Trends in Food Science & Technology 128 (2022) 102–117

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Review

Microfluidics at the interface of bacteria and fresh produce

Mohsen Ranjbaran [a,b], Mohit S. Verma [a,b,c,*]

[a] Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN 47907, USA
[b] Birck Nanotechnology Center, Purdue University, West Lafayette, IN 47907, USA
[c] Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN 47907, USA

ARTICLE INFO

Keywords:
Micropatterned surfaces
Cultivating devices
Biosensors
Nucleic acid amplification

ABSTRACT

Background: Understanding interactions between bacteria and fresh produce is of practical importance in the prevention and mitigation of foodborne and plant diseases. Microfluidic approaches, which usually operate at the same scale as produce–bacteria interactions, provide a platform for understanding interactions and detecting contamination.

Scope and Approach: Here, we review microfluidic approaches suitable for use in fresh produce research and industry, classifying them based on their applications. The microfluidic devices were either used to unravel underlying mechanisms of fresh produce contamination or to develop detection tools for bacteria present on the produce. Three classes of microfluidic devices are discussed based on their common designs, as well as their applications in both fresh produce safety and sustainable production.

Key Findings and Conclusions: The three classes of microfluidic devices are: (1) micropatterned surfaces (either with natural topography or simplified topography) used in the investigation of microbial attachment, infiltration, growth, and inactivation at the surface of the produce; (2) microfluidic cultivating devices, which consist of microcavities with a controlled environment for the growth and interaction of microbes and host; and (3) biosensing devices, which are portable analytical tools for the detection of microbial presence on produce. Across these three classes of devices, we illustrate that microfluidics can help understand the interactions between bacteria and produce as well as aid in the detection of nucleic acids from contaminant microbes in a portable format. The concise review of all microfluidic approaches presented here promises to promote future research and education in this area.

1. Introduction

With the ever-increasing popularity of fresh produce around the world, the number of foodborne illness outbreaks associated with fresh produce has been on the rise (Mostafidi, Sanjabi, Shirkhan, & Tamaskani Zahedi, 2020). *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* are major pathogens responsible for outbreaks of foodborne illnesses linked to fresh produce (CDC, 2021; Han et al., 2020). Once the fresh produce is contaminated, either in the field or during post-harvest operations, its full sanitization may not be possible, and it is no longer safe for consumption. To reduce the risk posed by pathogens, it is important to understand the pathway of contamination (Ranjbaran & Datta, 2019; Ranjbaran, Solhtalab, & Datta, 2020), develop preventive measures, and provide contamination detection tools and protocols (Choi, Yong, Choi, & Cowie, 2019). In addition to addressing food safety concerns, understanding produce–bacteria interactions and detecting microbial presence can also support the sustainable production of fresh produce. This benefit is possible through prevention and early detection of plant diseases to avoid outbreaks (Griesche & Baeumner, 2020). Also, water and soil quality as well as plant development can most likely be monitored and thereby managed. These measures can reduce the over-application of fertilizers and pesticides and improve the level of plant growth-promoting bacteria in the rhizosphere (Massalha, Korenblum, Malitsky, Shapiro, & Aharoni, 2017). Another potential advantage is to help manage and reduce food waste, whereby the microbial presence could be dealt with immediately (e.g., by redirecting contaminated product to a different production stream) before reaching the consumer.

Contamination of fresh produce by bacteria can take place as a result of bacterial attachment to the surface and/or infiltration into the produce tissue (Fig. 1a). Similar interactions also take place when beneficial bacteria colonize a plant surface. The contamination can be analyzed using several approaches. For instance, some of the common methods used in previous studies are shown in Fig. 1b, including plating methods (Ranjbaran et al., 2020), microscopy imaging (Harmon, Gray,

* Corresponding author at: Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN 47907, USA.
E-mail address: msverma@purdue.edu (M.S. Verma).

https://doi.org/10.1016/j.tifs.2022.07.014
Received 10 June 2021; Received in revised form 7 July 2022; Accepted 20 July 2022
Available online 30 July 2022

List of abbreviations

ATCC,	American Type Culture Collection
BFP,	Blue fluorescent protein
CDC,	Centers for Disease Control and Prevention
CFU,	Colony forming unit
DNA,	Deoxyribonucleic acid
EcoFAB,	Ecosystem Fabrication
FDA,	Food and Drug Administration
FTA,	Fast Technology Analysis
GFP,	Green fluorescent protein
HDA,	Helicase dependent amplification
ISDPR,	Isothermal strand displacement polymerization
LAMP,	Loop-mediated isothermal amplification
LAF,	Lateral flow assay
MST,	Microbial source tracking
PCR,	Polymerase chain reaction
PDMS,	Polydimethylsiloxane
PMMA,	Poly(methyl methacrylate)
PVC,	Polyvinyl chloride
POC,	Point-of-care
RCA,	Rolling circle amplification
RPA,	Recombinase polymerase amplification
rRNA,	Ribosomal ribonucleic acid
SDA,	Strand displacement amplification
SEM,	Scanning electron microscopy
TRIS,	Tracking root interactions system
TSB,	Tryptic soy broth
UV,	Ultraviolet
μPAD,	Microfluidic paper-based analytical devices

Young, & Schwab, 2020), microfluidic approaches (Ranjbaran & Datta, 2019), optical density assays (Terry, White, & Tigwell, 2005), and deoxyribonucleic acid (DNA) amplification techniques (Han et al., 2020). Application of these analytical approaches has led to the understanding of the contamination pathways, as well as detection and quantification of the bacterial populations on or within the fresh produce (Fig. 1c). Often, a combination of these approaches is used.

Microfluidics is the science and technology of manipulating liquids within channels that are tens to hundreds of micrometers wide (Whitesides, 2006). Microfluidics is useful for revealing the produce–bacteria interaction pathways, primarily because microfluidic devices work at the same scale at which the interaction (contamination) takes place. Microfluidics can also be applied to design portable analytical tools, such as biosensors, to detect bacteria at the point of need (e.g., for future applications in farms or processing plants). In general, microfluidic approaches can be used in three different classes for the analysis of produce–bacteria interactions:

(1) Micropatterned surfaces (Fig. 2a) are artificial surrogates of the produce surface and can be used to study how bacteria interact with the microstructures of the produce surface during attachment (Sirinutsomboon, Delwiche, & Young, 2011), infiltration (Ranjbaran & Datta, 2019), growth, and inactivation (Zhang et al., 2014).

(2) Microfluidic cultivating devices (Fig. 2b) provide controlled microenvironments for the host and bacterial cells to grow and interact within microcavities of various shapes and sizes (Burmeister & Grunberger, 2020). So far, they have been utilized to investigate and visualize rhizosphere interactions between roots and plant growth-promoting bacteria as well as bacterial pathogens (Massalha et al., 2017).

(3) Biosensing devices (Fig. 2c) are referred to as portable analytical tools that are applied to the detection of various microbial species. While there are various types of biosensing techniques available today (Choi et al., 2019), we review the most widely used ones in fresh produce research that utilize nucleic acids or antibodies as their biorecognition elements.

None of the available review papers (Choi et al., 2019; Mi et al., 2022; Weng & Neethirajan, 2017)—discussing microfluidics for food safety—primarily focus on fresh produce applications and provide practical guidelines for implementation in various stages of fresh produce production and processing. In this review, we highlight various microfluidic approaches in current or prospective use with the aim of understanding produce–bacteria interactions and promoting the on-site detection of bacterial presence on or within fresh produce. For the onsite detection, the major focus is to promote the design of portable fully-integrated biosensors that can be used by non-specialist personnel. All three classes (described above) are discussed in terms of their typical designs and applications. This first-ever concise classification should help promote further applications of microfluidic approaches in fresh produce safety and sustainable production by training food and biological scientists and engineers.

2. Micropatterned surfaces

Plant surfaces are covered with diverse types of microstructures. For example, on a leaf surface (see Fig. 3), stomata are micropores at the cuticle layer that facilitate gas exchange between the leaf interior and the outside environment (Ranjbaran et al., 2020), grooves are the valleys created between the epidermal cells, and trichomes are fine outgrowths that generally contribute to the reduction of water loss and prevent the entry of microorganisms (Ranjbaran & Datta, 2019). On a plant surface, there is a high diversity of these microstructures as well as variations of local hydrophobicity, causing difficulty in understanding the influence of the microstructures on microbial contamination. Artificial surfaces patterned with plant microstructures create platforms to minimize these complexities (Doan & Leveau, 2015) and function at prescribed hydrophobicity levels. These micropatterned surfaces have been made from various materials such as polydimethylsiloxane (PDMS) (Ranjbaran & Datta, 2019), agarose (Zhang et al., 2014), gelatin (Soffe, Altenhuber et al., 2019), and silicon (Sirinutsomboon et al., 2011). Below, we classify the micropatterned surfaces based on their topography and area of application in fresh produce microbial safety.

2.1. Typical designs for micropatterned surfaces

In Fig. 3, we classify the micropatterned surfaces based on their topography types. A device with a natural topography mimics the exact shapes of the microstructures available at the produce surface. Fig. 3a shows the three-dimensional optical microscopy images of stomata and grooves on a fresh spinach leaf and its PDMS replica. A two-step replica molding method is used to create the natural topography (Kumari et al., 2020; Zhang et al., 2014). In the first step, a PDMS stamp with reversed microstructure is provided. For this purpose, produce tissue (e.g., a piece of a leaf) that is already securely taped at the bottom of a dish is covered by a PDMS mixture. After curing the PDMS at a suitable temperature (e.g., 40 °C) and for a sufficient period (e.g., 12 h), the mold is peeled off the leaf and undergoes a surface chemical treatment (e.g., a coating with palladium nanoparticles) to create a nonadhesive surface. In the second step, the fabricated molds are used to create the final patterned surfaces with natural microstructures.

Another class of micropatterned surfaces uses simplified, highly ordered microstructures with a high degree of symmetry. For example,

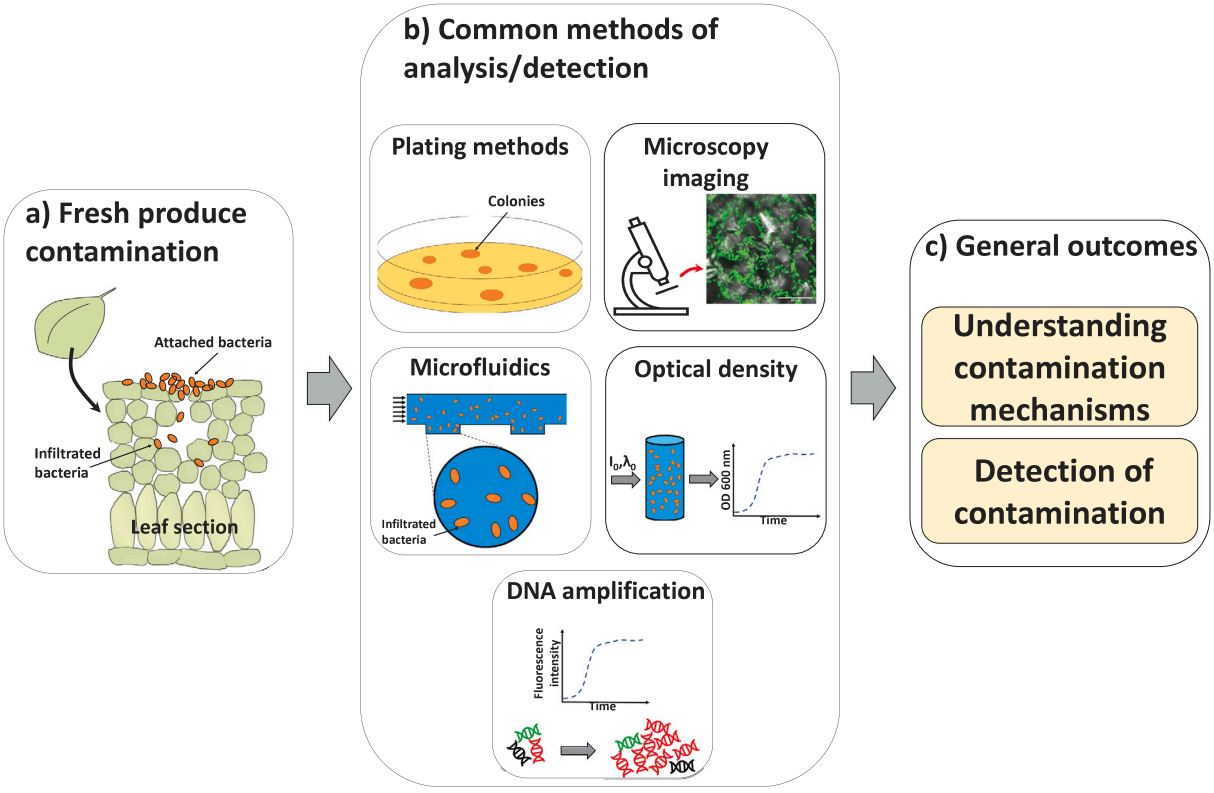

Fig. 1. An overview of various approaches used for understanding and detecting microbial contamination of fresh produce. (a) A schematic of bacterial attachment to and infiltration of leafy greens. The bacteria located at the leaf surface can infiltrate it through stomata, cracks, or wounds (Ranjbaran et al., 2020). (b) The common methods of analysis and detection that have been used in the area of fresh produce microbial safety. Plating methods refer to growing viable bacterial colonies on an agar gel containing growth medium at a prescribed incubation temperature. In microscopy imaging, the bacteria are usually tagged with a fluorescent protein to be distinguishable from the background substrate when they are seen through a fluorescence or confocal microscope. The inset microscopy image shown here illustrates the distribution of bacteria, tagged with green fluorescence protein, on a spinach leaf surface (Ranjbaran, 2019). In the schematic of a microfluidic device shown here, the bacterial behavior is analyzed as they pass through the device or deposit in the microwells. The optical density is used during spectrophotometry to detect the bacterial concentration within a liquid culture. In DNA amplification approaches, a target DNA is amplified and detected using fluorometric, colorimetric, or any other detection approach. (c) The two general outcomes that can be obtained from the analysis and detection methods..

stomata, grooves, and trichomes have been simplified to elliptical micropores, rectangular microchannels, and cylindrical microcolumns, respectively. Fig. 3b shows scanning electron microscopy (SEM) image of trichomes on a tomato leaf along with a silicon wafer patterned with microcolumns representing the trichomes (Sirinutsomboon et al., 2011). In recent studies, the microstructures were fabricated out of PDMS with a three-step fabrication process (e.g., Ranjbaran & Datta, 2019). In the first step, a photomask is prepared for each type of microstructure, typically using laser mask writer machines. In the second step, molds patterned with a reverse microstructure are made using a photolithography technique. A photoresist (liquid or solid) is coated on a silicon wafer and exposed to ultraviolet (UV) light passing through the photomask (from the first step). The thickness of the photoresist determines the height or depth of the microstructures on the final PDMS device. After developing the photoresist, the mold surface is treated to create a nonadhesive surface. In the third step, PDMS is cast on the molds to create the final device. See Table 1 for more details on the fabrication methods of both classes of micropatterned surfaces.

In the natural topography class, the microstructures match the real produce surface, providing the opportunity to study the effect of real microstrutures on microbial contamination. Understanding the sole effects of each microstructure type, size, and distribution on the contamination is possible using the simplified topography class. The simplified topography class can be more reproducible since it does not inherit its patterns from a natural leaf that has inherent variability and diversity. Fabrication of the natural topography devices is more challenging. These challenges include: (1) shriveling of the leaf due to excessive heat while curing the PDMS, resulting in inaccurate molding, (2) some leaves are fragile and limit the PDMS molding with high fidelity, (3) presence of moisture at the surface of the leaf, leading to inefficient curing of the PDMS over the entire leaf surface, and (4) after curing, some leaf residue may still stick to the PDMS (Soffe, Bernach, Remus-Emsermann, & Nock, 2019). To overcome these issues, one can try: (1) using a higher PDMS base to curing agent ratio to fabricate the leaf imprint, (2) slightly drying the leaf surface before pouring the PDMS on it, (3) curing the PDMS at a reasonably low temperature (around 40 °C–45 °C) for longer duration (around 12 h–20 h), and (4) placing the cured PDMS imprints in a leaf digestion solution to remove the leaf residues (Soffe, Bernach et al., 2019). Various applications of these micropatterned surfaces in understanding produce–bacteria interactions are described in Section 2.2.

2.2. Investigation of microbial contamination using micropatterned surfaces

Micropatterned surfaces have been utilized to study various phases of microbial contamination of plant leaves including bacterial attachment, infiltration, growth, and inactivation (Bernach, Soffe, Remus-Emsermann, & Nock, 2019; Doan, Antequera-Gómez et al., 2020b; Doan, Ngassam et al., 2020a; Ranjbaran & Datta, 2019; Sirinutsomboon et al., 2011; Soffe, Altenhuber et al., 2019; Soffe, Bernach et al., 2019; Zhang et al., 2014). An overview of the applications of micropatterned surfaces in investigations of plant–microbe interactions is provided in Table 1. In this section, representative examples of each application related to fresh produce safety are highlighted along with their notable results.

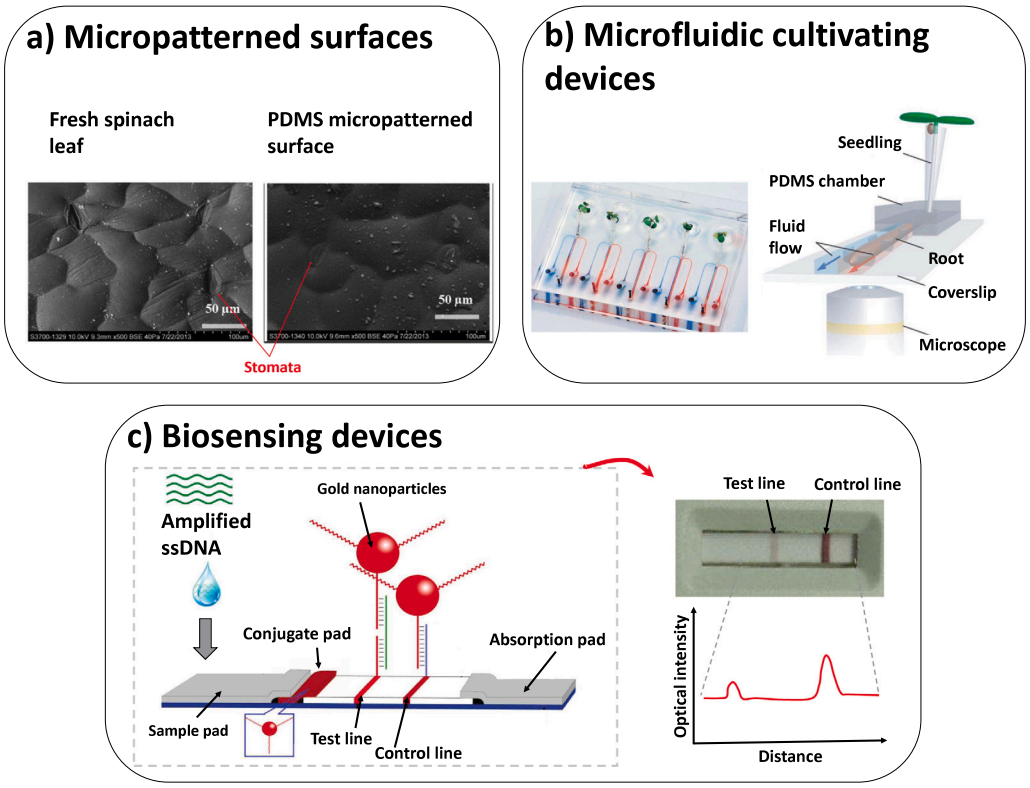

Fig. 2. An overview of various applications of microfluidics approaches to understanding and detecting microbial contamination of fresh produce.(a) micropatterned artificial surfaces, that are surrogates of the produce surface used to investigate bacterial interaction with the microstructures of the produce surface during attachment, infiltration, growth, and inactivation. The inset images were obtained from scanning electron microscopy of a fresh spinach leaf and the corresponding PDMS micropatterned surface. The images were reproduced from Zhang et al. (2014, copyright 2014 American Chemical Society) with permission from the publisher. (b) microfluidic cultivating devices, that provide controlled microenvironments for the bacteria and host to grow and interact within microcavities with various shapes and sizes. The inset image shows a microfluidic platform (dual-flow-RootChip) to grow Arabidopsis roots within microchannels. Using such a microfluidic platform, the live interaction of the plant root and bacteria can be observed using microscopy imaging through the transparent PDMS. The images were reproduced from Stanley et al. (2018, copyright 2017 John Wiley and Sons) with permission from the publisher. (c) biosensing devices, that are used to detect presence of microbial species in an environment. Here, the image shows a schematic of an aptamer-based lateral flow biosensor for detection of *E. coli* O157:H7. A sample containing the amplified single strand DNA (ssDNA) is added to the sample pad wherein it spreads through capillary action. At the conjugate pad, the ssDNA is captured by probe–gold nanoparticle (AuNP) conjugates through a complementary reaction between the probe on the AuNP surface and the ssDNA. The obtained ssDNA-probe-AuNP complex is then captured at the test line, by immobilized complementary strands of the target ssDNA, at which a color change indicates the detection of the target DNA. The excess probe-AuNP complex is either captured at the control line or absorbed at the absorption pad. The inset images were reproduced from Wu et al. (2015, copyright 2015 Elsevier) with permission from the publisher.

Sirinutsomboon et al. (2011) studied the effect of various surface microstructures on bacterial attachment to artificial plant leaves. The device included micropatterned silicon surfaces with simplified topography of various types, sizes, and spacing (see Fig. 3b), located vertically within a shaking beaker with a flow of bacterial culture over it (Fig. 4a). The bacterial strain was *E. coli* O137:H41, obtained from field cabbage contaminated by sewage water and then tagged with green fluorescent protein (GFP). The beaker was shaken for 48 h and the micropatterned surfaces were washed with distilled water for 5 s afterward to remove the unattached bacteria. The authors showed that microstructure geometry and position affected bacterial attachment. For example, the level of attached bacteria in an area with an array of trichomes was about half of that for the stomata or grooves. The bacterial attachment was observed to be higher within the area 2.5–5.0 μm away from stomatal pores. This work was a piece of evidence for the potential application of micropatterned surfaces (discussed in Section 2.3) in fresh produce safety assessment. A recent review of the effects of surface micro- and nanostructures on the bacterial attachment to a surface can be seen in Cheng, Feng, and Moraru (2019).

Ranjbaran and Datta (2019) studied the effect of evaporation of sessile droplets, located on plant leaves, on bacterial infiltration into the stomatal pores. The device included an artificial leaf surface, made out of PDMS, patterned with stomatal features of different sizes and spacing, with a simplified topography. The patterned surface was mounted on a coverslip. During the experiments, a 1-μl droplet containing a known concentration of GFP-tagged *E. coli* RP437 was placed on a micropatterned surface and allowed to evaporate in approximately 15 minutes (Fig. 4a). Given the transparency of the PDMS surfaces, the evaporation-driven bacterial transport and infiltration were observed, from the bottom, using an inverted confocal microscope. It was shown that larger size and wider spacing of the micropores (i.e., stomatal features), as well as a more hydrophilic surface, led to more infiltration. Also, it was highlighted that a stick–slip behavior of the contact line of the droplet contributed to more infiltration into stomata. This was because the sticking of the contact line to the stomatal pores increased the time scale at which evaporation-driven internal flows could transport bacteria into the stomata that facilitated their infiltration. This work showed that even very specific pathways of contamination (i.e., in this case, evaporation-driven infiltration) can be explored in detail using micropatterned surfaces, which contributes to the development of mitigation strategies to avoid produce contamination.

Zhang et al. (2014) fabricated patterned surfaces, mimicking spinach leaves, with natural topography (Fig. 3a) and applied them to study bacterial growth at the leaf surface. The device was fabricated out of agarose with or without 10% tryptic soy broth supplements (TSB as nutrient source). The bacterial strain was *E. coli* tagged with blue fluorescent protein (BFP). In their experiments, a 1-cm^2 area of the patterned surface was inoculated with 100 μl of the bacterial suspension. The samples were then incubated at 25 °C for 24 h. The bacterial

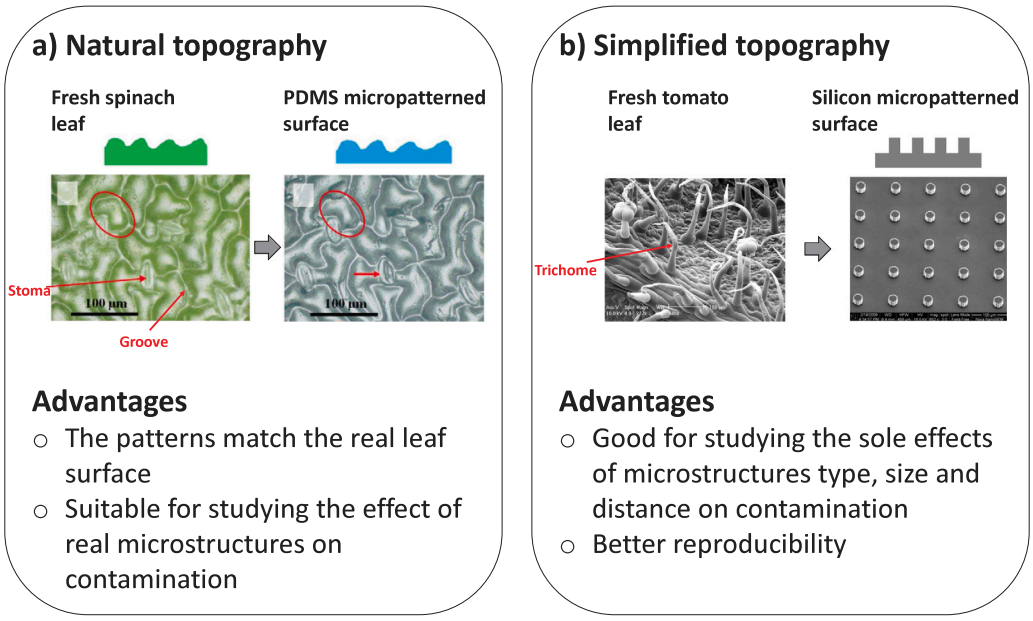

Fig. 3. Classification of micropatterned surfaces used for analysis of plant–microbe interactions, based on the type of surface topography. (a) 3D microscopy images of natural topography on fresh spinach leaf and the corresponding micropatterned surface made from polydimethylsiloxane (PDMS). The inset images were reproduced from Zhang et al. (2014, copyright 2014 American Chemical Society), with permission from the publisher. (b) Scanning electron microscopy images of trichomes at a fresh tomato leaf, and simplified trichomes at a silicon surface. The inset images were reproduced from Sirinutsomboon, Delwiche, and Young (2011, copyright 2011 Elsevier), with permission from the publisher. The advantages of the natural and simplified topography types are highlighted.

Table 1
An overview of the applications of micropatterned surfaces in investigating plant–microbe processes with potential applications in fresh produce microbial safety and sustainable production.

Device category	Mold fabrication	Mold treatment	Device fabrication	Microorganisms	Investigated process	Test time	Reference
Simplified topography	–	–	Photolithography followed by silicon etching	*Escherichia coli* O137:H41	Attachment to fabricated leaf microstructures	48 h	Sirinutsomboon et al. (2011)
	Photolithography on photoresists sheets	FOTS deposition	PDMS casting on molds	*E. coli* RP437	Retention and infiltration on fabricated microstructures during sessile droplets evaporation	15 min	Ranjbaran and Datta (2019)
	Photolithography on photoresists sheets followed by template PDMS casting	FOTS deposition	Casting agarose solution, gelatin solution, or PDMS on the PDMS template	*Pantoea agglomerans*	Survival on leaf replicas of different materials	0–72 h	Soffe, Altenhuber, Bernach, Remus-Emsermann, and Nock (2019)
Natural topography	Casting PDMS on spinach leaves	Application of palladium nanoparticles	Casting PDMS or agarose solution on the molds	*E. coli*	Growth and inactivation on the devices	12 h	Zhang et al. (2014)
	Casting PDMS on a root surface	FOTS treatment	Casting PDMS on the molds	*Ralstonia solanacearum* K60	Colonization of on the tomato root replica	Overnight	Kumari et al. (2020)
	Casting PDMS on a leaf and digesting leaf residue from the PDMS	FOTS deposition	Casting PDMS on the molds	*P. agglomerans* 299R and *Sphingomonas melonis* Fr1	Distribution on *A. thaliana* leaf replica	Overnight	Soffe, Altenhuber et al. (2019)
	Casting PDMS on a leaf	Surface wash and UV treatment	Casting PDMS on the molds	*E. coli* ATCC 700728 and *Bacillus amyloliquefaciens* spores	Attachment and retention on spinach and lettuce leaves replicas	~ 6 min	Doan, Ngassam et al. (2020a)
	Casting PDMS on a leaf	Surface wash and UV treatment	Casting PDMS on the molds	*E. coli* O157:H7	Resistance to splash dispersal and inactivation on spinach replicas	72 h	Doan, Antequera-Gómez, Parikh, and Leveau (2020b)

FOTS is (1H,1H,2H,2H-Perfluorooctyl)trichlorosilane; PDMS is polydimethylsiloxane; UV is ultraviolet light.

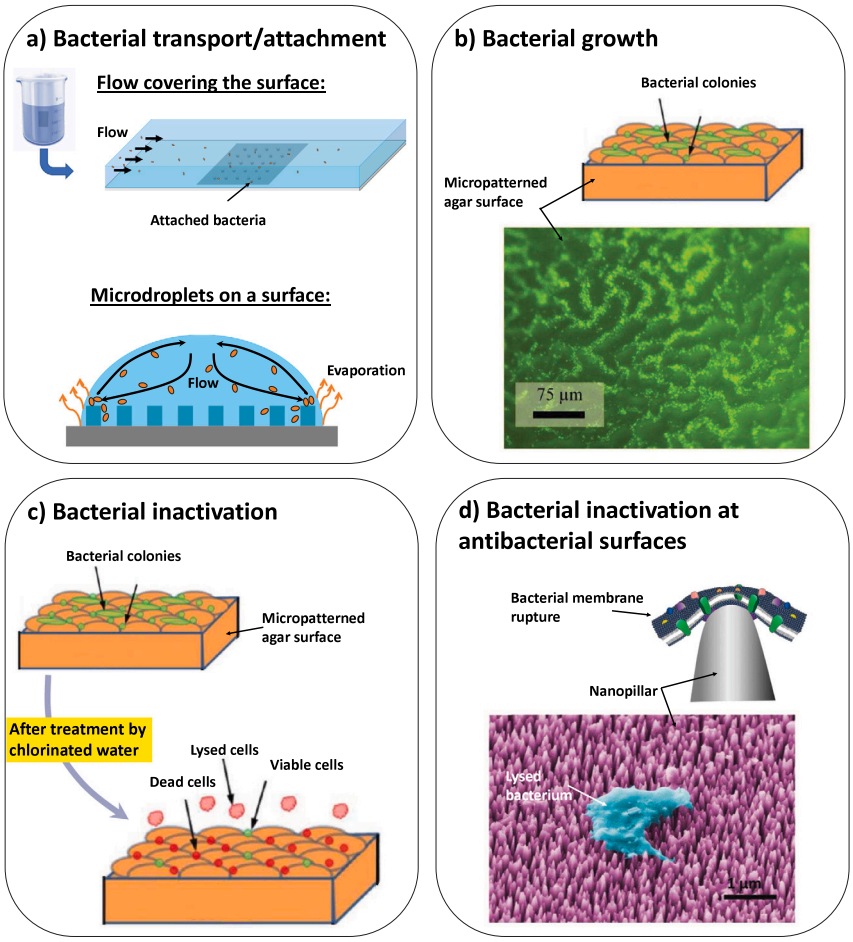

Fig. 4. Classification of micropatterned surfaces that are or can be used for fresh produce microbial safety. (a) Investigation of bacterial attachment and transport. The top image in this panel illustrates the experimental setup of Sirinutsomboon et al. (2011) in which a micropatterned surface was placed vertically within a shaking beaker containing an *E. coli* O137:H4 culture to investigate bacterial attachment to the simplified microstructures. The bottom image in this panel illustrates the experimental setup of Ranjbaran and Datta (2019) in which a microdroplet from an *E coli* RP437 culture was left to evaporate on a micropatterned surface to investigate bacterial transport with internal flow and their interactions with the microstructures. (b) Investigation of bacterial growth and biofilm formation. The inset shows a fluorescence image of green-stained *E. coli* on an agar surface patterned with natural topography of a spinach leaf surface, after 6 h of incubation at 25 °C (Zhang et al., 2014). (c) Investigation of bacterial inactivation using biocidal treatments. The schematic illustrates how presence of microstructures on a patterned agar surface can lead to inefficacy of the surface sanitation with chlorinated water. Inset images in (b) and (c) have been reproduced from Zhang et al. (2014, copyright 2014 American Chemical Society), with permission from the publisher. (d) Investigation of bacterial inactivation on antibacterial surfaces. The scanning electron microscopy image shows how nanopillar features cause mechanical lysis of a *Staphylococcus aureus* cell. The inset images have been reproduced from Linklater, Juodkazis, and Ivanova (2017, copyright 2017 The Royal Society of Chemistry), with permission from the publisher. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

growth was examined using plating methods as well as fluorescence microscopy imaging every 2 h during the experimental duration (Fig. 4b). The authors showed that when the agarose-based surfaces contained 10% TSB, the level of bacterial growth on the patterned and unpatterned surfaces was not significantly different. Using agarose-based devices without TSB, the researchers highlighted that stomata and grooves on the patterned surfaces could offer significant protection to bacterial cells against dehydration (Fig. 4b). These findings suggest that there is a need for developing surface washing procedures that can efficiently target the bacteria hidden in the leaf microstructures.

Zhang et al. (2014) further used their micropatterned agarose-based devices, without TSB supplements, to investigate bacterial inactivation on plant leaves. The bacteria on the devices were stained at 22 °C for 15 min using live/dead bacterial viability stains, showing the live bacteria as green and dead bacteria as red (Fig. 4c). After 12 h of incubation of bacteria on the devices, 200 mg/l chlorinated water was applied to the surface for 1 min. It was shown that the topography of plant surfaces influences the efficiency of the biocide treatment. On unpatterned surfaces, most of the bacterial cells were killed and no viable cells were observed within microscopy images obtained after the treatment. However, on patterned surfaces, viable cells were still observed in the valleys (i.e., stomata and grooves), reflecting the steric protection of the bacterial cells provided by the leaf microstructures against biocidal treatment.

Another potential application of surface patterning in fresh produce safety makes use of antibacterial nanopatterned surfaces (Linklater, Juodkazis, & Ivanova, 2017) capable of mechanical bacterial inactivation (Fig. 4d). The bactericidal effects of nanopatterned surfaces were inspired by the surface topography of cicada (*Psaltoda claripennis*) wings, which are inherently antibacterial (Linklater, Juodkazis, & Ivanova, 2017). So far, the antibacterial nanopatterned surfaces have been utilized in various biomedical applications, such as the fabrication of medical implants (Linklater, Nguyen, Bhadra, Juodkazis, & Ivanova, 2017), and they can also be applied in food packaging and processing materials (Zhou et al., 2021). For fresh produce applications, the nanopatterns can be incorporated at the surfaces that are in contact with the produce during post-harvest operations to minimize contamination. When a bacterial cell rests on the nanopatterns, the nanopillars cause substantial stress on the cell wall architecture, leading to an increase in the internal turgor pressure of the cell. Rupturing

is caused by extensive stretching of the cell membrane between each nanopillar. A comprehensive review of the underlying mechanisms of mechano-bactericidal actions of nanostructured surfaces can be found in Linklater et al. (2021). The antibacterial nanopatterned surfaces have been made from various materials, from which black silicon (e.g., Nguyen et al., 2019), graphene (e.g., Selim, Mo, Hao, Fatthallah, & Chen, 2020) and titanium-based (e.g., Wandiyanto et al., 2019) designs were the most prevalent. Other materials for time-efficient, scalable, precise, and low-cost fabrication of bactericidal nanopatterned surfaces include PDMS (Heckmann & Schiffman, 2020), poly(methyl methacrylate) (PMMA) (Rosenzweig et al., 2019), and polyvinylsiloxane (Green et al., 2017). With these polymers, a large number of replicates can be fabricated from one master mold.

2.3. Potential applications of micropatterned surfaces in the fresh produce industry

The current technology of fabrication of micropatterned surfaces can be further extended to develop innovative risk assessment tools for future industrial applications. Low-cost, disposable, and easy-to-use commercialized artificial leaves can potentially help the fresh produce industry in (1) establishing a background level of microbial contamination of the produce in the field due to exposure to unsanitized irrigation water or fecal matter, (2) understanding whether a certain cooling/washing practice can promote contamination at the produce surface or inside its tissue, (3) tuning cooling/washing operating conditions to minimize microbial attachment and infiltration, (4) reliable selection from various microbial inactivation strategies to sanitize produce surfaces of various roughness and hydrophobicity, and (5) tuning controllable operating conditions in an inactivation process to achieve the highest efficacy of microbial reduction.

These potential applications can be implemented during growing, harvesting, and post-processing of various fresh produce, such as leafy greens. For example, the artificial leaves can be left in the field, during growing season, for a certain time period to collect the possible sources of contamination. Then, the collected samples can be used for upcoming nucleic acid testing to detect pathogens. Micropatterned surfaces can also be used by companies that produce disinfectants for fresh produce applications. Using these devices, the efficacy of a certain disinfectant can be assessed by considering the produce surface's microstructures and wettability. In practice, the application of the micropatterned surfaces requires access to the lab environment and microscopy facilities. Small farms as well as large producers can benefit from this technology through collaboration with land grant universities and other research institutes. Such collaborations can be steered by intermediate organizations such as The Center for Produce Safety (CPS) that works with both the stakeholders and the research sectors.

Polymeric devices (e.g., PDMS-based, plastics) might be suitable for industrial needs. For instance, a PDMS-based device can mimic any microstructure on the produce surface and, therefore, it can be made for various produce types, ages, sizes, etc. Further, fabrication does not require permanent access to high-tech microfabrication facilities. It only requires pouring and curing the PDMS on the leaves/molds, which can be made once and re-used, and which is feasible for rolling-out purposes.

The antibacterial nanopatterned surfaces can be used during various steps of fresh produce process operations. Surfaces that are in permanent contact with the produce during processing can be coated with bactericidal nanostructures to minimize the bacterial colony formation and disease spread. Following Zhou et al. (2021), future industrial application of the antibacterial nanopatterned surfaces in fresh produce safety requires: (1) an understanding of how various influencing design parameters (e.g., shape and density of the nanostructures) can affect strain-dependent bactericidal efficacy and whether these structures can damage the produce itself; (2) an investigation of which type of material is the safest and most cost-effective for fabrication of produce processing and packaging surfaces; and (3) an understanding of how the bactericidal efficacy can be improved by incorporating other technologies, such as chemical surface modification.

3. Microfluidic cultivating devices

Fresh produce contamination may include various inter-kingdom interactions among bacterial, plant, and fungal species (Burmeister & Grunberger, 2020; Stanley, Grossmann, Solvas, & deMello, 2016). Understanding these interactions helps provide preventive measures to minimize produce contamination. Microfluidic cultivating devices provide a platform to investigate these complex interactions in controlled environments. So far, these devices have been extensively used in understanding of bacteria–bacteria interactions (Burmeister et al., 2019; van Vliet, Hol, Weenink, Galajda, & Keymer, 2014), as well as bacterial interactions with various higher organisms including fungi (Stanley et al., 2014), plants (Aufrecht et al., 2018; Massalha et al., 2017; Stanley et al., 2018), and mammalian cells (Ellett et al., 2019). In this section, some of the notable applications of these devices that are relevant to fresh produce safety research are discussed by highlighting their typical designs and method of use. More comprehensive information and classification about microfluidic cultivating devices can be found in Burmeister and Grunberger (2020).

3.1. Typical designs of cultivating devices

Microwells are among the popular designs to study bacteria–bacteria interactions. The simplest microwell design possessed disconnected habitats wherein the bacterial species interact with each other in a mixed culture within each microwell (Fig. 5a) (Hansen et al., 2016). In another design, the microwells were connected through narrow corridors, facilitating bacterial transport from one microwell to another. This design was used to study competence among bacterial species to occupy a fresh habitat (van Vliet et al., 2014). The microwell design may resemble microbial colonies on a leaf surface. Therefore, it can be an appropriate tool to explore phyllosphere microbial interactions. Microwells separated by nanoporous membranes were also used to study chemical signaling among various species. In such a design, only the chemicals, and not bacterial cells, were able to transport (via diffusion) across microwells. This provides a platform to explore the importance of chemical interactions (e.g., gradients in signaling molecules, metabolites, and nutrients) for habitat competition, in the absence of the physical interactions (e.g., spacial exclusion) (van Vliet et al., 2014).

In addition, microdroplets (Fig. 5a) have also been used as platforms to assay microbial interactions and growth. In a typical application of this microfluidic system, the droplets serve as enclosed environments, with a controlled nutrient condition, for the bacteria to grow and interact within. One approach to generating droplets containing multicomponent species was loading individual species into separate droplets, grouping the droplets in enclosed cavities, and finally merging them via alternating-current electric field to form the final microbial community (Kehe et al., 2019).

Microchambers are another class of microfluidic cultivating devices for the investigation of bacteria–bacteria interactions (Fig. 5a) (Burmeister et al., 2019). These devices have enabled observation of cellular interactions with full spatio-temporal resolution because the chambers are shallow enough to provide a 2D distribution of bacterial cells that can be tracked at a single-cell resolution. The chambers can be separated via nanoporous membranes to only allow diffusion of the metabolite across chambers (see Fig. 5a). One potential application of the above devices is to study various interactions of foodborne pathogens with natural microbiota that exist at the surface of fresh produce, assisting in developing strategies to improve fresh produce safety.

The cultivating devices have also been applied to study interactions of bacteria with higher organisms (Burmeister & Grunberger, 2020), among which plants and fungi are of interest. Investigating root–bacteria interactions has been the most popular in this area (Aufrecht et al., 2018; Massalha et al., 2017; Parashar & Pandey, 2011; Stanley

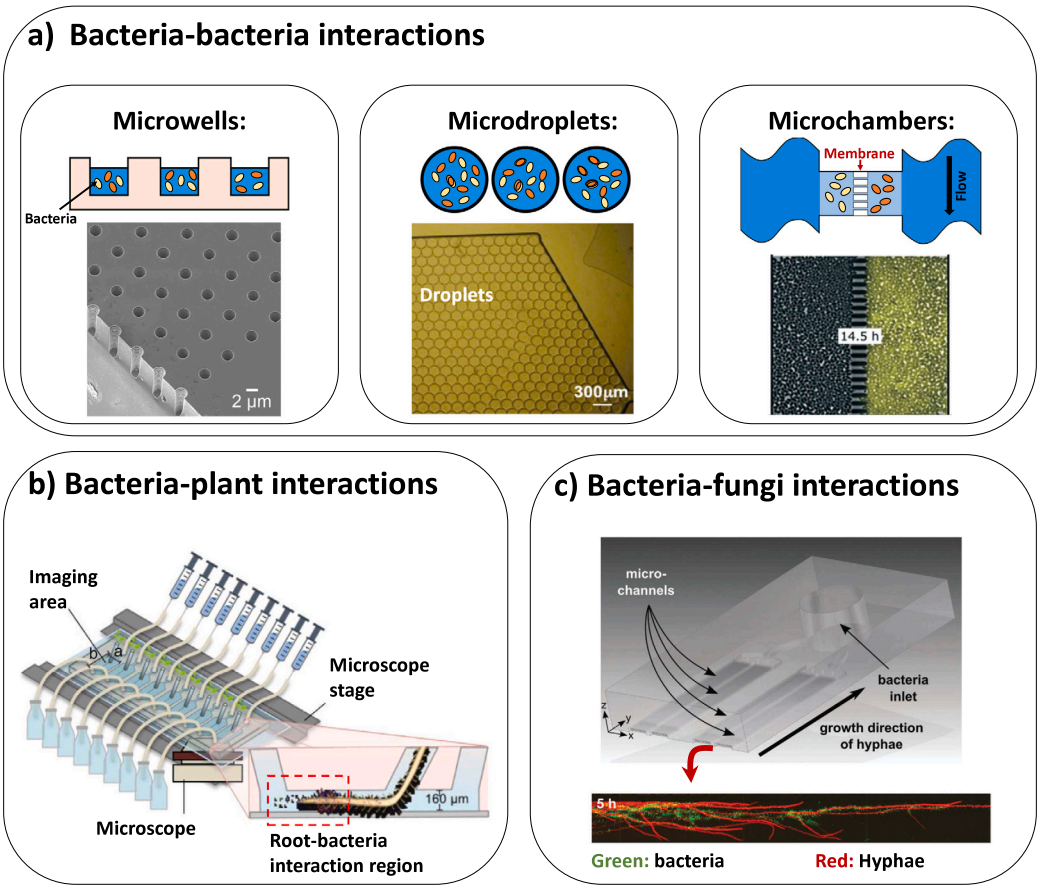

Fig. 5. A schematic of microfluidic cultivating systems. (a) Investigation of bacteria–bacteria interactions, that can be performed using various designs. A microwell device provides discontinued habitats wherein bacteria can grow and interact inside microwells. The inset shows a scanning electron microscopy image of an array of microwells on a silicon substrate with 2 µm in diameter. Microdroplets provide an enclosed environment with prescribed nutrient condition wherein bacteria can grow and interact. The inset image shows microdroplets filling a large chamber in a microfluidic device. Microchambers can provide a 2D distribution of bacteria within the device enabling observation of cellular interactions with full spatio-temporal resolution at single-cell level. Microscopy images in (a) have been reproduced from Hansen et al. (2016) (left), Park, Kerner, Burns, and Lin (2011) (middle). Rightmost microscopy image in (a) has been reproduced with publisher's permission from Burmeister et al. (2019, copyright 2019 The Royal Society of Chemistry). (b) Investigation of bacteria–plant interactions. The image shows the tracking root interactions system (TRIS) device that provides growth medium for nine seedlings. The root of the plant elongates within transparent microchannels whose interactions with the bacteria can be monitored using a microscope. This image was reproduced with publisher's permission from Massalha et al. (2017). (c) Investigation of bacteria–fungi interactions. The image shows a microfluidic device in which the basidiomycete *Coprinopsis cinerea* elongates its hyphae in transparent microchannels wherein its interactions with *Bacillus subtilis* was monitored using microscopy imaging. The image was reproduced with publisher's permission from Stanley et al. (2014, copyright 2014 Oxford University Press).

et al., 2018). In a typical design of the microfluidic device, the plant seedlings were placed in ports that were in touch with the open air while the roots were allowed to grow into transparent microchannels (usually made out of PDMS), supplied with nutrient environments (Fig. 5b). The bacteria (usually fluorescently labeled) were then introduced to the microchannels and being monitored using microscopy during their interactions with the plant root. A similar design including transparent microchannels for the growth of hyphae was used to study fungi–bacteria interactions (Fig. 5c) (Stanley et al., 2014). The fungal and bacterial inocula were introduced from different inlets to the microchannels and their interactions were investigated. Recently, the Ecosystem Fabrication (EcoFAB) protocols have provided methods for the production of such laboratory ecosystems designed to investigate plant–microbe interactions (Gao et al., 2018). Following EcoFAB guidelines, the future microfluidic cultivating devices used for fresh produce safety and sustainable production are expected to be: (1) observable, by allowing for monitoring and measuring inter-species and biochemical interactions at high precision, (2) reproducible, by allowing for the applicability in dynamic biological situations, (3) controllable, by allowing for the calibration of defined conditions for the microenvironment and the species, and (4) ecosimilar, by allowing for reproduction of the key behavior observed in the real system. In Sections 3.2 and 3.3, we discuss various applications of the above cultivating devices in investigating inter-species interactions.

3.2. Investigation of bacteria–bacteria interactions

Microbial contamination of fresh produce may include various interactive processes among microbial communities. Examples of these interactive processes are quorum sensing (Nagy et al., 2014), cross-feeding (Burmeister et al., 2019; Connell, Ritschdorff, Whiteley, & Shear, 2013), and habitat competition dynamics (van Vliet et al., 2014) that can be investigated using cultivating devices.

Spoilage of food products, including fresh produce, is known to be attributed to quorum sensing among microbial communities. In quorum sensing, gene regulations dependent on cell population density benefit the bacterial community growing within a host. The spoilage of vegetables and fruit is often caused by the pectinolytic activity of bacteria (e.g., members of the *Pseudomonadaceae* or *Enterobacteriaceae* families) at high cell densities. Because spoilage is a phenomenon requiring high levels of microbial populations, it might be regulated by quorum sensing (Skandamis & Nychas, 2012). Also, quorum sensing is an important factor in the colonization of foodborne pathogens at the surface and within fresh produce tissue. Microfluidic cultivating devices have the potential to be used in revealing the effects of quorum sensing on fresh produce safety and quality, although they have not yet been utilized in this context. An example of the application of these devices in the investigation of cell–cell signaling in bacterial communities is

Table 2
An overview of the applications of microfluidic cultivating devices in investigating plant/fungi–microbe processes with potential applications in fresh produce microbial safety and sustainable production.

Device category	Mold fabrication	Mold treatment	Device fabrication	Microorganisms	Plants/fungi	Investigated process	Test time	Reference
Microchannels	Photolithography on SU-8 photoresists	–	Casting PDMS on the molds	*Phytophthora sojae*	*Arabidopsis*	Root-microbe interactions	<198 h	Parashar and Pandey (2011)
	Photolithography on SU-8 photoresists	–	Casting PDMS on the molds	*Bacillus subtilis* and *Escherichia coli*	*Arabidopsis thaliana*	Root-microbe interactions, and pathogen exclusion from roots	96–120 h	Massalha et al. (2017)
	Photolithography	Silanization with chlorotrimethyl-silane under vacuum	Casting PDMS on the molds	*B. subtilis*	Basid-iomycete *Coprinopsis cinerea*	Hyphae-microbe interactions	<8 h	Stanley et al. (2014)
	Photolithography on a silicon wafer	–	Casting PDMS on the molds	Two plant growth promoting bacterial species isolated from the endosphere and rhizosphere of *Populus deltoides*	*Arabidopsis thaliana*	Root-microbe interactions	<96 h	Aufrecht et al. (2018)

PDMS is polydimethylsiloxane.

given by Nagy et al. (2014), where researchers studied the interaction of two physically separated but chemically coupled *E. coli* strains. Their device consisted of microchambers that were connected via porous membranes. They showed that bacterial populations exhibited dynamic spatial rearrangements as a result of secretion, diffusion, and sensing of metabolic products and/or signaling molecules.

Cross-feeding—a biological interaction in which the growth of one bacterial species depends on the nutrients, substrates, or growth factors provided by the other species—has been studied using cultivating devices. Using a device consisting of two adjacent microchambers separated by nanoporous membranes (Fig. 5a), Burmeister et al. (2019) investigated commensal interactions between L-lysine-producing *Corynebacterium glutamicum* and an L-lysine auxotrophic variant of the same species. They revealed that spatially separated cultivation of both strains led to the growth of the auxotrophic strain as a result of secreted L-lysine supplied by the producer strain. Using a 3D printing technique to generate distinct but chemically interactive bacterial communities, Connell et al. (2013) were able to show that *Staphylococcus aureus* (which is naturally susceptible to β-lactam-based antibiotics) would be sheltered from ampicillin toxicity when embedded within a microcolony of *Pseudomonas aeruginosa* actively producing β-lactamases. Using similar devices, one can address microbial interactions that may benefit the fresh produce industry. For example, investigation of the interaction of natural microbiota on a produce surface with foodborne pathogens may lead to creation of engineered organisms—under the regulations related to the genetically modified organisms (GMOs)—that prevent or decrease the pathogen colonization on the produce surface.

Using a cultivating device consisting of one-dimensional arrays of microwells linked by connectors, van Vliet et al. (2014) studied habitat competition of two neutrally labeled strains of *E. coli*. In their setup, the bacteria invaded the habitat from opposing sides, and differential fluorescent labeling allowed the researchers to monitor bacterial competence in the colonization of the habitat. Their results highlighted the crucial roles of chemical interactions between populations in determining the outcome of habitat colonization. Microfluidic cultivating devices have the potential to investigate how native microbial communities associated with fresh produce affect the colonization of foodborne human pathogens on fresh produce surfaces. An example of such microbial interactions can be seen in the work by Massalha et al. (2017) where researchers utilized a microfluidic cultivating device (discussed further in Section 3.3) to show that colonization of *Bacillus subtilis* at the roots of *Arabidopsis thaliana* caused the active exclusion of *E. coli* cells from the root surface.

3.3. Investigation of bacteria–plant and bacteria–fungi interactions

Fresh produce safety and plant pathology involve various interkingdom interactions (e.g., pathogenicity, symbiosis) among different species, e.g., bacteria–plant (Aufrecht et al., 2018; Massalha et al., 2017; Parashar & Pandey, 2011; Stanley et al., 2018) and bacteria–fungi (Stanley et al., 2014; Millet et al., 2019) interactions. These interactions can be investigated using cultivating devices. An overview of the applications of microfluidic cultivating devices in investigations of plant–microbe and fungi–microbe interactions is provided in Table 2, and representative examples are discussed below.

Concerning bacteria–plant interactions, most studies have investigated the interactions between roots and bacteria (Fig. 5b). Using a microfluidic device to monitor the root system, Massalha et al. (2017) investigated the intimate interaction of *B. subtilis* with *A. thaliana* roots at a high resolution. Their microfluidic device, called a tracking root interactions system (TRIS), included nine independent chambers for growing seedlings and monitoring of the root growth in real time. Their results showed a distinct chemotactic behavior of *B. subtilis* toward the root elongation zone, followed by rapid colonization of the area after 6 h of interactions. Moreover, they investigated bacterial preferences between the wild-type and mutant root genotypes using a double-channel TRIS device to simultaneously track two root systems in one chamber. They showed that a significant accumulation of *B. subtilis* around a hairless mutant root (*cpc/try*) was 30% higher than that around a wild-type root. Using a microfluidic device to culture plants with beneficial growth-promoting bacteria, Aufrecht et al. (2018) visualized and quantified their interactions. They showed that, after four days, the populations of the two bacterial species under study on the *A. thaliana* root's visible surface were independent of the inoculum concentration. They suggested that the plant limits the population of bacteria in its rhizosphere through mechanisms such as attenuating the quantity and type of root-available exudates. For the sustainable production of fresh produce, such microfluidic studies can help determine the optimum quantity of beneficial microorganisms in the soil.

In the area of bacteria–fungi interactions, Stanley et al. (2014) investigated the interaction between the basidiomycete *Coprinopsis cinerea* and the bacterium *B. subtilis* using a transparent microchannel cultivating device (Fig. 5c) that restricted the hyphal growth to one plane inside a shallow cultivation channel. They observed that the hyphae stopped growing with the formation of extracellular, cytoplasm-filled blebs after contact with the wild-type *B. subtilis* strain.

Table 3
An overview of the applications of biosensing devices in the detection of microbial contamination on plants/plant-based extracts, with potential applications in fresh produce microbial safety and sustainable production.

Platform	Assay type	Detection method	Sample	Microorganism	Limit of detection	Detection time	Reference
In-tube assays							
	LAMP	Colorimetric	Tomato plant	*Pseudomonas syringae* pv. *tomato*	$1.05 \times 10^3\ CFU/ml$	1 hr	Chen et al. (2020)
	LAMP	Colorimetric	Pea plant	*P. syringae*	$2.5 \times 10^2\ CFU/ml$	20 min	Kant et al. (2021)
	LAMP	Fluorometric	Strawberry plant	*Xanthomonas fragariae*	$1 \times 10^2\ CFU/ml$	20 min	Getaz et al. (2017)
	LAMP	Colorimetric	Rice leaves	*X. oryzae* pv. *oryzae*	$2 \times 10^3\ CFU/ml$	40 min	Buddhachat et al. (2021)
Paper-based devices							
	Enzyme-linked immunosorbent assay	Colorimetric	Chinese cabbage	*Escherichia coli* O157:H7	$1 \times 10^4\ CFU/ml$	<3 hr	Pang et al. (2018)
	Aptamer-based assay	Colorimetric	Apple juice	*E. coli* O157:H7	$10\ CFU/ml$	<1 hr	Wu et al. (2015)
	LAMP	Colorimetric	Spinach leaf	*E. coli* ATCC 25922	$1 \times 10^3\ CFU/ml$	1 hr	Choi et al. (2016)
	Helicase dependent amplification of DNA	Colorimetric	Fruit juice	*Salmonella typhimurium*	$1 \times 10^3\ CFU/ml$	1 hr	Tang, Yang, Gong et al. (2017b)
	Immunoassay using immobilized antibodies	Colorimetric	Lettuce leaf	*E. coli* O157:H7, *S. typhimurium*	$1.87 \times 10^5\ CFU/10g$, $1.47 \times 10^5\ CFU/10g$	30 min	Shin et al. (2018)
	Immunoassays using immobilized antibodies	Colorimetric	Fruits/leaves of almond, peach, apricot	*X. arboricola* pv. *pruni*	$1 \times 10^4\ CFU/ml$	–	Lopez-Soriano et al. (2017)
Chip-based devices							
	LAMP	Optical turbidity	Apple juice	*E. coli* O157:H7	$1\ CFU/ml$	1 hr	Li et al. (2017)
	Immunoassays using immobilized antibodies on poly(carboxybetaine acrylamide) coatings	Surface plasmon resonance	Cucumber extracts	*E. coli* O157:H7, *Salmonella sp.*	$57\ CFU/ml$, $7.4 \times 10^3\ CFU/ml$	<80 min	Vaisocherová-Lísalová et al. (2016)
	Immunoassays using immobilized antibodies on amino functionalized SBA-15 platforms	Electrochemical	Walnut plant	*X. arboricola*	$1.5 \times 10^2\ CFU/ml$	30 min	Regiart et al. (2017)
Other devices							
	Immunoassays using immobilized antibodies on cotton threads	Colorimetric	Lettuce leaf	*S. enterica* serotype *Enteritidis*	$5 \times 10^3\ CFU/ml$	10 min	Choi, Nilghaz, Chen, Chou, and Lu (2018)
	Immunoassays using immobilized antibodies on graphene wrapped copper (II) assisted cysteine (rGO-CysCu), self-assembled on gold electrodes	Electrochemical	Fruit juice	*E. coli* O157:H7	$3.8\ CFU/ml$	–	Pandey et al. (2017)

LAMP is loop-mediated isothermal amplification; DNA is deoxyribonucleic acid.

3.4. Potential applications of microfluidic cultivating devices in fresh produce industry

Several plant tissue-on-chip devices have been used for various research purposes, but not for industrial applications. Similar to organ-on-chip technologies that have found their applications outside research areas (e.g., Emulate, 2017), the plant tissue-on-chip, which can benefit fresh produce safety and plant disease control, needs to be pushed toward industrial applications. A potential industrial application of the plant tissue-on-chip devices could be during the development of plant probiotic fertilizers. These devices can be used to analyze how a certain probiotic product—which contains plant growth promoting bacteria such as *Lactobacillus casei*—affects the growth of various target plants. Similarly, farmers and large producers who would like to use such probiotics can take advantage of plant tissue-on-chip technology during decision making for investments on a certain probiotic product. A key driver to commercialize the plant tissue-on-chip is the adoption of the technology by major agricultural companies and universities, which in turn requires overcoming key restraints such as cost and complexity of the devices. Since the application of plant tissue-on-chip devices requires lab environments and microscopy facilities, it is not anticipated that small farms can directly use them as part of their decision-making tools. However, they can still benefit from

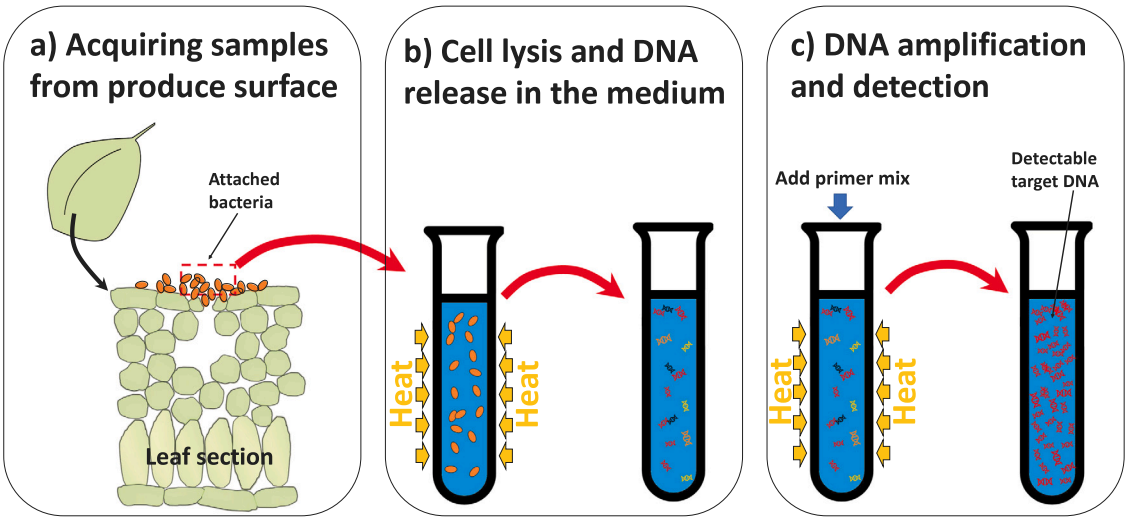

Fig. 6. A schematic showing how DNA amplification approaches can be used to detect microbial contamination of fresh produce. (a) Bacterial sample from the surface of the produce can be obtained using different approaches; For example, by swabbing of the surface and resuspension in a liquid medium, or by surface washing, microfiltration of wash water, and resuspension of the captured bacteria by the filter membrane, in a liquid medium. (b) The bacterial sample is then heated up to lyse the cells and release their DNA in the liquid medium. Afterwards, (c) by adding a primer mix and other reagents, the target DNA (in red) is amplified when heating the DNA solution. For example, in the case of the loop-mediated isothermal amplification (LAMP), the heating temperature is 65 °C. The amplification can be detected using various detection methods, such as a colorimetric method.

these technologies through collaboration with land grant universities and other research institutes.

In the next section, we discuss biosensing devices as another class of microfluidic applications in produce safety.

4. Biosensing devices

The detection of microbial presence on fresh produce can help ensure produce safety as it moves from field to fork. Within the context of microbial safety of fresh produce, the biosensors present two broad application areas: namely, the detection of foodborne pathogens, and tracking sources of fecal contamination (known as microbial source tracking, MST). In pathogen detection on fresh produce, prevalence has resulted in more work on *E. coli* and *Salmonella* spp., and less on *L. monocytogenes* (Soni, Ahmad, & Dubey, 2018)—a topic for future research. From the viewpoint of sustainable agriculture, biosensors have been utilized in the detection of plant diseases during their growth period. This section explores typical elements and design characteristics of biosensors, and their applications in the detection of foodborne and plant pathogens as well as microbial source tracking. The comprehensive reviews available in the literature provide more information on the applications of biosensors in food and water safety (Choi et al., 2019; Thavarajah et al., 2020) and sustainable agriculture (Griesche & Baeumner, 2020).

4.1. Typical elements and designs of biosensing devices

A biosensor is a combination of a biorecognition element, which recognizes a target analyte, and a transducer, which converts the recognition event into a detectable signal. Common biorecognition elements for the detection of bacteria (the target analyte) include phages (Wang et al., 2021a; Wang, Kanach, Han, & Applegate, 2021b), enzymes (Kurbanoglu, Erkmen, & Uslu, 2020), ionic surfactants (Verma, Tsuji et al., 2016b; Verma, Wei et al., 2016a), antibodies (Asal, Ozen, Sahinler, & Polatoglu, 2018), aptamers (Yoo, Jo, & Oh, 2020), and nucleic acids (Leonardo, Toldra, & Campas, 2021; Mohan et al., 2021). Among the aforementioned biorecognition elements, nucleic acids and antibodies seem to be the most widely used in the detection of bacterial pathogens. Despite the popularity of both of these biorecognition elements, the synthesis of primers needed for detecting nucleic acids is simple, rapid, and inexpensive compared to the production of antibodies. Fig. 6 shows the typical steps by which nucleic acid testing can be used in the detection of microbial contamination of fresh produce. Bacterial samples from the surface of the produce are obtained (using aseptic swabbing or surface washing) and transferred to a liquid medium. Then, the bacterial solution is processed (e.g., by heating, sonicating, or using chemical methods) to lyse the cells and release their DNA. The target DNA is amplified using a DNA amplification technique that includes the addition of a primer mix and other reagents (such as enzymes, buffers, and salts) to the solution and heating it with a prescribed thermal pattern. The amplification of the DNA leads to changes in the solution such as precipitation of magnesium phosphate and a drop in the pH. The most widely-used transducing elements have been colorimetric (Choi et al., 2019), fluorescence-based (Camarca et al., 2021), and electrochemical (Zhang, Zhou, & Du, 2021); however, other types such as surface plasmon resonance, surface-enhanced Raman scattering, and chemiluminescence have also been applied in biosensors for food safety (Choi et al., 2019).

The most common method in nucleic acid amplification is the polymerase chain reaction (PCR). Although PCR is an established method, it requires precise cycling of temperature to separate the double-stranded DNA, anneal two primers (a forward and a reverse primer), and elongate the templates using DNA polymerase. Repeating these three steps requires precise control over the temperature of the reaction, which is achieved using a thermocycler. Because thermocyclers are often sophisticated, bulky, and expensive, PCR is not regarded as a preferred nucleic acid amplification technique in portable biosensors. Instead, isothermal amplification techniques, which operate at a single temperature, alleviate the need for a thermocycler. The most widely used of these techniques is loop-mediated isothermal amplification (LAMP) (developed by Notomi et al., 2000), which operates at 65 °C. LAMP is fast (operating within a few minutes, as shown in Table 3) with high specificity because the reaction occurs when six distinct regions on the target DNA are recognized by a set of four primers. Due to its promising sensitivity and short response time, LAMP has been extensively used in pathogen detection in food (Huang et al., 2020; Kim & Oh, 2021; Shang et al., 2020). Isothermal amplification techniques provide the opportunity to develop and commercialize portable nucleic acid-based biosensors for on-site detection of target microorganisms on fresh produce. A comprehensive review of several isothermal amplification techniques, including LAMP, rolling circle amplification (RCA), recombinase polymerase amplification (RPA), helicase dependent amplification (HDA),

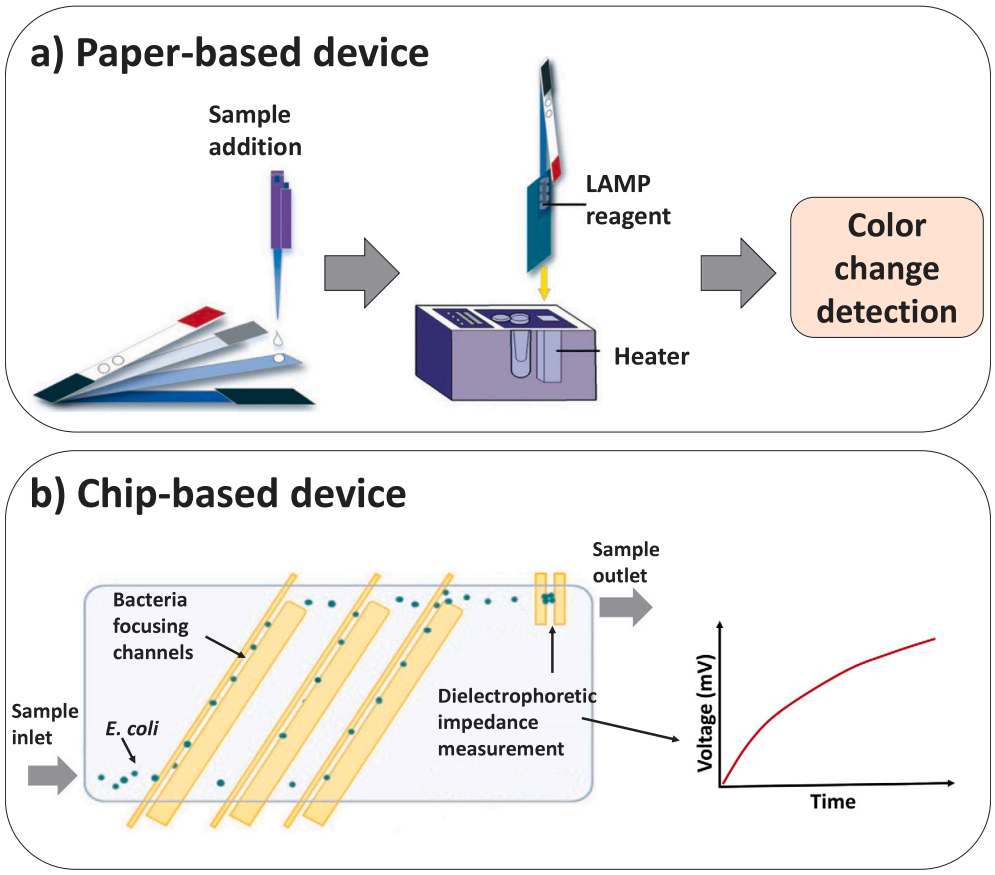

Fig. 7. A schematic of the two major classes of biosensors used to detect microbial contamination of fresh produce. (a) A paper-based device receives the sample containing lysed bacteria and performs LAMP reaction, leading to a detectable color change at the reaction site. The device is composed of four hydrophobic layers to control the sample flow toward the DNA amplification sites. The loop mediated isothermal amplification (LAMP) is performed by addition of the LAMP reagents to the sample and heating the device using a closed heating compartment. After amplification, the target DNA is detected using a color change on a lateral flow strip. Images in (a) have been reproduced with publisher's permission from Choi et al. (2016, copyright 2016 The Royal Society of Chemistry). (b) A chip-based device receives the sample containing *E. coli* cells and measures the dielectrophoretic impedance as a representative for bacterial presence. After entrance of the bacterial sample into the device, they pass through a set of bacteria focusing channels where the bacterial concentration is increased using positive dielectrophoresis before they each the sensing zone. Image in (b) has been reproduced with publisher's permission from Kim et al. (2015, copyright 2015 Elsevier).

strand displacement amplification (SDA), and isothermal strand displacement polymerization (ISDPR), along with their applications in food safety, can be found in Leonardo et al. (2021).

While various types of biosensing platforms are available, paper-based (Fig. 7a) and chip-based devices (Fig. 7b) have been the most popular in food safety applications. Microfluidic paper-based analytical devices (μPADs) and lateral flow assay (LFA) paper strips are among the most widely-used point-of-care (POC) devices in food safety analysis (Choi et al., 2019). Paper is usually made up of cellulose, which enables ease of fabrication and proper mixing of reagents with the samples. It enables storage of functional biomolecules (such as DNA and proteins) that can be utilized in nucleic acid extraction since it is thermally stable and can be heated up to 300 °C (Wong, Cabodi, Rolland, & Klapperich, 2014). Paper also offers biodegradability and disposability. Paper-based devices have been fabricated in different designs, such as using multiple layers (Choi et al., 2016) or a sliding strip (Verma et al., 2018). A comprehensive review of various fabrication methods, device designs, and applications of μPADs is provided in Noviana et al. (2021). Chip-based devices have also been popular in designing sample-to-answer devices used in food safety analysis. Using these devices, precise manipulation and control of small amounts of samples can be achieved. Popular materials for the fabrication of chip-based devices include PDMS and PMMA. In comparison, the above characteristics make paper an inexpensive and reliable platform for the fabrication of portable biosensors for on-farm detection of produce contamination. Comprehensive reviews in the application of these platforms in POC diagnostic devices are available in Choi et al. (2019) and Zhang, Bi, Liu, and Qiao (2018). An overview of application of various biosensing devices in detection of microbial contamination on plant-based samples is provided in Table 3. In the next two sections, representative investigations are discussed in more detail.

4.2. Investigation of pathogen detection

Detection of microbial contamination can benefit the entire production chain of fresh produce. Several investigations in biosensor developments have aimed at the detection of foodborne pathogens (i.e., with a food safety perspective) and a number of investigations have explored the detection of plant pathogens (i.e., from the viewpoint of sustainable agriculture). Here, the main efforts have been in increasing sensitivity, specificity, multiplexing capability, and in designing fully-integrated portable sample-to-answer devices. Below, the notable studies in these areas will be reviewed with emphasis placed on the proof-of-concept investigations that can be extended to fresh produce research.

Being a fully-integrated sample-to-answer device is an important expected feature for field-deployable detection tools. Such a device should combine various necessary steps in detection of contamination into a user-friendly system. Despite their importance, the number of reported fully-integrated devices for food safety application are small. Choi et al. (2016) combined the three main steps in nucleic acid

testing—DNA extraction, DNA amplification (using LAMP), and colorimetric detection—into an integrated four-layered paper-based device. In their biosensors, a Fast Technology Analysis (FTA) card and a glass fiber membrane were integrated into a lateral flow strip for nucleic acid extraction and amplification. Hydrophobic polyvinyl chloride (PVC) layers were initially embedded between the paper matrices, creating valves to control the fluid flow from the nucleic acid extraction zone to the amplification zone and lateral flow strip. The device was tested for detection of *E. coli* ATCC 25922 in several media, including drinking water and spinach leaf extracts, with detection limits of 1×10^1 CFU/ml and 1×10^3 CFU/ml, respectively. The worst detection limit in spinach extract was attributed to the requirement for pre-processing steps (e.g., filtration) to remove the residue of spinach leaves before the detection, resulting in the loss of bacteria. This suggests that, instead of preparing leaf extracts, another method of bacterial sampling from fresh produce is needed (e.g., using a small volume of liquid to extract bacteria from the surface of leaves). This change in bacterial sampling is of high practical importance for on-site microbial detection, where there is a lack of equipment for sample homogenization and preparation.

For efficient pathogen detection in the field or during post-processing operations, the biosensors must offer a multiplexing capability (i.e., the ability for simultaneous detection of multiple target pathogens in one or more samples using a single device) and an acceptable usability (i.e., simple and minimal operation steps for the user). For multiplexed detection of foodborne pathogens from contaminated lettuce, Shin et al. (2018) developed a multiplexed LFA device consisting of four test lines, with four different capture antibodies, to detect the presence of *E. coli* O157:H7 and *S. typhimurium*. Their device consisted of a stationary bottom part that contained the test strips and a rotating top part that contained the sample pads and their corresponding absorbent pads. Detection occurred in the presence of specific gold-nanoparticle-conjugated antibodies (AuNP-Ab) for each target analyte. These authors showed that the detection limits for *E. coli* O157:H7 and *S. typhimurium* in lettuce samples were 1.87×10^5 and 1.47×10^5 CFU/10g, respectively (corresponding to 2.88×10^5 and 1.13×10^5 CFU/ml). Since these detection limits were not meaningful for practical detection of those bacteria—as few as 10 CFU of *E. coli* O157:H7 can cause infection in humans—the bacterial samples were enriched before being tested by the device. The researchers showed that a 6 h of enrichment was sufficient to detect as few as 1 bacterium in 1 g of lettuce (i.e., about 5 bacteria per single lettuce leaf). While this research was focused on fresh produce safety, there are various other works done in other applications. For example, an LFA strip capable of detecting FITC-, hex-, and digoxin-tagged LAMP amplicons, corresponding to three main toxin genes of *P. aeruginosa* (i.e., *ecfX*, *ExoS* and *ExoU* genes) was developed for environmental and drinking water applications (Chen et al., 2016).

Biosensing tools have also been used for the detection of plant pathogens (Griesche & Baeumner, 2020) that have implications for the sustainable production of fresh produce. Getaz et al. (2017) designed a fast and specific LAMP assay for detection of *Xanthomonas fragariae* on strawberry leaves. The specificity of the assay was tested on various strains of *X. fragariae* and other *Xanthomonas* species. A detection limit of 1×10^2 CFU/ml was achieved, with an assay time of 7–20 min, providing a high-throughput and user-friendly method for on-site detection and screening of plant materials. In another study, Buddhachat et al. (2021) used a colorimetric LAMP assay for the detection of *X. oryzae* pv. *oryzae* in rice, showing a high performance (above 80%) of target bacteria detection in naturally infected leaf samples harvested from a rice field. Lopez-Soriano et al. (2017) designed an LFA for the detection of *X. arboricola* pv. *pruni* in symptomatic field samples. Their LFA strips included a test line with immobilized 2626.1-HT antibody and a control line. In each test, the paper strips were introduced to a solution containing a bacterial suspension (obtained from suspending the plant tissues in phosphate-buffered saline) and a carbon nanoparticles suspension conjugated with polyclonal 2626.1-WC antibodies. After 10 min of LFA, the test line was distinguishable by the naked eye, in the case of bacterial presence. The detection limit was 1×10^4 CFU/ml. Other examples of biosensing tools for detection of plant pathogens are listed in Table 3.

4.3. Investigation of microbial source tracking (MST)

MST aims to develop tools to recognize hosts (i.e., sources) of fecal contamination in food and the environment. In fresh produce risk assessment, these tools can have a large potential impact, because fresh produce is usually consumed raw and the contaminants could reach the consumer. To track the sources of fecal contamination, two different classes of MST have been used: namely, library-dependent (i.e., application of metagenomic sequencing) and library-independent methods (Fu & Li, 2014). The latter is of interest here. Within library-independent methods of MST, host-specific genetic markers are directly detected using a DNA amplification technique. Usually, PCR has been used for this purpose. The studied biomarkers have been animal-specific viruses (Hundesa, Maluquer de Motes, Bofill-Mas, Albinana-Gimenez, & Girones, 2006) and bacteria (Jiang et al., 2018). See Garcia-Aljaro, Blanch, Campos, Jofre, and Lucena (2018) for more detailed information about various fecal biomarkers. Among bacterial biomarkers, the members of the order *Bacteroidales* are the most appropriate for MST studies. This is because they are limited to warm-blooded animals, are dominant members of gut microflora, and cannot grow in the ambient aerobic environment. An example of the application of bacterial biomarkers in monitoring fecal contamination in fresh produce production is in Ravaliya et al. (2014). They investigated the utility of an MST method based on *Bacteroidales* 16S ribosomal ribonucleic acid (16S rRNA) gene sequences as a means of identifying sources of fecal contamination in growing tomatoes, jalapeño peppers, and cantaloupe. They conducted quantitative PCR assays on rinses of the above fresh produce, source and irrigation water, and harvester hand rinses. It was shown that 39% of samples were positive for a universal *Bacteroidales* marker. Among the positive samples, 46% were positive for one of the three human-specific markers (BFD, HF183, BVulg) and none were positive for a bovine-specific marker (BoBac).

As noted in Section 4.1, PCR cannot be used in portable biosensors for on-site detection of biomarkers, such as *Bacteroidales*. Therefore, attention has alternatively been devoted to isothermal amplification techniques such as LAMP (Jiang et al., 2018) and HDA (Kolm et al., 2019). Currently, there is no study reporting the application of isothermal amplification techniques in the detection of fecal contamination of fresh produce. Most of the studies have been done in water contamination detection. For example, Jiang et al. (2018) developed a portable nucleic acid diagnostic platform for rapid in-field identification of human fecal contamination of water. Their assays were based on LAMP of human-associated *Bacteroides* HF183 genetic markers from crude samples, followed by a fluorescence visualization of the results. Their assay could detect as few as 17 copies/ml of human-associated HF183 targets in sewage-contaminated water with no off-target signal from canine or feline feces.

4.4. Challenges in the development of biosensors for on-farm applications in the fresh produce industry

Fresh produce safety and sustainable production can benefit from biosensors for the detection of foodborne and plant pathogens, as well as biomarkers of fecal contamination. A portable biosensor can be used as part of fresh produce harvest operations by continuous monitoring of the bacterial load on harvester machines that are in contact with the produce. If contamination by pathogenic bacteria—such as *S. enterica*, Shiga toxin producing *E. coli*, *L. monocytogenes*, or *Bacteroidetes* as biomarkers of fecal contamination—is detected, the most recent harvest (e.g., within the last hour) can be discarded and the harvest machine can go through a sanitation process. This kind of hourly data on the

contamination of the harvesters and produce can prevent foodborne illnesses and also help prevent millions of dollars of losses as a result of the recall of a certain crop from the market due to foodborne contamination.

Designing a fully-integrated sample-to-answer detection tool for on-farm use involves various challenges—mainly, increasing the functionality of the tool and ensuring its multiplexing capability. In an ideal design, an ordinary user (e.g., a farmer or a technician in a post-processing facility) would provide a sample of the produce to the device (e.g., by swabbing or washing the produce surface) and follow some easy instructions (e.g., twisting a valve or pushing a button) to get a response, such as a color change, indicating the presence or absence of some bacterial strains. Not only can this user-friendly device be used by large producers as part of their produce safety assessments, but also by small farms. However, such an ideal tool does not yet exist commercially. The emergence of isothermal DNA amplification techniques, such as LAMP, has now made it possible to perform nucleic acid testing on the farm using a heater that operates at 65 °C (Pascual-Garrigos et al., 2021). Paper can be used as a platform to run multiple LAMP assays at a time and to store the reagents (e.g., primer sets) for various target microorganisms. It has been used in various applications such as SARS-CoV-2 diagnostics (e.g., Davidson et al., 2021) and protocols for fabrication of paper-based LAMP assays have been introduced (Wang et al., 2021). The anticipated cost of the paper-based LAMP assays was reported to be approximately $10 per assay (Davidson et al., 2021), which might be further decreased by mass fabrication. Despite these improvements, it is still necessary to resolve many issues, such as providing for the efficient delivery of a sufficient amount of the DNA template to the LAMP reaction sites, eliminating uneven heat transfer to the reaction sites, improving the limit of detection of the LAMP assays, and inhibiting unwanted reactions that may interfere with the LAMP-induced response. Overcoming these challenges will pave the way toward the industrial application of paper-based LAMP biosensors as reliable decision-making tools in the field.

5. Conclusions

As a unique characteristic of the current review, this paper classifies various microfluidic approaches that have been used (or could be used) in understanding fresh produce–bacteria interactions and facilitating the detection of bacterial presence on or within the produce. This classification scheme includes: (1) micropatterned surfaces that are artificial surrogates of the produce surface, (2) microfluidic cultivating devices that include microcavities with controlled environments in which the microbes and host can grow and interact with each other, and (3) biosensing devices that are portable analytical tools used in the detection of microbial presence. Simplified topography micropatterned surfaces are suitable to investigate the effect of each microstructure type, size, and distribution on the contamination. Surfaces patterned with natural topography of the leaf provide the opportunity to study the effect of real microstructures on the microbial contamination. However, their fabrication may be more challenging due to shriveling the leaf during mold preparation, presence of moisture at the leaf surface, and sticking leaf residue to the final PDMS device. Micropatterned surfaces can potentially serve the fresh produce industry as novel risk assessment tools in selecting various operating conditions that enhance microbial safety of the produce. Popular designs for the microfluidic cultivating devices used to investigate bacteria–bacteria interactions are microwells, microdroplets, and microchambers. All designs provide controlled microenvironments for the bacterial species to grow and interact, and they can be monitored by the researchers under a microscope. Several interactive processes within bacterial communities can be investigated, including quorum sensing, cross-feeding, and habitat competition dynamics. Plant–bacteria interactions can also be studied using microfluidic cultivating devices. These microdevices can hold plant seedlings in special ports where the root growth and interactions with bacteria can be monitored through transparent microchannels, usually made from PDMS. The biosensing devices have been used in detection of foodborne pathogens, plant diseases, and sources of fecal contamination on fresh produce. Isothermal DNA amplification techniques (such as LAMP) have provided the opportunity to bring the nucleic acid testing on farm. Paper-based devices (known as μPADs) can be utilized in storing reagents in a dry state and performing the reactions at the point of need. While fresh produce industry needs on-farm easy-to-use fully-integrated diagnostic tools capable of multiplex detection by non-specialist users, such devices do not yet exist commercially. This necessitates more research focused on resolving the major challenges in developing such devices.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

Acknowledgments

This review paper is partially funded by Purdue University, USA. It is also funded in part by CPS, USA AWARD NUMBER: 2021CPS12, CDF, USA Agreement No: 20-0001-054-SF USDA, USA Cooperative Agreement No. USDA-AMS-TM-SCBGP-G-20-0003. Any opinions, findings, conclusions, or recommendations expressed in this publication or audiovisual are those of the author(s) and do not necessarily reflect the views of The Center for Produce Safety, the California Department of Food and Agriculture, or the Agricultural Marketing Service of the U.S. Department of Agriculture (USDA). The work upon which this project entitled "Field evaluation of microfluidic paper-based analytical devices for microbial source tracking" was funded in whole or in part through a subrecipient grant awarded to The Center for Produce Safety through the California Department of Food and Agriculture 2020 Specialty Crop Block Grant Program and the USDA's Agricultural Marketing Service. This manuscript has been edited by Editing Press Inc.

References

Asal, M., Ozen, O., Sahinler, M., & Polatoglu, I. (2018). Recent developments in enzyme, DNA and immuno-based biosensors. *Sensors*, *18*, 1924.

Aufrecht, J. A., Timm, C. M., Bible, A., Morrell-Falvey, J. L., Pelletier, D. A., Doktycz, M. J., et al. (2018). Quantifying the spatiotemporal dynamics of plant root colonization by beneficial bacteria in a microfluidic habitat. *Advanced Biosystems*, *2*, Article 1800048.

Bernach, M., Soffe, R., Remus-Emsermann, M. N. P., & Nock, V. (2019). Micropatterning of hybrid polydimethylsiloxane for replica leaves. *Japanese Journal of Applied Physics*, *58*, Article SDDK01.

Buddhachat, K., Ritbamrung, O., Sripairoj, N., Inthima, P., Ratanasut, K., Boonsrangsom, T., et al. (2021). One-step colorimetric LAMP (cLAMP) assay for visual detection of *Xanthomonas oryzae* pv. oryzae in rice. *Crop Protection*, *150*, Article 105809.

Burmeister, A., & Grunberger, A. (2020). Microfluidic cultivation and analysis tools for interaction studies of microbial co-cultures. *Current Opinion in Biotechnology*, *62*, 106–115.

Burmeister, A., Hilgers, F., Langner, A., Westerwalbesloh, C., Kerkhoff, Y., Tenhaef, N., et al. (2019). A microfluidic co-cultivation platform to investigate microbial interactions at defined microenvironments. *Lab on A Chip*, *19*, 98–110.

Camarca, A., Varriale, A., Capo, A., Pennacchio, A., Calabrese, A., Giannattasio, C., et al. (2021). Emergent biosensing technologies based on fluorescence spectroscopy and surface plasmon resonance. *Sensors*, *21*(906).

Centers for Disease Control and Prevention (CDC) (2021). List of selected multistate foodborne outbreak investigations. Available at https://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html.

Chen, Y., Cheng, N., Xu, Y., Huang, K., Luo, Y., & Xu, W. (2016). Point-of-care and visual detection of *P. aeruginosa* and its toxin genes by multiple LAMP and lateral flow nucleic acid biosensor. *Biosensors and Bioelectronics*, *81*, 317–323.

Cheng, Y., Feng, G., & Moraru, C. I. (2019). Micro- and nanotopography sensitive bacterial attachment mechanisms: A review. *Frontiers in Microbiology*, *10*(191).

Choi, J. R., Hu, J., Tang, R., Gong, Y., Feng, S., Ren, H., et al. (2016). An integrated paper-based sample-to-answer biosensor for nucleic acid testing at the point of care. *Lab Chip*, *16*, 611–621.

Choi, J. R., Nilghaz, A., Chen, L., Chou, K. C., & Lu, X. (2018). Modification of thread-based microfluidic device with polysiloxanes for the development of a sensitive and selective immunoassay. *Sensors and Actuators B (Chemical)*, *260*, 1043–1051.

Choi, J. R., Yong, K. W., Choi, J. Y., & Cowie, A. C. (2019). Emerging point-of-care technologies for food safety analysis. *Sensors*, *19*(817).

Connell, J. L., Ritschdorff, E. T., Whiteley, M., & Shear, J. B. (2013). 3D printing of microscopic bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 18380–18385.

Davidson, J. L., Wang, J., Maruthamuthu, M. K., Dextre, A., Pascual-Garrigos, A., Mohan, S., et al. (2021). A paper-based colorimetric molecular test for SARS-CoV-2 in saliva. *Biosensors and Bioelectronics: X*, *9*, Article 100076.

Doan, H. K., Antequera-Gómez, M. L., Parikh, A. N., & Leveau, J. H. J. (2020b). Leaf surface topography contributes to the ability of escherichia coli on leafy greens to resist removal by washing, escape disinfection with chlorine, and disperse through splash. *Frontiers in Microbiology*, *11*, 1485.

Doan, H. K., & Leveau, J. H. J. (2015). Artificial surfaces in phyllosphere microbiology. *Phytopathology*, *105*(8), 1036–1042.

Doan, H. K., Ngassam, V. N., Gilmore, S. F., Tecon, R., Parikh, A. N., & Leveau, J. H. J. (2020a). Topography-driven shape, spread, and retention of leaf surface water impacts microbial dispersion and activity in the phyllosphere. *Phytobiomes Journal*, *4*, 268–280.

Ellett, F., Jalali, F., Marand, A. L., Jorgensen, J., Mutlu, B. R., Lee, J., et al. (2019). Microfluidic arenas for war games between neutrophils and microbes. *Lab on A Chip*, *19*, 1205–1216.

Emulate (2017). FDA signs collaborative agreement with emulate, inc. to use organs-on-chips technology as a toxicology testing platform for understanding how products affect human health and safety. Available at https://www.emulatebio.com/press/fda-collab-agreement-emulate.

Fu, L. L., & Li, J. R. (2014). Microbial source tracking: A tool for identifying sources of microbial contamination in the food chain. *Critical Reviews in Food Science and Nutrition*, *54*(6), 699–707.

Gao, J., Sasse, J., Lewald, K. M., Zhalnina, K., Cornmesser, L. T., Duncombe, T. A., et al. (2018). Ecosystem fabrication (EcoFAB) protocols for the construction of laboratory ecosystems designed to study plant-microbe interactions. *Journal of Visualized Experiments*, *134*, Article e57170.

Garcia-Aljaro, C., Blanch, A. R., Campos, C., Jofre, J., & Lucena, F. (2018). Pathogens, faecal indicators and human-specific microbial source-tracking markers in sewage. *Journal of Applied Microbiology*, *126*, 701–717.

Getaz, M., Buhlmann, A., Schneeberger, P. H. H., Van Malderghem, C., Duffy, B., Maes, M., et al. (2017). A diagnostic tool for improved detection of *Xanthomonas fragariae* using a rapid and highly specific LAMP assay designed with comparative genomics. *Plant Pathology*, *66*, 1094–1102.

Green, D. W., Lee, K. K. H., Watson, J. A., Kim, H. Y., Yoon, K. S., Kim, E. J., et al. (2017). High quality bioreplication of intricate nanostructures from a fragile gecko skin surface with bactericidal properties. *Scientific Reports*, *7*(41023).

Griesche, G., & Baeumner, A. J. (2020). Biosensors to support sustainable agriculture and food safety. *Trends in Analytical Chemistry*, *128*, Article 115906.

Han, L., Wang, K., Ma, L., Delaquis, P., Bach, S., Feng, J., et al. (2020). Viable but nonculturable *Escherichia coli* O157:H7 and *Salmonella enterica* in fresh produce: Rapid determination by loop-mediated isothermal amplification coupled with a propidium monoazide treatment. *Applied and Environmental Microbiology*, *86*(7), e02566–19.

Hansen, R. H., Timm, A. C., Timm, C. M., Bible, A. N., Morrell-Falvey, J. L., Pelletier, D. A., et al. (2016). Stochastic assembly of bacteria in microwell arrays reveals the importance of confinement in community development. *PLoS ONE*, *11*(5), Article e0155080.

Harmon, J. B., Gray, H. K., Young, C. C., & Schwab, K. J. (2020). Microfluidic droplet application for bacterial surveillance in fresh-cut produce wash waters. *PLoS ONE*, *15*(6), Article e0233239.

Heckmann, T. S., & Schiffman, J. D. (2020). Spatially organized nanopillar arrays dissimilarly affect the antifouling and antibacterial activities of *Escherichia coli* and *Staphylococcus aureus*. *ACS Applied Nano Materials*, *3*, 977–984.

Huang, T., Li, L., Liu, X., Chen, Q., Fang, X., Kong, J., et al. (2020). Loop-mediated isothermal amplification technique: principle, development and wide application in food safety. *Analytical Methods*, *12*, 5551.

Hundesa, A., Maluquer de Motes, C., Bofill-Mas, S., Albinana-Gimenez, N., & Girones, R. (2006). Identification of human and animal adenoviruses and polyomaviruses for determination of sources of fecal contamination in the environment. *Applied Environmental Microbiology*, *72*, 7886–7893.

Jiang, Y. S., Riedel, T. E., Popoola, J. A., Morrow, B. R., Cai, S., Ellington, A. D., et al. (2018). Portable platform for rapid in-field identification of human fecal pollution in water. *Water Research*, *131*, 186–195.

Kehe, J., Kulesa, A., Ortiz, A., Ackerman, C. M., Thakku, S. G., Sellers, D., et al. (2019). Massively parallel screening of synthetic microbial communities. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(26), 12804–12809.

Kim, M., Jung, T., Kim, Y., Lee, C., Woo, K., Seol, J. H., et al. (2015). A microfluidic device for label-free detection of *Escherichia coli* in drinking water using positive dielectrophoretic focusing, capturing, and impedance measurement. *Biosensors and Bioelectronics*, *74*, 1011–1015.

Kim, J. H., & Oh, S. W. (2021). Pretreatment methods for nucleic acid–based rapid detection of pathogens in food: A review. *Food Control*, *121*, Article 107575.

Kolm, C., Martzy, R., Fuhrer, M., Mach, R. L., Krska, R., Baumgartner, S., et al. (2019). Detection of a microbial source tracking marker by isothermal helicase-dependent amplification and a nucleic acid lateral-flow strip test. *Scientific Reports*, *9*(393).

Kumari, P., Ginzburg, N., Sayas, T., Saphier, S., Bucki, P., Miyara, S. B., et al. (2020). A biomimetic platform for studying root-environment interaction. *Plant Soil*, *447*, 157–168.

Kurbanoglu, S., Erkmen, C., & Uslu, B. (2020). Frontiers in electrochemical enzyme based biosensors for food and drug analysis. *Trends in Analytical Chemistry*, *124*, Article 115809.

Leonardo, S., Toldra, A., & Campas, M. (2021). Biosensors based on isothermal DNA amplification for bacterial detection in food safety and environmental monitoring. *Sensors*, *21*(602).

Li, T., Zhu, F., Guo, W., Gu, H., Zhao, J., Yan, M., et al. (2017). Selective capture and rapid identification of *E. coli O157: H7* by carbon nanotube multilayer biosensors and microfluidic chip-based LAMP. *RSC Advances*, *7*, 30446–30452.

Linklater, D. P., Baulin, V. A., Juodkazis, S., Crawford, R. J., Stoodley, P., & Ivanova, E. P. (2021). Mechano-bactericidal actions of nanostructured surfaces. *Nature Reviews*, *19*, 8–22.

Linklater, D. P., Juodkazis, S., & Ivanova, E. P. (2017). Nanofabrication of mechano-bactericidal surfaces. *Nanoscale*, *9*, 16564–16585.

Linklater, D. P., Nguyen, H. K. D., Bhadra, C. M., Juodkazis, S., & Ivanova, E. P. (2017). Influence of nanoscale topology on bactericidal efficiency of black silicon surfaces. *Nanotechnology*, *28*, Article 245301.

Lopez-Soriano, P., Noguera, P., Gorris, M. T., Puchades, R., Maquieira, A., Marco-Noales, E., et al. (2017). Lateral flow immunoassay for on-site detection of xanthomonas arboricola pv. pruni in symptomatic field samples. *PLoS One*, *12*, Article e0176201.

Massalha, H., Korenblum, E., Malitsky, S., Shapiro, O. H., & Aharoni, A. (2017). Live imaging of root–bacteria interactions in a microfluidics setup. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(17), 4549–4554.

Mi, F., Hu, C., Wang, Y., Wang, L., Peng, F., Geng, P. F., et al. (2022). Recent advancements in microfuidic chip biosensor detection of foodborne pathogenic bacteria: a review. *Analytical and Bioanalytical Chemistry*, *414*, 2883–2902.

Millet, L. J., Aufrecht, J., Labbé, J., Uehling, J., Vilgalys, R., Estes, M. L., et al. (2019). Increasing access to microfluidics for studying fungi and other branched biological structures. *Fungal Biology and Biotechnology*, *6*(1).

Mohan, S., Pascual-Garrigos, A., Brouwer, H., Pillai, D., Koziol, J., Ault, A., et al. (2021). Loop-mediated isothermal amplification for the detection of Pasteurella multocida, Mannheimia haemolytica, and Histophilus somni in bovine nasal samples. *ACS Agricultural Science and Technology*, *1*(2), 100–108.

Mostafidi, M., Sanjabi, M. R., Shirkhan, F., & Tamaskani Zahedi, M. (2020). A review of recent trends in the development of the microbial safety of fruits and vegetables. *Trends in Food Science and Technology*, *103*, 321–332.

Nagy, K., Sipos, O., Gombai, E., Kerenyi, A., Valkai, S., Ormos, P., et al. (2014). Interaction of bacterial populations in coupled microchambers. *Chemical and Biochemical Engineering*, *28*, 225–231.

Nguyen, D. H. K., Loebbe, C., Linklater, D. P., Xu, X., Vrancken, N., Katkus, T., et al. (2019). The idiosyncratic self-cleaning cycle of bacteria on regularly arrayed mechano-bactericidal nanostructures. *Nanoscale*, *11*, 16455–16462.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., et al. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, *28*(12), Article e63.

Noviana, E., Ozer, T., Carrell, C. S., Link, J. S., McMahon, C., Jang, I., et al. (2021). Microfluidic paper-based analytical devices: From design to applications. *Chemical Reviews*, http://dx.doi.org/10.1021/acs.chemrev.0c01335.

Pandey, C. M., Tiwari, I., Singh, V. N., Sood, K., Sumana, G., & Malhotra, B. D. (2017). Highly sensitive electrochemical immunosensor based on graphene-wrapped copper oxide-cysteine hierarchical structure for detection of pathogenic bacteria. *Sensors and Actuators B (Chemical)*, *238*, 1060–1069.

Pang, B., Zhao, C., Li, L., Song, X., Xu, K., Wang, J., et al. (2018). Development of a low-cost paper-based ELISA method for rapid *Escherichia coli* O157: H7 detection. *Analytical Biochemistry*, *542*, 58–62.

Parashar, A., & Pandey, S. (2011). Plant-in-chip: Microfluidic system for studying root growth and pathogenic interactions in Arabidopsis. *Applied Physics Letters*, *98*, Article 263703.

Park, J., Kerner, A., Burns, M. A., & Lin, X. N. (2011). Microdroplet-enabled highly parallel co-cultivation of microbial communities. *PLoS ONE*, *6*(2), Article e17019.

Pascual-Garrigos, A., Maruthamuthu, M. K., Ault, A., Davidson, J. L., Rudakov, G., Pillai, D., et al. (2021). Onfarm colorimetric detection of *Pasteurella multocida*, Mannheimia haemolytica, and *Histophilus somni* in crude bovine nasal samples. *Veterinary Research*, *52*, 126, 2021.

Ranjbaran, M. (2019). *Pathways of bacterial infiltration into plant leaves: mechanistic modeling, microbiological and microfabrication experiments* PhD dissertation, Ithaca, NY: Cornell University.

Ranjbaran, M., & Datta, A. K. (2019). Retention and infiltration of bacteria on a plant leaf driven by surface water evaporation. *Physics of Fluids*, *31*, Article 112106.

Ranjbaran, M., Solhtalab, M., & Datta, A. K. (2020). Mechanistic modeling of light-induced chemotactic infiltration of bacteria into leaf stomata. *PLoS Computational Biology*, *16*(5), Article e1007841.

Ravaliya, K., Gentry-Shields, J., Garcia, S., Heredia, N., de Aceituno, A. F., Bartz, F. E., et al. (2014). Use of *Bacteroidales* microbial source tracking to monitor fecal contamination in fresh produce production. *Applied and Environmental Microbiology*, *80*(2), 612–617.

Regiart, M., Rinaldi-Tosi, M., Aranda, P. R., Bertolino, F. A., Villarroel-Rocha, J., Sapag, K., et al. (2017). Development of a nanostructured immunosensor for early and in situ detection of *Xanthomonas arboricola* in agricultural food production. *Talanta*, *175*, 535e541.

Rosenzweig, R., Perinbam, K., Ly, V. K., Ahrar, S., Siryaporn, A., & Yee, A. F. (2019). Nanopillared surfaces disrupt *Pseudomonas aeruginosa* mechanoresponsive upstream motility. *ACS Applied Materials and Interfaces*, *11*, 10532–10539.

Selim, S., Mo, P. J., Hao, Z., Fatthallah, N. A., & Chen, X. (2020). Blade-like structure of graphene oxide sheets decorated with cuprous oxide and silicon carbide nanocomposites as bactericidal materials. *Journal of Colloid and Interface Science*, *578*, 698–709.

Shang, Y., Sun, J., Ye, Y., Zhang, J., Zhang, Y., & Sun, X. (2020). Loop-mediated isothermal amplification-based microfluidic chip for pathogen detection. *Critical Reviews in Food Science and Nutrition*, *60*(2), 201–224.

Shin, J. H., Hong, J., Go, H., Park, J., Kong, M., Ryu, S., et al. (2018). Multiplexed detection of foodborne pathogens from contaminated lettuces using a handheld multistep lateral flow assay device. *Journal of Agricultural and Food Chemistry*, *66*, 290–297.

Sirinutsomboon, B., Delwiche, M. J., & Young, G. M. (2011). Attachment of Escherichia coli on plant surface structures built by microfabrication. *Biosystems Engineering*, *108*, 244–252.

Skandamis, P. N., & Nychas, G-J. E. (2012). Quorum sensing in the context of food microbiology. *Applied and Environmental Microbiology*, *78*(16), 5473–5482.

Soffe, R., Altenhuber, N., Bernach, M., Remus-Emsermann, M. N. P., & Nock, V. (2019). Comparison of replica leaf surface materials for phyllosphere microbiology. *PLoS ONE*, *14*(6), Article e0218102.

Soffe, R., Bernach, M., Remus-Emsermann, M. N. P., & Nock, V. (2019). Replicating Arabidopsis model leaf surfaces for phyllosphere microbiology. *Scientific Reports*, *9*(14420).

Soni, D. K., Ahmad, R., & Dubey, S. K. (2018). Biosensor for the detection of *Listeria monocytogenes*: emerging trends. *Critical Reviews in Microbiology*, *44*(5), 590–608.

Stanley, C. E., Grossmann, G., Solvas, X. C. I., & deMello, A. J. (2016). Soil-on-a-chip: Microfluidic platforms for environmental organismal studies. *Lab on A Chip*, *16*(228).

Stanley, C. E., Shrivastava, J., Brugman, R., Heinzelmann, E., van Swaay, D., & Grossmann, G. (2018). Dual-flow-RootChip reveals local adaptations of roots towards environmental asymmetry at the physiological and genetic levels. *New Phytologist*, *217*, 1357–1369.

Stanley, C. E., Stockli, M., van Swaay, D., Sabotic, J., Kallio, P. T., Kunzler, M., et al. (2014). Probing bacterial–fungal interactions at the single cell level. *Integrative Biology*, *6*(935).

Tang, R., Yang, H., Gong, Y., You, M., Liu, Z., Choi, J. R., et al. (2017b). A fully disposable and integrated paper-based device for nucleic acid extraction, amplification and detection. *Lab on A Chip*, *17*, 1270–1279.

Terry, L. A., White, S. F., & Tigwell, L. J. (2005). The application of biosensors to fresh produce and the wider food industry. *Journal of Agricultural and Food Chemistry*, *53*, 1309–1316.

Thavarajah, W., Verosloff, M. S., Jung, J. K., Alam, K. K., Miller, J. D., Jewett, M. C., et al. (2020). A primer on emerging field-deployable synthetic biology tools for global water quality monitoring. *Npj Clean Water*, *3*(18).

Vaisocherová-Lísalová, H., Víšová, I., Ermini, M. L., pringer, T., Song, X. C., Mrázek, J., et al. (2016). Low-fouling surface plasmon resonance biosensor for multi-step detection of foodborne bacterial pathogens in complex food samples. *Biosensors and Bioelectronics*, *80*, 84–90.

van Vliet, S., Hol, F. J. H., Weenink, T., Galajda, P., & Keymer, J. E. (2014). The effects of chemical interactions and culture history on the colonization of structured habitats by competing bacterial populations. *BMC Microbiology*, *14*(116).

Verma, M. S., Tsaloglou, M.-N., Sisley, T., Christodouleas, D., Chen, A., Milette, J., et al. (2018). Sliding-strip microfluidic device enables ELISA on paper. *Biosensors and Bioelectronics*, *99*, 77–84.

Verma, M. S., Tsuji, J. M., Hall, B., Chen, P. Z., Forrest, J., Jones, L., et al. (2016b). Towards point-of-care detection of polymicrobial infections: Rapid colorimetric response using a portable spectrophotometer. *Sensing and Bio-Sensing Research*, *10*, 15–19.

Verma, M. S., Wei, S. C., Rogowski, J. L., Tsuji, J. M., Chen, P. Z., Lin, C.-W., et al. (2016a). Interactions between bacterial surface and nanoparticles govern the performance of chemical nose biosensors. *Biosensors and Bioelectronics*, *83*, 115–125.

Wandiyanto, J. V., Truong, V. K., Al Kobaisi, M., Juodkazis, S., Thissen, H., Bazaka, O., et al. (2019). The fate of osteoblast-like MG-63 cells on pre-infected bactericidal nanostructured titanium surfaces. *Materials*, *12*(1575).

Wang, J., Dextre, A., Pascual-Garrigos, A., Davidson, J. L., Maruthamuthu, M. K., McChesney, D., et al. (2021a). Fabrication of a paper-based colorimetric molecular test for SARS-CoV-2. *MethodsX*, *8*, Article 101586.

Wang, J., Kanach, A., Han, R., & Applegate, B. (2021b). Application of bacteriophage in rapid detection of Escherichia coli in foods. *Current Opinion in Food Science*, *39*, 43–50.

Weng, X., & Neethirajan, S. (2017). Ensuring food safety: Quality monitoring using microfluidics. *Trends in Food Science & Technology*, *65*, 10–22.

Whitesides, G. M. (2006). The origins and the future of microfluidics. *Nature*, *442*, 368–373.

Wong, S. Y., Cabodi, M., Rolland, J., & Klapperich, C. M. (2014). Evaporative concentration on a paper-based device to concentrate analytes in a biological fluid. *Analytical Chemistry*, *86*(24), 11981–11985.

Wu, W., Zhao, S., Mao, Y., Fang, Z., Lu, X., & Zeng, L. (2015). A sensitive lateral flow biosensor for Escherichia coli O157:H7 detection based on aptamer mediated strand displacement amplification. *Analytica Chimica Acta*, *861*, 62–68.

Yoo, H., Jo, H., & Oh, S. S. (2020). Detection and beyond: Challenges and advances in aptamer-based biosensors. *Materials Advances*, *1*, 2663–2687.

Zhang, D., Bi, H., Liu, B., & Qiao, L. (2018). Detection of pathogenic microorganisms by microfluidics based analytical methods. *Analytical Chemistry*, *90*, 5512–5520.

Zhang, B., Luo, Y., Pearlstein, A. J., Aplin, J., Liu, Y., Bauchan, G. R., et al. (2014). Fabrication of biomimetically patterned surfaces and their application to probing plant-bacteria interactions. *Applied Materials and Interfaces*, *6*, 12467–12478.

Zhang, Z., Zhou, J., & Du, X. (2021). Electrochemical biosensors for detection of foodborne pathogens. *Micromachines*, *10*(222).

Zhou, C., Koshani, R., O'Brien, B., Ronholm, J., Cao, X., & Wang, Y. (2021). Bio-inspired mechano-bactericidal nanostructures: A promising strategy for eliminating surface foodborne bacteria. *Current Opinion in Food Science*, *39*, 110–119.