell surface, was found to be responsible for strong hydrophobic interactions between yeast cells and abiotic surfaces, making *C. glabrata* cells attach firmly to hydrophobic substrates. This study showed that CFM and SCFS, the two AFM-based techniques, could provide a molecular basis for fungal biofilm formation, which was critical for the development of an effective biofilm control measure in food industry.

Fungal spores, like the seeds in the plant world, are units of sexual or asexual reproduction serving a purpose for fungal dispersal and survival (Dijksterhuis, 2019). Direct surface measurements of fungal spores can give us more information about spore physical properties during spore germination and outgrowth. One researcher used chemically functionalized AFM probes (i.e., OH- and CH_3-terminated probes) to characterize the spore surface properties (morphology and molecular interactions) of *Phanerochaete chrysosporium*, one saprophytic fungus with great degradation capacity to aromatic polymer lignin (Dufrêne, 2000). This study might be one of the first attempts to use AFM to map spore's topography and measure the adhesion forces between the spore surface and functionalized probes. The direct visualization demonstrated that the spores of *P. chrysosporium* were in clubbed patterns with a lateral diameter of around 10 nm, and the absence of adhesive forces indicated that the spore surface was homogeneously hydrophilic, which contributed to fungal spore's protection and dispersion roles (Dufrêne, 2000).

6.5.3 Viruses

Viruses are acellular microorganisms, and a wide variety of them can cause foodborne outbreaks due to their adhesive nature on food products or food contact surfaces, such as norovirus and hepatitis A virus (Bosch, Pintó, & Guix, 2016). Therefore, understanding the underlying mechanisms of virus adhesion can offer us clues for developing effective virus control protocols to reduce the disease transmission. AFM-based approach in characterizing virus adhesion is gaining ever-increasing popularity. For example, some researchers used AFM to investigate the adhesion features of three bacteriophages (Ms2, Qβ and GA) on abiotic surfaces, and found the interfacial properties and the

hydrophobic/hydrophilic balance of both phages and attachment substrates all affected virions adhering behavior and led to different adhesion capacities (Dika, Ly-Chatain, Francius, Duval, & Gantzer, 2013).

Moreover, other researchers evaluated virus adhesion force on different food contact surfaces and found Ms2 exhibited more adhesion force to polyvinyl chloride (PVC) than to glass under AFM examination (Shim et al., 2017); however, this strong adhesion on PVC could be broken off and weakened when surfactant (e.g., sodium dodecyl sulfate) was incorporated during attachment. The attachment and detachment observations shown in this study could help deepen our understanding of the physicochemical determinants of virus adhesion and potentially enable us to come up with corresponding ways to interrupt virus adhesion to food contact surfaces.

6.6 Combined use of atomic force microscopy with other techniques

Although AFM has been proved to be an invaluable tool to obtain information about the surface morphology and physical properties of microorganisms and macromolecular interactions, there are still some limitations of this "powerful" technique, such as slow scanning speed and difficulty in analyzing soft materials (Yang et al., 2007). Therefore, in order to obtain more comprehensive and detailed information of microorganisms, AFM combined with other complementary techniques seems to be a promising way, offering new possibilities to provide a full characterization of samples. And, in turn, this combination could also solve some key problems of other techniques with the help of AFM.

6.6.1 Atomic force microscopy and infrared combination

Several studies suggest that AFM combined with infrared (IR) spectroscopy could provide both physical and chemical probing of a single bacterium cell in the nanoscale range, overcoming spatial resolution limitation of conventional IR spectroscopy, whose spatial resolution is restricted to several microns (Deniset-Besseau, Prater, Virolle, & Dazzi, 2014; Roman, Wrobel, Panek, Paluszkiewicz, & Kwiatek, 2019). Considering the size of most bacteria fail to reach that minimum threshold (e.g., the diameter of *S. aureus* is only around 400 nm), IR spectroscopy alone is inapplicable to the analysis at single-cell or intracellular level. Fortunately, the combined AFM−IR approach can improve resolution greatly (about 20 nm

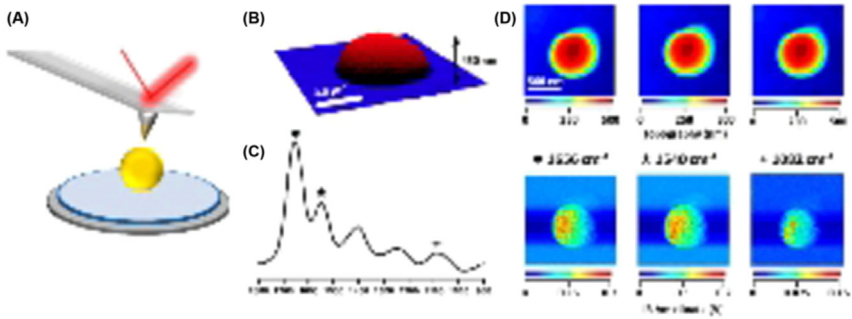

Figure 6.5 An overview of the atomic force microscopy (AFM)−IR working for single-bacterium studies. (A) Setting diagram of the AFM−IR with the AFM cantilever located on the top of a single bacterial cell for probing; (B) AFM height profile of a single *Staphylococcus aureus* cell; (C) AFM−IR spectrum obtained from the middle part of the cell presented in (B); and (D) AFM−IR image of single *S. aureus* cell. First row: height images recorded at the same time with each IR map; Second row: each IR map, representing the corresponding absorbance intensity of marked band (1656, 1540, and 1082 cm^{-1}) in (C). *All subgraphs are reproduced with permission from the American Chemical Society (From Kochan, K., Nethercott, C., Perez-Guaita, D., Jiang, J. H., Peleg, A. Y., Wood, B. R., & Heraud, P. (2019). Detection of antimicrobial resistance-related changes in biochemical composition of Staphylococcus aureus by means of atomic force microscopy-infrared spectroscopy.* Analytical Chemistry, 91*(24), 15397−15403. https://doi.org/10.1021/acs.analchem.9b01671).*

resolution), providing single-cell images and corresponding absorbance intensity of selected wavenumber values from the same chosen spot (Kochan, Peleg, Heraud, & Wood, 2020).

An overview of the working of AFM−IR is given in Fig. 6.5. In brief, the photothermal expansion response generated by the biological sample after absorbing the tuned pulses of IR radiation is the key step for AFM−IR measurement, which can be detected by AFM cantilever for morphology characterization and then trigger the following AFM−IR spectrum collection and multivariate data analysis for absorbance intensity of selected band. The AFM−IR spectrum showed several bands, indicating different kinds of intracellular biological components based on the different position of their functional groups (Fig. 6.5C). More importantly, no localized biochemical differences were found in all of the tested bacteria, demonstrating that a single spectrum collected from one sample can be an indicator of its composition as a whole. This example shows that the AFM−IR method enables detection of biological components at or below the bacterial surface by obtaining information of specific marker bands, which cannot be achieved by each used alone (Kochan et al., 2019).

6.6.2 Atomic force microscopy and Raman spectroscopy combination

The combination of AFM and tip-enhanced Raman spectroscopy (TERS) has received more and more attention recently, as it can provide both morphological and chemical fingerprints simultaneously at the micro- and nanoscale, which is an outstanding advantage in microbiology study (Liu et al., 2012; Rusciano, Zito, Pesce, & Sasso, 2017). Some researchers first used this combined method to investigate *Bacillus subtilis* spores, finding that the TERS-based imaging maps were related to AFM-phase maps, which meant the distribution of biological components in the complicated spore system could be identified and discriminated (Rusciano et al., 2014). Considering *B. subtilis* spores are hard to be inactivated under adverse environmental conditions and several related foodborne outbreaks have been reported (Kramer & Gilbert, 1989), understanding the physicochemical properties of *B. subtilis* spores by using this combined approach could provide important guidance to food decontamination.

6.7 Conclusion and future trends

Since its invention in 1986, AFM has provided a major thrust to microbiological research and its applications, such as characterizing the surface properties as well as manipulating molecular interactions of food microorganisms, contribute a lot to food safety. In this chapter, we have discussed recent progress in the application of AFM for the multiparametric and multifunctional characterization of microbiological systems, which can provide deeper insight into the food microbiology.

As the surface analysis tool, AFM allows high-resolution imaging of cell-surface structures under real-time monitoring conditions close to their native state. Examples include the observation of single cell's surface dynamic changes after environmental treatments and structural differentiation during biofilm development. Besides, many surface physical properties could be quantified by force measurements, such as the adhesion and aggregation behaviors, the stiffness of cell walls, and the surface charge and hydrophobicity. More importantly, through tip functionalization with biomolecules, the studies of molecular interactions and recognitions can be implemented, considering AFM as the only force-measuring method that enables functionalized probe to map the spatial distribution of single molecular binding sites on cellular surfaces. Besides characterizing

foodborne pathogens, imaging the structural and physicochemical properties of probiotics' surfaces with AFM is also helpful in deepening our understanding on the probiotic mechanisms, contributing to a better application in food processing.

Future research could focus more on how to improve the scanning rate of AFM, as current time resolution is still a limiting factor (Shibata, Yamashita, Uchihashi, Kandori, & Ando, 2010). Moreover, the combination of AFM with other complementary optical techniques, such as infrared spectroscopy, Raman spectroscopy, or confocal fluorescence microscopy, has been used recently to obtain more comprehensive information about the structure–function relationship of microorganisms. We expect that in future studies, AFM-based method combined with other modern approaches or suitable functionalized tips could have a broader range of utility in the food microbiology area, to address food safety problems and challenges from the perspective of the structure and function of microbial cell surfaces.

Acknowledgements

This study was funded by Applied Basic Research Project (Agricultural) Suzhou Science and Technology Planning Programme (SNG2020061), Natural Science Foundation of Jiangsu Province (BK20181184), Singapore Ministry of Education Academic Research Fund Tier 1 (A-8000469-00-00), and an industry grant supported by Shenzhen Zhiyun Optoelectronics Co., Ltd. (R-143-000-A24-597).

Declaration of competing interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with this manuscript. We have no financial and personal relationships with other people or organizations that can inappropriately influence our work.

References

Alsteens, D., Gaub, H. E., Newton, R., Pfreundschuh, M., Gerber, C., & Müller, D. J. (2017). Atomic force microscopy-based characterization and design of biointerfaces. *Nature Reviews Materials*, *2*(5). Available from https://doi.org/10.1038/natrevmats.2017.8.

Araújo, G. R. D. S., Viana, N. B., Gómez, F., Pontes, B., & Frases, S. (2019). The mechanical properties of microbial surfaces and biofilms. *Cell Surface*, *5*. Available from https://doi.org/10.1016/j.tcsw.2019.100028.

Bhat, S. V., Booth, S. C., Vantomme, E. A. N., Afroj, S., Yost, C. K., & Dahms, T. E. S. (2015). Oxidative stress and metabolic perturbations in *Escherichia coli* exposed to sub-lethal levels of 2,4-dichlorophenoxyacetic acid. *Chemosphere*, *135*, 453–461. Available from https://doi.org/10.1016/j.chemosphere.2014.12.035.

Bosch, A., Pintó, R. M., & Guix, S. (2016). Foodborne viruses. *Current Opinion in Food Science*, *8*, 110–119. Available from https://doi.org/10.1016/j.cofs.2016.04.002.

Braga, P.C., & Ricci, D. (2004). Atomic force microscopy: Biomedical methods and applications (Vol. 242).

Chen, L., Liu, Q., Zhao, X., Zhang, H., Pang, X., & Yang, H. (2022). Inactivation efficacies of lactic acid and mild heat treatments against *Escherichia coli* strains in organic broccoli sprouts. *Food Control*, *133*, 108577. Available from https://doi.org/10.1016/j.foodcont.2021.108577.

Chen, L., Zhang, H., Liu, Q., Pang, X., Zhao, X., & Yang, H. (2019). Sanitising efficacy of lactic acid combined with low-concentration sodium hypochlorite on *Listeria innocua* in organic broccoli sprouts. *International Journal of Food Microbiology*, *295*, 41–48. Available from https://doi.org/10.1016/j.ijfoodmicro.2019.02.014.

Chen, L., Zhao, X., Wu, J., Liu, Q., Pang, X., & Yang, H. (2020). Metabolic characterisation of eight *Escherichia coli* strains including "Big Six" and acidic responses of selected strains revealed by NMR spectroscopy. *Food Microbiology*, *88*. Available from https://doi.org/10.1016/j.fm.2019.103399.

Cui, Y., Oh, Y. J., Lim, J., Youn, M., Lee, I., Pak, H. K., … Park, S. (2012). AFM study of the differential inhibitory effects of the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) against Gram-positive and Gram-negative bacteria. *Food Microbiology*, *29*(1), 80–87. Available from https://doi.org/10.1016/j.fm.2011.08.019.

De Sa Peixoto, P., Roiland, C., Thomas, D., Briard-Bion, V., Le Guellec, R., Parayre, S., … Guyomarc'h, F. (2015). Recrystallized S-layer protein of a probiotic propionibacterium: Structural and nanomechanical changes upon temperature or pH shifts probed by solid-state NMR and AFM. *Langmuir*, *31*(1), 199–208. Available from https://doi.org/10.1021/la503735z.

Deniset-Besseau, A., Prater, C. B., Virolle, M. J., & Dazzi, A. (2014). Monitoring TriAcylGlycerols accumulation by atomic force microscopy based infrared spectroscopy in Streptomyces species for biodiesel applications. *Journal of Physical Chemistry Letters*, *5*(4), 654–658. Available from https://doi.org/10.1021/jz402393a.

Dijksterhuis, J. (2019). Fungal spores: Highly variable and stress-resistant vehicles for distribution and spoilage. *Food Microbiology*, *81*, 2–11. Available from https://doi.org/10.1016/j.fm.2018.11.006.

Dika, C., Ly-Chatain, M. H., Francius, G., Duval, J. F. L., & Gantzer, C. (2013). Non-DLVO adhesion of F-specific RNA bacteriophages to abiotic surfaces: Importance of surface roughness, hydrophobic and electrostatic interactions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *435*, 178–187. Available from https://doi.org/10.1016/j.colsurfa.2013.02.045.

Dos Santos Morais, R., El-Kirat-Chatel, S., Burgain, J., Simard, B., Barrau, S., Paris, C., … Gaiani, C. (2020). A fast, efficient and easy to implement method to purify bacterial pili from *Lacticaseibacillus rhamnosus* GG based on multimodal chromatography. *Frontiers in Microbiology*, *11*. Available from https://doi.org/10.3389/fmicb.2020. 609880.

Dufrêne, Y. F. (2000). Direct characterization of the physicochemical properties of fungal spores using functionalized AFM probes. *Biophysical Journal*, *78*(6), 3286–3291. Available from https://doi.org/10.1016/S0006-3495(00)76864-0.

El-Kirat-Chatel, S., Beaussart, A., Derclaye, S., Alsteens, D., Kucharíková, S., Van Dijck, P., & Dufrêne, Y. F. (2015). Force nanoscopy of hydrophobic interactions in the fungal pathogen *Candida glabrata*. *ACS Nano*, *9*(2), 1648–1655. Available from https://doi.org/10.1021/nn506370f.

Günther, T. J., Suhr, M., Raff, J., & Pollmann, K. (2014). Immobilization of microorganisms for AFM studies in liquids. *RSC Advances*, *4*(93), 51156–51164. Available from https://doi.org/10.1039/c4ra03874f.

He, Y., Zhao, X., Chen, L., Zhao, L., & Yang, H. (2021). Effect of electrolysed water generated by sodium chloride combined with sodium bicarbonate solution against *Listeria innocua* in broth and on shrimp. *Food Control*, *127*, 108134. Available from https://doi.org/10.1016/j.foodcont.2021.108134.

Heaton, J. C., & Jones, K. (2008). Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: A review. *Journal of Applied Microbiology*, *104*(3), 613–626. Available from https://doi.org/10.1111/j.1365-2672.2007.03587.x.

Heeney, D. D., Gareau, M. G., & Marco, M. L. (2018). Intestinal Lactobacillus in health and disease, a driver or just along for the ride? *Current Opinion in Biotechnology*, *49*, 140–147. Available from https://doi.org/10.1016/j.copbio.2017.08.004.

Hinterdorfer, P., & Dufrêne, Y. F. (2006). Detection and localization of single molecular recognition events using atomic force microscopy. *Nature Methods*, *3*(5), 347–355. Available from https://doi.org/10.1038/nmeth871.

Hyldgaard, M., Sutherland, D. S., Sundh, M., Mygind, T., & Meyer, R. L. (2012). Antimicrobial mechanism of monocaprylate. *Applied and Environmental Microbiology*, *78*(8), 2957–2965. Available from https://doi.org/10.1128/AEM.07224-11.

Kant, A., Palva, A., von Ossowski, I., & Krishnan, V. (2020). Crystal structure of lactobacillar SpaC reveals an atypical five-domain pilus tip adhesin: Exposing its substrate-binding and assembly in SpaCBA pili. *Journal of Structural Biology*, *211*(3), 107571. Available from https://doi.org/10.1016/j.jsb.2020.107571.

Kochan, K., Nethercott, C., Perez-Guaita, D., Jiang, J. H., Peleg, A. Y., Wood, B. R., & Heraud, P. (2019). Detection of antimicrobial resistance-related changes in biochemical composition of Staphylococcus aureus by means of atomic force microscopy-infrared spectroscopy. *Analytical Chemistry*, *91*(24), 15397–15403. Available from https://doi.org/10.1021/acs.analchem.9b01671.

Kochan, K., Peleg, A. Y., Heraud, P., & Wood, B. R. (2020). Atomic force microscopy combined with infrared spectroscopy as a tool to probe single bacterium chemistry. *Journal of Visualized Experiments*, *2020*(163), 1–18. Available from https://doi.org/10.3791/61728.

Koonin, E. V., & Wolf, Y. I. (2008). Genomics of bacteria and archaea: The emerging dynamic view of the prokaryotic world. *Nucleic Acids Research*, *36*(21), 6688–6719. Available from https://doi.org/10.1093/nar/gkn668.

Kramer, J.M., & Gilbert, R.J. (1989). Bacillus cereus and other Bacillus species. Foodborne Bacterial Pathogens (Vol. 19, pp. 21–70).

Kuyukina, M. S., Ivshina, I. B., Korshunova, I. O., & Rubtsova, E. V. (2014). Assessment of bacterial resistance to organic solvents using a combined confocal laser scanning and atomic force microscopy (CLSM/AFM). *Journal of Microbiological Methods*, *107*, 23–29. Available from https://doi.org/10.1016/j.mimet.2014.08.020.

Lee, K. G., Pillai, S. R., Singh, S. R., & Willing, G. A. (2008). The investigation of protein A and Salmonella antibody adsorption onto biosensor surfaces by atomic force microscopy. *Biotechnology and Bioengineering*, *99*(4), 949–959. Available from https://doi.org/10.1002/bit.21644.

Lim, J., Lee, K. M., So, H. K., Nam, S. W., Yoo, J. O., Hyun, S. Y., … Park, S. (2008). Nanoscale characterization of *Escherichia coli* biofilm formed under laminar flow using atomic force microscopy (AFM) and scanning electron microscopy (SEM). *Bulletin of the Korean Chemical Society*, *29*(11), 2114–2118. Available from https://doi.org/10.5012/bkcs.2008.29.11.2114.

Liu, Q., Jin, X., Feng, X., Yang, H., & Fu, C. (2019). Inactivation kinetics of *Escherichia coli* O157:H7 and *Salmonella typhimurium* on organic carrot (*Daucus carota* L.) treated with low concentration electrolyzed water combined with short-time heat treatment. *Food Control*, *106*, 106702. Available from https://doi.org/10.1016/j.foodcont.2019. 06.028.

Liu, Q., Wei, L., Wang, J., Peng, F., Luo, D., Cui, R., ... Li, Y. (2012). Cell imaging by graphene oxide based on surface enhanced Raman scattering. *Nanoscale*, *4*(22), 7084–7089. Available from https://doi.org/10.1039/c2nr32525j.

Medinger, R., Nolte, V., Pandey, R. V., Jost, S., Ottenwälder, B., Schlötterer, C., & Boenigk, J. (2010). Diversity in a hidden world: Potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. *Molecular Ecology*, *19*(1), 32–40. Available from https://doi.org/10.1111/j.1365-294X.2009.04478.x.

Núñez, M. E., Martin, M. O., Chan, P. H., & Spain, E. M. (2005). Predation, death, and survival in a biofilm: Bdellovibrio investigated by atomic force microscopy. *Colloids and Surfaces B: Biointerfaces*, *42*(3–4), 263–271. Available from https://doi.org/10.1016/j.colsurfb.2005.03.003.

Papaioannou, E., Giaouris, E. D., Berillis, P., & Boziaris, I. S. (2018). Dynamics of biofilm formation by *Listeria monocytogenes* on stainless steel under mono-species and mixed-culture simulated fish processing conditions and chemical disinfection challenges. *International Journal of Food Microbiology*, *267*, 9–19. Available from https://doi.org/10.1016/j.ijfoodmicro.2017.12.020.

Park, S. H., Cheon, H. L., Park, K. H., Chung, M. S., Choi, S. H., Ryu, S., & Kang, D. H. (2012). Inactivation of biofilm cells of foodborne pathogen by aerosolized sanitizers. *International Journal of Food Microbiology*, *154*(3), 130–134. Available from https://doi.org/10.1016/j.ijfoodmicro.2011.12.018.

Rodriguez, A., Autio, W. R., & McLandsborough, L. A. (2008). Effects of contact time, pressure, percent relative humidity (%RH), and material type on Listeria biofilm adhesive strength at a cellular level using atomic force microscopy (AFM). *Food Biophysics*, *3*(3), 305–311. Available from https://doi.org/10.1007/s11483-008-9085-4.

Roman, M., Wrobel, T. P., Panek, A., Paluszkiewicz, C., & Kwiatek, W. M. (2019). Nanoscale AFM-IR spectroscopic imaging of lipid heterogeneity and effect of irradiation in prostate cancer cells. *Nanotechnology*, *30*(42). Available from https://doi.org/10.1088/1361-6528/ab31dd.

Rusciano, G., Zito, G., Isticato, R., Sirec, T., Ricca, E., Bailo, E., & Sasso, A. (2014). Nanoscale chemical imaging of bacillus subtilis spores by combining tip-enhanced raman scattering and advanced statistical tools. *ACS Nano*, *8*(12), 12300–12309. Available from https://doi.org/10.1021/nn504595k.

Rusciano, G., Zito, G., Pesce, G., & Sasso, A. (2017). Cell imaging by spontaneous and amplified Raman spectroscopies. *Journal of Spectroscopy*, *2017*. Available from https://doi.org/10.1155/2017/2193656.

Schaer-Zammaretti, P., & Ubbink, J. (2003). Imaging of lactic acid bacteria with AFM—Elasticity and adhesion maps and their relationship to biological and structural data. *Ultramicroscopy*, *97*(1–4), 199–208. Available from https://doi.org/10.1016/S0304-3991(03)00044-5.

Schuster, B., & Sleytr, U. B. (2015). Relevance of glycosylation of S-layer proteins for cell surface properties. *Acta Biomaterialia*, *19*, 149–157. Available from https://doi.org/10.1016/j.actbio.2015.03.020.

Shibata, M., Yamashita, H., Uchihashi, T., Kandori, H., & Ando, T. (2010). High-speed atomic force microscopy shows dynamic molecular processes in photoactivated bacteriorhodopsin. *Nature Nanotechnology*, *5*(3), 208–212. Available from https://doi.org/10.1038/nnano.2010.7.

Shim, J., Stewart, D. S., Nikolov, A. D., Wasan, D. T., Wang, R., Yan, R., & Shieh, Y. C. (2017). Differential MS2 interaction with food contact surfaces determined by atomic force microscopy and virus recovery. *Applied and Environmental Microbiology*, *83*(24). Available from https://doi.org/10.1128/AEM.01881-17.

Spacova, I., O'Neill, C., & Lebeer, S. (2020). Lacticaseibacillus rhamnosus GG inhibits infection of human keratinocytes by *Staphylococcus aureus* through mechanisms involving cell surface molecules and pH reduction. *Beneficial Microbes*, *11*(7), 703–715. Available from https://doi.org/10.3920/BM2020.0075.

Surveillance for Foodborne Disease Outbreaks. (2019). Centers for Disease Control and Prevention (CDC).

Toca-Herrera, J. L., Moreno-Flores, S., Friedmann, J., Pum, D., & Sleytr, U. B. (2004). Chemical and thermal denaturation of crystalline bacterial S-layer proteins: An atomic force microscopy study. *Microscopy Research and Technique*, *65*(4–5), 226–234. Available from https://doi.org/10.1002/jemt.20127.

Touhami, A., Hoffmann, B., Vasella, A., Denis, F. A., & Dufrêne, Y. F. (2003). Aggregation of yeast cells: Direct measurement of discrete lectin-carbohydrate interactions. *Microbiology*, *149*(10), 2873–2878. Available from https://doi.org/10.1099/mic.0.26431-0.

Tyagi, A. K., & Malik, A. (2010). In situ SEM, TEM and AFM studies of the antimicrobial activity of lemon grass oil in liquid and vapour phase against *Candida albicans*. *Micron*, *41*(7), 797–805. Available from https://doi.org/10.1016/j.micron.2010. 05.007.

Van der Aa, B. C., Asther, M., & Dufrêne, Y. F. (2002). Surface properties of *Aspergillus oryzae* spores investigated by atomic force microscopy. *Colloids and Surfaces B: Biointerfaces*, *24*(3–4), 277–284. Available from https://doi.org/10.1016/S0927-7765(01)00277-6.

Volle, C. B., Ferguson, M. A., Aidala, K. E., Spain, E. M., & Núñez, M. E. (2008a). Quantitative changes in the elasticity and adhesive properties of *Escherichia coli* ZK1056 prey cells during predation by *Bdello vibrio* bacteriovorus 109. *Journal of Langmuir*, *24*(15), 8102–8110. Available from https://doi.org/10.1021/la8009354.

Volle, C. B., Ferguson, M. A., Aidala, K. E., Spain, E. M., & Núñez, M. E. (2008b). Spring constants and adhesive properties of native bacterial biofilm cells measured by atomic force microscopy. *Colloids and Surfaces B: Biointerfaces*, *67*(1), 32–40. Available from https://doi.org/10.1016/j.colsurfb.2008.07.021.

Wong, S. S., Joselevich, E., Woolley, A. T., Cheung, C. L., & Lieber, C. M. (1998). Covalently functionalized nanotubes as nanometresized probes in chemistry and biology. *Nature*, *394*(6688), 52–55. Available from https://doi.org/10.1038/27873.

Wright, C. J., Shah, M. K., Powell, L. C., & Armstrong, I. (2010). Application of AFM from microbial cell to biofilm. *Scanning*, *32*(3), 134–149. Available from https://doi.org/10.1002/sca.20193.

Yang, H., Wang, Y., Lai, S., An, H., Li, Y., & Chen, F. (2007). Application of atomic force microscopy as a nanotechnology tool in food science. *Journal of Food Science*, *72*(4), R65–R75. Available from https://doi.org/10.1111/j.1750-3841.2007.00346.x.

Zhang, J., & Yang, H. (2017). Effects of potential organic compatible sanitisers on organic and conventional fresh-cut lettuce (*Lactuca sativa* Var. Crispa L). *Food Control*, *72*, 20–26. Available from https://doi.org/10.1016/j.foodcont.2016.07.030.

Zhao, L., Zhang, Y., & Yang, H. (2017). Efficacy of low concentration neutralised electrolysed water and ultrasound combination for inactivating *Escherichia coli* ATCC 25922, *Pichia pastoris* GS115 and *Aureobasidium pullulans* 2012 on stainless steel coupons. *Food Control*, *73*, 889–899. Available from https://doi.org/10.1016/j.foodcont.2016.09.041.

Zhao, L., Zhao, M. Y., Phey, C. P., & Yang, H. (2019). Efficacy of low concentration acidic electrolysed water and levulinic acid combination on fresh organic lettuce (*Lactuca sativa* Var. Crispa L.) and its antimicrobial mechanism. *Food Control*, *101*, 241–250. Available from https://doi.org/10.1016/j.foodcont.2019.02.039.

Zhao, X., Chen, L., Zhao, L., He, Y., & Yang, H. (2020). Antimicrobial kinetics of nisin and grape seed extract against inoculated *Listeria monocytogenes* on cooked shrimps: Survival and residual effects. *Food Control*, *115*, 107278. Available from https://doi.org/10.1016/j.foodcont.2020.107278.