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Mini-Review

Human Pathogenic Microorganisms in Fresh Produce Production: Lessons Learned When Plant Science Meets Food Safety

Xingchen Zhao [1,2,*], Leo Van Overbeek [3], Catherine M. Burgess [4], Nicola Holden [5], Fiona Brennan [6], Gro S. Johannessen [7], Ana Allende [8], Monica Höfte [9], Bart Cottyn [10], Joël F. Pothier [11], Adam Schikora [12], Mieke Uyttendaele [1,*]

[1] Food Microbiology and Food Preservation Research Unit, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium
[2] Research Group for Food Microbiology and Hygiene, National Food Institute, Technical University of Denmark, 2800 Kongens Lyngby, Denmark
[3] Wageningen University & Research, Wageningen Plant Research, P.O. Box 16, 6700 AA Wageningen, The Netherlands
[4] Food Safety Department, Teagasc Food Research Centre Ashtown, Dublin, Ireland
[5] Scotland's Rural College, Ferguson Building, Craibstone Estate, AB21 9YA Aberdeen, UK
[6] Department of Soils, Environment and Landuse, Teagasc, Johnstown Castle, Wexford, Ireland
[7] Norwegian Veterinary Institute, Section for Food Safety and Animal Health Research, P.O.Box 64, 1431 Ås, Norway
[8] Research Group on Microbiology and Quality of Fruits and Vegetables, Food Science & Technology Department, CEBAS-CSIC, 30100 Murcia, Spain
[9] Laboratory of Phytopathology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium
[10] Plant Sciences Unit, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), 9810 Merelbeke, Belgium
[11] Environmental Genomics and Systems Biology Research Group, Institute of Natural Resource Sciences, Zurich University of Applied Sciences (ZHAW), Wädenswil, Switzerland
[12] Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11/12, 38104 Braunschweig, Germany

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ABSTRACT

To enhance control of human pathogenic microorganisms in plant production systems, an EU COST Action (HUPLANTcontrol CA16110) was initiated, bringing together microbiologists in food, environmental, and plant microbial ecology. This article summarizes the outcomes of multiple workshops and the four main lessons learned: (i) many terminologies need further explanation to facilitate multidisciplinary communication on the behavior of human pathogens from preharvest plant production to postharvest food storage, (ii) the complexity of bacterial taxonomy pushes microbial hazard identification for greater resolution of characterization (to subspecies, or even strain level) needing a multimethod approach, (iii) hazard characterization should consider a range of factors to evaluate the weight of evidence for adverse health effects in humans, including strain pathogenicity, host susceptibility, and the impact of the plant, food, or human gut microbiome, (iv) a wide diversity of microorganisms in varying numbers and behaviors coexist in the plant microbiome, including good (beneficial for plant or human health), bad (established human or plant pathogens), or ugly (causing spoilage or opportunistic disease). In conclusion, active listening in communication and a multiperspective approach are the foundation for every successful conversation when plant science meets food safety.

To achieve the Sustainable Development Goals, the One Health approach has been jointly proposed by the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the World Organization for Animal Health (OIE) (WHO et al., 2019). One Health is a collaborative, transdisciplinary, and multisectoral approach that aims to sustainably balance and optimize the health of humans, animals, and ecosystems (One Health High-Level Expert Panel (OHHLEP), 2022). However, there are some barriers to successfully implementing the One Health approach, including the lack of cooperation among One Health actors and the lack of harmonized One Health terminology across disciplines and sectors (OHHLEP, 2022).

The increased focus on human pathogens in edible plant-based foods (Barak & Schroeder, 2012; Fletcher et al., 2013) urged the need for a multidisciplinary network to promote cooperation between microbiologists in food and plant sciences, together with relevant public and industrial stakeholders. An EU COST Network Action on the Control of Human Pathogenic Microorganisms in Plant Production Systems (CA16110 HUPLANTcontrol, 2017–2021) was initiated. Due to their

* Corresponding authors at: Food Microbiology and Food Preservation Research Unit, Department of Food Technology, Safety and Health, Faculty of Bio-Science Engineering, Coupure Links 653, 9000 Ghent, Belgium.
E-mail addresses: xizhao@food.dtu.dk (X. Zhao), mieke.uyttendaele@ugent.be (M. Uyttendaele).

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training and disciplinary experience, microbiologists from these different research areas have different viewpoints on microbes. Plant and soil microbiologists mainly have a background in agricultural production, plant pathology, and usually view plant-associated microorganisms from an environmental microbial ecology perspective. They handle microorganisms more often in a positive manner as agents that can help plants suppress diseases, stimulate plant growth, promote stress resistance, and positively influence crop yield and quality, although they also focus on studies on nonbeneficial microorganisms associated with plant diseases present in soils, seeds, and plants. Food microbiologists often have a background in food or veterinary science including food safety and usually look at food-associated microorganisms in a targeted approach, focusing on specific spoilage organisms or human pathogens. They handle microorganisms more often in a negative manner, as agents associated with food deterioration and foodborne illness and refer to them as unwanted contamination. However, they are also interested in microorganisms associated with beneficial impacts on food production, such as during fermentation studies or when looking for protective (probiotic) cultures. Primarily, however, the focus within food microbiology is on the microorganisms of interest themselves, rather than on interactions within complex microbial communities.

Human pathogens such as *Salmonella* spp., Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* are occasionally present in edible crop production and can persist, posing significant food safety risks. *Salmonella* spp., and STEC are zoonotic pathogens (animal reservoir) but can be disseminated in the environment and introduced in plant production via fecal pollution of water or soil (Thomas et al., 2024; Van Overbeek et al., 2014). *Salmonella* can attach to and might internalize within produce, surviving through various stages from farm to table (Jacobsen & Bech, 2012; Zarkani & Schikora, 2021). STEC can persist in soil and on plants, with certain subpopulations demonstrating enhanced tolerance to environmental stresses (Murphy et al., 2024; Ramos et al., 2021). *L. monocytogenes* has an environmental reservoir but is also a zoonotic pathogen. It can be found in a wide range of animals, decaying vegetation, water, and soil. Once introduced in the food supply chain, it particularly persists in humid and cold conditions in food processing environments, increasing the likelihood of contamination of finished products, for example via contaminated equipment (Sullivan et al., 2022; Townsend et al., 2021). Stringent good agricultural and processing practices need to be implemented to mitigate the presence of these human pathogens and ensure food safety (Gil et al., 2015). Both food microbiologists and plant microbiologists share concerns regarding Human Pathogenic Microorganisms (HPMOs) in the agri-food chain. It is clear that both disciplines need to effectively collaborate to elaborate control measures from farm to fork in order to prevent foodborne illness originating from fresh produce. Food microbiologists, and microbial food safety as a discipline, need to communicate with plant microbiologists and to be updated on the views and research emerging from the discipline of plant-microbiome interactions, and vice versa. It was one of the objectives of the EU COST Action HUPLANTcontrol to facilitate the multidisciplinary collaboration by developing a common understanding of the terminology used.

The EU COST Action HUPLANTcontrol initiated several workshops aiming to:

- clarify the terminology used in the respective research fields,
- discuss the identification of members of plant microbial communities that may pose a negative impact on human health, their behavior in the plant or plant-derived food microbiome,
- control HPMOs in plant production systems.

When plant science meets food safety, four main lessons learned during the HUPLANTcontrol workshops are presented in this paper. The scope of microorganisms of food safety concern was in this context limited to bacterial pathogens; thus, Archaea or eukaryotic organisms such as pathogenic molds and yeasts (fungi), parasites, or viruses were not considered.

Lesson #1: Dialogue is needed to understand the terminology of each other's discipline

All conversations within the HUPLANTcontrol EU COST Action started from the definition of a pathogen (see Glossary Box). The use of the single term 'pathogen' generates confusion among plant and food microbiologists, as a pathogen often refers to a 'plant pathogen (a phytopathogen)' for plant science, but to a 'human pathogen' for food safety. In this paper, the focus is on human bacterial pathogens and not plant pathogens. The epidemiological triad suggests that every microorganism can be a human pathogen if there is a susceptible human host, and the context or environmental factors enable the susceptible host to come into contact with the microbial agent (van Seventer & Hochberg, 2017). Often, further classification of the pathogen is inherently linked to the susceptibility of the host for infection (Casadevall & Pirofski, 2018, 2002). As such, human pathogens are usually classified into established human pathogens and opportunistic human pathogens (see Glossary Box). The established human pathogens are those that present a substantial public health burden to the society and can affect any individual in the general population. Opportunistic human pathogens are those microorganisms that are normally commensal in the human body or do not pose any harm. Opportunistic pathogens can cause disease when the host's resistance is altered, e.g. immunocompromised individuals who have become more susceptible to infection due to medical conditions or treatments, or due to age or pregnancy (Berg et al., 2014).

Glossary box Some definitions (in alphabetical order)

Established human pathogen: microorganisms involved in causing consistently human infectious disease in an individual, also when these persons have no prior underlying disease or disturbed health condition. They can cause widespread occurrence of an infectious disease in a community (an outbreak or an epidemic).

Human pathogen: microorganisms involved in causing human infectious disease, as confirmed by isolation of the disease-causing microorganism from the patient (microbiological evidence) or by culture-independent diagnostic tests (CIDTs) such as DNA-based techniques including PCR (Polymerase Chain Reaction) or next-generation sequencing approaches.

Foodborne pathogen: foodborne pathogens (e.g. viruses, bacteria, parasites) are biological agents that can cause a foodborne illness event (Bintsis, 2017).

Hazard: A biological, chemical, or physical agent in, or condition of, food that has the potential to cause an adverse health effect (CAC, 1999).

Microbiota: the living organisms of an ecosystem or a particular area (Berg et al., 2020).

Opportunistic human pathogens: microorganisms that do not usually cause disease in a healthy, immunocompetent host, but that take advantage of certain situations in which conditions are favorable for the microorganism to dominate (e.g. in case of dysbiosis or disturbed and low diversity of the gut microbiota) or to express virulence factors (Brown et al., 2012).

Pathogen: a microorganism with the potential to cause disease to its (susceptible) host, with the severity of the disease symptoms being referred to as virulence (Balloux & van Dorp, 2017).

Pathogenicity: the capacity of a microbe to cause disease / damage in a host (Casadevall & Pirofski, 1999).

Phyllosphere: the parts of a plant above the ground, usually the leaf surface, that are regarded as a habitat for microorganisms (Tokas et al., 2023).

Phytopathogen (Plant pathogen): microorganism involved in causing plant diseases. They are one of the major causes of negative crop yield and sales.

Qualified Presumption of Safety (QPS) assessment: the assessment of the safety of biological agents (microorganisms) that the European Food and Safety Authority (EFSA) is required to do in the applications it receives for market authorization of feed additives, food additives, food enzymes, food flavorings, novel foods,

and plant protection products ("regulated products") (EFSA, 2023b).

Risk: a function of the probability of an adverse health effect and the severity of that effect as a consequence of a hazard in food (CAC, 1999).

Strong epidemiological evidence: statistically significant association between exposure and being a case in a well-conducted analytical epidemiologic study, or convincing descriptive evidence (EFSA, 2014a).

Strong microbiological evidence: the identification of an indistinguishable causative agent in a human case and in a food, a food component, or its environment that is unlikely to have been contaminated coincidentally, or after the event, or the identification of a causative agent, such as a toxin or bio-active amine, in the food vehicle, combined with strongly indicative clinical symptoms and onset of illness in outbreak cases that are strongly indicative/pathognomonic to the causative agent (EFSA, 2014a).

Virulence factor: a component of a pathogen that causes damage to the host. It enables a pathogen to colonize, replicate, and disseminate within a host, in part by subverting or eluding host defenses (Casadevall & Pirofski, 1999; Cross, 2008).

Virulence: the relative capacity of a microbe to cause damage, with the term 'relative' being a necessary component of the definition, as there are no absolute measures of virulence. Virulence is only expressed in a susceptible host and thus its measurement is dependent on the host system used (Casadevall, 2017; Casadevall & Pirofski, 2002).

Furthermore, human illness due to specific pathogens can be attributed to specific foods (of animal or plant-based origin) but also to other sources like environmental exposure (e.g. waterborne), direct contact with animals, and human-to-human contact (EFSA, 2008). In the HUPLANTcontrol EU COST Action, the focus was on HPMOs with a foodborne transmission route linked with edible plant production. *Salmonella* spp., STEC, and *L. monocytogenes* present significant public health concerns when it comes to foodborne illnesses originating from fresh produce. For example, notwithstanding *Salmonella* spp., and STEC being zoonotic pathogens, it has been established that also often foods of nonanimal origin are responsible for foodborne salmonellosis or STEC infection. Attribution models estimating the sources of foodborne salmonellosis and STEC infections suggest that produce (approximately 30–35%) contributes nearly as much to infections as chicken (around 34%) in the case of salmonellosis, and as beef (about 30–40%) in the case of STEC infections (Pires et al., 2019; Rose et al., 2025). *L. monocytogenes* cases are mainly linked to a variety of ready-to-eat foods including fresh meat (e.g. steak tartare), seafoods (e.g. smoked fish), raw milk, dairy products but also a wide range of raw (fresh-cut) vegetables or fruits (Filipello et al., 2020; Mughini-Gras et al., 2025). The definition of a human pathogen as a foodborne pathogen is based on the (repeated) reporting of a microbial agent in a foodborne outbreak or in individual reported cases. Strong epidemiological or microbiological evidence supports linking the human pathogen with the type of food involved, the transmission route, or the source of pathogen contamination during food processing or primary production (EFSA, 2014a). Table 1 provides an overview of some foodborne outbreaks linked to fresh produce involving *Salmonella* spp., STEC, and *L. monocytogenes*.

Frequently terminology needs further explanation to facilitate multidisciplinary communication. There is a nuance in the understanding of some terms linked to the nature of the discipline. This is because plant microbiologists from an intrinsic scientific interest more often take a 'qualitative' approach, focusing on research related to 'who is out there (and their relative abundance)?', 'what are they doing?', and 'what are the mechanisms of their behavior and adaptation?'; whereas food microbiologists from an applied science-based approach are more likely to have a 'quantitative' approach, focusing on 'who is out there (and the numbers/dose of exposure)?', 'how can we eliminate, reduce, or control them?'. Plant microbiologists try to exploit and steer the microbial community in a beneficial manner from an ecological point of view, while food safety scientists try to avoid or minimize the risk to public health by targeted reduction of specific pathogens. Box 1 shows examples of two pairs of different terms presenting similar meanings but slightly different understandings in the two disciplines, i.e. 'microbial community' and 'persistence' (often used by plant microbiologists) versus 'microbial contamination' and 'survival' of human pathogens (similar terms often used in microbial food safety).

Box 1 When plant science meets food safety: definition of 'microbial contamination' versus 'microbial community' and 'persistence' versus 'survival'

Contamination: This is the presence of a substance/agent where it should not be or at concentrations above the background (Chapman, 2007).

- From a food safety perspective, microbial contamination refers to the undesired introduction of a defined (group of) human pathogenic microorganism(s) into or on plants and crops, intermediate (minimal) processed products or packaging material, production (post-)harvest equipment, and production areas.
- In the context of a microbial community, contamination would refer to nonindigenous occurring microorganisms, microorganisms for which the host is not its main reservoir or for which the (inert abiotic or biological/plant) surface or environment is not a natural environment or ecological niche for the microorganism.
  - o *Note: the term 'contamination' has not been often used in plant science, because the extraneous microorganisms could persist, adapt and colonize on/in the plant (definition below), and as such could be considered a member of the microbial community.*

Persistence: This is tied up with the fitness of an organism for a given environment. Stochastic factors (e.g. heterogeneity of the cell population in terms of tolerance toward environmental factors or growth phase and growth rate) as well as some intrinsic characteristics of the pathogen (e.g. abundance, virulence, or fitness factors in a strain) influence the persistence. Moreover, bacterial cells may change (adapt) physiologically and therefore persist under otherwise less favorable conditions (e.g. viable nonculturable form or in biofilms).

- From a food safety perspective, the term 'persistence' is often used to describe the survival of microorganisms in a food production (either preharvest or postharvest) environment.
  - o *Note: to describe the behavior of foodborne pathogens in the food matrix, the term 'survival' is more often used to determine the dynamic change of a (human) pathogen over time. Because the pathogen has been considered a contaminant, prevention or intervention strategies are often used to reduce and preferably inactivate it. Survival is monitored quantitatively to estimate the corresponding risk to public health of its (residual) presence in food according to the dose-response relationship.*
- In plant science, the term 'persistence' is used in a (slightly) different context. Persistence in ecological terms indicates that the microorganism can survive within the microenvironment but is grossly unable to colonize the niche. The term 'growth' or 'reproduction' is an important criterion for colonization, but not for persistence (Onofri, 2011).

Lesson #2: Bacterial diversity, versatility, and the use of genomics exert pressure on taxonomical classification toward hazard identification

The concept of bacterial 'species' is notoriously ambiguous. In the pregenomic era, bacterial species definition was historically based on

Table 1
A selection of EU multicountry or US multistate foodborne outbreaks of human pathogens *Salmonella*, pathogenic Shiga-toxin producing *Escherichia coli* (STEC), and *Listeria monocytogenes* linked to fresh produce

Pathogen	Fresh produce item	Geographical location	Year	Number of Cases	Number of Hospitalizations	Number of Deaths	Reference(s)
Salmonella (multiple serotypes)	Sprouted seeds	EU (9 countries, mainly Norway, Sweden, Finland and Germany)	2023–2025	509	100	0	ECDC and EFSA (2025)
Salmonella Typhimurium	Cucumbers	USA	2024	113	28	0	FDA (2025)
Salmonella Strathcona	Small tomatoes	EU (16 countries, mainly Italy, Germany, Austria, France) and UK	2023–2024	232	0	0	ECDC and EFSA (2024)
Salmonella Sundsvall	Cantaloupes	USA	2023	407	158	6	FDA (2024)
Salmonella Senftenberg	(possibly) cherry-like tomatoes	EU (12 countries, mainly Germany, France, Finland, Sweden) and USA	2022–2023	92	12	1	ECDC and EFSA (2023)
Salmonella Typhimurium	Packaged leafy greens	USA	2021	11	4	0	FDA (2022a)
STEC O157:H7	Spinach	USA	2021	15	4	0	FDA (2022b)
STEC O157:H7	Romaine lettuce	USA	2018	210	96	5	FDA (2019)
STEC O104:H4	Sprouted seeds	EU (mainly Germany and France) and Switzerland, US and Canada	2011	4022	778	50	EFSA (2011)
Listeria monocytogenes	Fresh-cut leafy greens	USA	2021	28	26	4	CDC (2022b, 2022a)
Listeria monocytogenes	Frozen (mixed) vegetables	EU (Austria, Denmark, Finland, Sweden and the United Kingdom)	2015–2018	47	9	16	ECDC and EFSA (2018)
Listeria monocytogenes	Fresh Whole Cantaloupe	USA	2011	147	143	33	CDC, 2012

phenotypic information, or medical and economic relevance. From the latter half of the 20th century to the early part of the 21st century, genotypic approaches led to an updated species definition: "a prokaryotic species is considered to be a group of strains that are characterized by a certain degree of phenotypic consistency, showing 70% of DNA–DNA binding and over 97% of 16S ribosomal RNA (rRNA) gene-sequence identity" (Gevers et al., 2005). Soon later, whole genome sequencing (WGS) delivered a new taxonomic metric, i.e. average nucleotide identity (ANI). An ANI value of ≥95% corresponding to the traditional 70% DNA-DNA hybridization threshold has been defined as the species boundary (Goris et al., 2007), which remains in use today. This brings the new concept of 'genomospecies' which is a phylogenetic definition of species based on a genomic method, i.e. ANI. However, ANI-based genomospecies delineation may lead to greater taxonomic ambiguity and contradiction, as traditionally used phenotype-based species names may be inconsistent with genome evolution (Pettengill et al., 2016; Smith et al., 2018a, 2018b; Tettelin & Medini, 2020). Therefore, some microbial taxonomists use a comprehensive/hybrid approach to define a bacterial species, where a bacterial species represents "a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity with respect to many independent characteristics and is diagnosable by a discriminative phenotypic property" (Rosselló-Mora & Amann, 2001). Notably, there is no official classification of prokaryotes, but the names given to prokaryotes are regulated by the International Code of Nomenclature of Bacteria (Bacteriological Code) and its successors (International Code of Nomenclature of Prokaryotes) (Parte et al., 2020). The List of Prokaryotic names with Standing in Nomenclature (LPSN) can be found on the website: https://lpsn.dsmz.de/based on the principles of (1) valid publication, (2) legitimacy, and (3) priority of publication.

The complexity to define a (bacterial) species has implications in facilitating cross-disciplinary communication of scientific findings among microbiologists, but also with nonmicrobiologists, stakeholders, and public health management that lack expertise in bacterial systematics or molecular biology. However, in a regulatory environment or diagnostic laboratories, there is often a temptation to allocate human pathogens to the species level. For instance, the application of the Qualified Presumption of Safety (QPS) assessment in a regulatory policy by EU governments that imposes controls and restrictions on the use or application of defined bacterial agents (Box 2; *Bacillus thuringiensis* as an example); or routine diagnostic laboratories active in monitoring and surveillance seeking to apply simple, rapid (and cost-effective) detection and identification methods in sampling and testing of products (or production environment) for the presence of human pathogens. It is clear that a 'species' can often contain both nonpathogenic as well as human pathogenic strains, such as *Escherichia coli* (Box 3). Therefore, there is a need to be able to characterize bacterial isolates to greater resolution, i.e. to the subspecies, infra-subspecies (e.g. serovars/serotypes) (EFSA, 2025), or nowadays to the strain level using whole genome sequencing (WGS) of isolates (e.g., in foodborne outbreak investigation). The identification of certain pathogenicity islands or defined virulence, toxin, or antibiotic resistance genes may enable conclusions about the pathogenic potential or increased public health concern of the bacterial carrier. However, horizontal gene transfer, the loss of virulence genes during subculturing etc. may occur. Therefore, identifying other properties of the bacterial pathogen by other techniques will still be necessary for reliable diagnosis.

Box 2 Why is the species *Bacillus thuringiensis* not on the QPS list as a biopesticide?
During the QPS assessment, a microorganism must meet the following criteria: (1) Its taxonomic identity must be well-defined. (2) The available body of knowledge must be sufficient to establish its safety. (3) Its lack of pathogenic properties must be established and substantiated. (4) Its intended use must be clearly described.

Overall, *B. thuringiensis* as a species does not meet the first three criteria very well. Firstly, the taxonomy and nomenclature of *Bacillus cereus* group members (including *B. thuringiensis*) are collapsing. For historical reasons, the taxonomy and the definition of these so-called classical species within the *B. cereus* group are predominantly based on phenotypes, related to their medical and economic significance, which are mainly associated with plasmid elements (Kolstø et al., 2002; Rasko et al., 2005). Relying strictly on a phenotypic-based taxonomic classification at the species level can lead not only to misclassification of isolates but also to an inaccurate assessment of a given isolate's virulence potential. The above reasons suggest that describing the taxonomy of the *B. cereus* group by phenotypically based species names alone is not adequate, but due to traditional and pragmatic reasons, the classical species names are still being used today. The classification and taxonomic separation of the *B. cereus* group needs to be supported by genetic evidence with modern molecular biology tools. However, numerous genomospecies thresholds (92.5–97% ANI) for *B. cereus sensu lato* species classification have been used by researchers (Carroll et al., 2020, 2022; Liu et al., 2017; Miller et al., 2016), sometimes resulting in the same isolate or strain being assigned to different species using different taxonomic frameworks (Carroll et al., 2022; Zervas et al., 2020). Thus, it is better to unambiguously identify commercial *B. thuringiensis* biopesticide strains at the strain level using WGS (EFSA, 2016; Fichant et al., 2022; Zhao et al., 2022). Secondly, *B. thuringiensis* is a spore-forming bacterium that is widely distributed in the environment (i.e., soil and plant), and some strains have been authorized and safely used as commercial biopesticides for decades (Raymond & Federici, 2017). However, the few outbreaks that have been reported related to *B. thuringiensis* warrant attention and fuel the debate for its position as a foodborne pathogen (Bonis et al., 2021; EFSA, 2016; Raymond & Federici, 2017). Thirdly, it has been reported that many *B. thuringiensis* isolates carry the enterotoxin and *cytK-2* genes, and express other virulence factors (Johler et al., 2018; Schwenk et al., 2020; Zhao et al., 2022). Thus, the reasons mentioned above explain why *B. thuringiensis* is not on the list of QPS status evaluated and published by EFSA (EFSA, 2023b, 2025).

Box 3 *Escherichia coli*: a paradigm as a versatile bacterial species

Escherichia coli exhibits considerable versatility and comprises a variety of nonpathogenic variants, as well as pathogenic variants that have been identified to cause intestinal or extraintestinal diseases in humans and many animal hosts (Leimbach et al., 2013). *E. coli* clearly exhibits fitness in multiple niches and can become naturalized in the environment. Most *E. coli* strains are not harmful and are an important part of the normal microbiota of the human gut, where they can be beneficial to humans (Bentley & Meganathan, 1982; Hudault, 2001; Martinson & Walk, 2020). The better-studied pathogenic *E. coli* is divided into two large groups: the intestinal pathogenic *E. coli* and the extraintestinal pathogenic *E. coli*, depending on the site of infection. Both are further divided into different pathotypes that cause distinct diseases (Kaper et al., 2004). In food safety, the most prominent intestinal pathogenic *E. coli* causing foodborne outbreaks is Shiga toxin-producing *E. coli* (STEC), but enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and diffusely adherent *E. coli* (DAEC) are also important (Kaper et al., 2004; Leimbach et al., 2013).

STEC may cause a range of symptoms varying from noncomplicated diarrhea, bloody diarrhea and hemolytic uremic syndrome (HUS). In some cases, the infection can lead to death. STEC always possess Shiga toxin genes (*stx*) which encode for Shiga toxins. There are two major types of Shiga toxins, namely Stx1 and Stx 2, and the *stx* genes have several subtypes, with four for stx_1 and at least 12 for stx_2 (EFSA, 2020).

It is also worth mentioning that due to the development of phylogenetic studies, the relationship between *Shigella* species and *E. coli* (e.g. EIEC) needs to be reconsidered. *Shigella*, a bacteria responsible for bacillary dysentery, was recognized in the late 19th century. Early phenotyping experiments revealed that it was closely related to *E. coli,* and DNA–DNA reassociation studies in the 1970s showed that *Shigella* species could not be distinguished from *E. coli* strains. Recent molecular studies have further validated the genetic relatedness of the *Shigella* species and EIEC. There has been a consensus that the *Shigella* species do indeed fall within the definition of the *E. coli* species, but still, the nomenclature of the genus *Shigella* and species included has been retained for historical and medical reasons (Lan & Reeves, 2002; Parks et al., 2021; The Editors, 2000).

Lesson #3: A combination of genomic, functional, and epidemiological data is needed for microbial hazard characterization

Many elements should be considered in the characterization of the bacterial hazard that affects the ability of the bacterium to cause disease in humans via food transmission. When undertaking hazard characterization for foodborne pathogens, a range of factors need to be considered to evaluate the weight of evidence for adverse health effects in humans, needing a multimethod approach (Fig. 1). Overall, all these pieces of information, ranging from genomic data to data from infection models in cellular microbiology and real-life data from foodborne outbreaks, are used to describe the likelihood of infection and disease in human hosts, thus moving from the hazard to the risk (Glossary Box 1) (FAO/WHO, 2021).

Pathogenicity and virulence (refer to Glossary Box) of bacterial hazards have often been addressed and evaluated. At the most basic level, genetic content describes whether virulence factors are present or not, as well as their predicted function and how they may impact the host. To determine whether the virulence factors are likely to be active in a particular environmental niche, or a susceptible host, their gene expression first needs to be determined, coupled with production of the gene product (e.g. protein) (Thomas & Wigneshweraraj, 2014). This determines the activity of a particular virulence factor. To test the function of virulence factors and understand the human host-pathogen interaction, model systems of increasing complexity are used (Fig. 1) (López-Jiménez & Mostowy, 2021). Many *in vitro* models are used, from layers of a single cell type right up to reconstructed artificial organs (Feaugas & Sauvonnet, 2021; Law et al., 2013), from static to dynamic simulation models of the gastrointestinal tract with or without higher complexity of competing microbiota present (e.g. SHIME®) (Roussel et al., 2020). Specific measurements of pathogenicity in *in vivo* models including animal (Palmer & Slauch, 2017) and human volunteer studies (Mawer, 1988) can also be performed, but have drawbacks. Due to ethical reasons and improving animal welfare, the use of animal tests is reduced (Festing & Lovell, 1996) and volunteer studies are quite costly and difficult to perform (Roestenberg et al., 2018).

Casadevall (2017) introduced the concept and mathematical calculation of pathogenic potential, taking into account the contributions of both the (human) host and the microbe (i.e. the bacterial strain) to microbial pathogenesis (Casadevall, 2017). In the case of foodborne established human pathogens such as *Salmonella* spp., STEC, and *L. monocytogenes*, information on host susceptibility and dose-response relationship can be generated from available epidemiological and microbiological data from foodborne outbreaks supported with strong evidence (FAO/WHO, 2021, 2002). Besides, the impact of the type of food/vehicle on the overall likelihood of infection and severity of disease can also be considered. Characteristics such as intrinsic or extrinsic physio-chemical factors, composition, and structure of the (edible) plant or plant-based food, but also competing microbiota present and external climatic or storage conditions, will influence the growth and

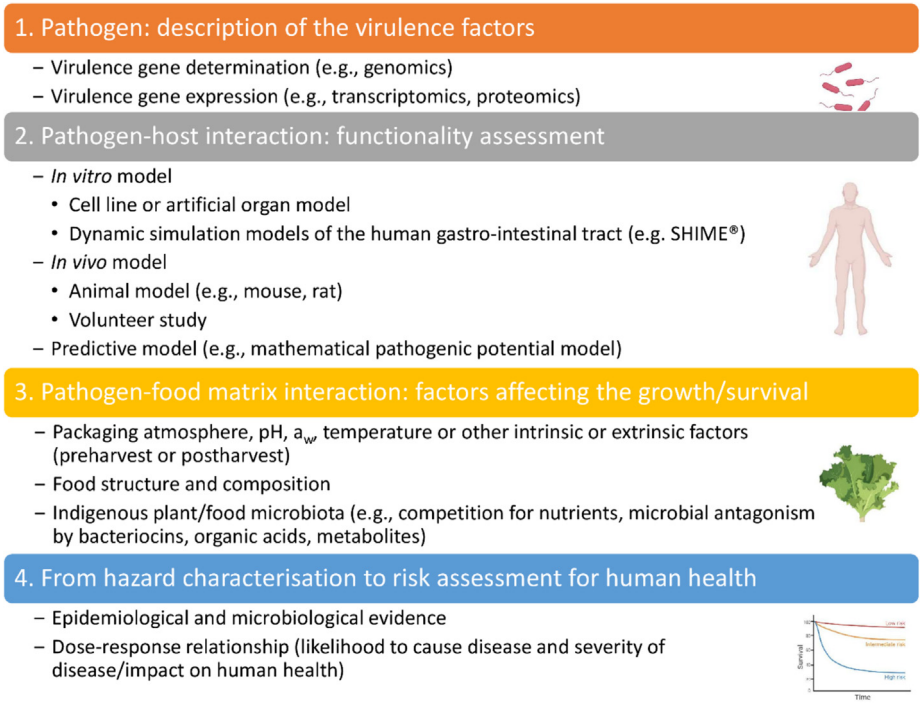

Figure 1. Different factors to evaluate the weight of evidence for adverse health effects in humans when undertaking hazard characterization for foodborne pathogens.

persistence of the pathogen (and thus its concentration) both pre- and postharvest and thus the dose to which consumers are exposed. In addition, after food consumption, there might be effects of food composition on foodborne pathogen infectious dose and host susceptibility (Fig. 1) (Ponder, 2017). For example, survival of *Salmonella* during gastric passage is known to be improved by low water activity and high-fat content (Morasi et al., 2022). It is also hypothesized that prior exposure to stressors within the food product, including sublethal exposures to organic acids or thermal processing, may affect the virulence of a pathogen and may protect them from the human body's defense system (Horn & Bhunia, 2018). However, in minimally processed (fresh-cut prepacked) edible plant-based food (e.g. leafy vegetables), such stressful preservation factors are usually not applicable, although, in some countries, leafy vegetables or other fresh produce are exposed to oxidizing sanitizers in water. More importantly, these types of raw or minimally processed plant-based foods usually have a high intrinsic microbial load, but also a diverse microbiome composition. The activities of one bacterial species (or group) may therefore influence the growth and activities of others. As such, there is a need to consider the 'HPMO – plant microbiome interaction'. This is addressed and discussed in the following section (lesson 4).

Lesson #4: Microbes are everywhere: the good, the bad, and the ugly in the plant microbiome

Plants are naturally associated with a diverse community of microorganisms including bacteria, fungi, viruses, archaea, protozoa, etc. (Dastogeer et al., 2020; Trivedi et al., 2020). The phyllosphere community of leafy vegetables mainly comprises bacteria belonging to the phylum Proteobacteria, followed by Actinobacteria, Bacteroidetes, and Firmicutes (Müller et al., 2016; Sohrabi et al., 2023; Trivedi et al., 2020). Pseudomonadales, Enterobacterales, Bacillales, and Rhizobiales were the most abundant bacterial orders on leafy vegetables such as lettuce, spinach, and rocket (Brandl et al., 2023; Darlison et al., 2019). Fresh produce including minimally processed vegetables, such as ready-to-eat salad or fruit mixes, undergo only limited processing steps, typically including trimming, shredding, washing, and packaging, with the primary aim of preserving visual appeal and sensory quality (EFSA, 2014b; Garrett, 1999; Gómez-López et al., 2008). As a result, the production process does not include any specific control measure targeting microbial inactivation, and the final product retains an elevated microbial load (often $>10^{6-7}$ colony-forming units per gram for total aerobic plate counts (TAPC)). Traditionally, food microbiologists have considered TAPC alongside counts of specific spoilage organisms such as yeast or molds, as indicators of the quality of fresh, perishable foods (Brackett, 1993; Erkmen, 2022; Uyttendaele, 2018). These practices originate historically from the quality assessment of animal-based products, such as dairy and meat products, where it is generally accepted that a lower microbial load correlates with a higher-quality product. For instance, in meat, most surface contamination is removed during the dehiding of carcasses, and further contamination during slaughter is minimized through Good Hygiene Practices (GHP). In dairy products, pasteurization or other heat treatments reduce microbial loads, making elevated counts an indication of insufficient thermal processing or postprocess contamination. However, while microbiological guidelines based on plate counts are widely accepted and applied for quality assessment of animal-based products, their relevance for fresh produce is questionable. Unlike animal-based or heat-treated foods, fresh fruits and vegetables inherently carry a high and variable microbial load due to environmental exposure during growth and harvest (Berg et al., 2020; Jackson et al., 2013; Medina-Martínez et al., 2015). Additionally, fresh produce does not undergo any processing steps that significantly reduce microbial populations. Most importantly, their quality is primarily influenced by physiological and enzymatic processes, such as dehydration, enzymatic browning, or wilting, rather than microbial proliferation. Consequently, a high microbial load on fresh produce does not necessarily indicate a lower quality product, and achieving lower microbial counts is not the best indicator of freshness in fresh produce. Moreover, the edible plant microbiome (also assumed to be the microbiome of raw or minimally processed plant-based foods), as a part of our diet, has been recognized as a transient contributor to the gut microbiome and a stimulus to the human immune system (Berg et al., 2015; Soto-Giron et al., 2021) (Fig. 2). Thus, the high microbial load on

raw or minimally processed fresh produce can be "potentially" considered overall as 'good bugs'.

The microorganisms living in association with plants are grossly divided into three functional groups by plant microbiologists: (i) those that cause diseases in plants, referred to as 'phytopathogens'; (ii) those that contribute to plant growth and establishment, e.g. by nutrient acquisition, growth support and/or disease suppression, referred to as 'plant mutualists or plant beneficial microorganisms'; and (iii) those that have no obvious interaction with plants, and this group is often referred to as 'commensals' (Trivedi et al., 2020; Wiesmann et al., 2022). The first group, phytopathogens, are considered 'bad bugs' by plant microbiologists (Mendes et al., 2013). Phytopathogenic fungi (*Botrytis*, *Alternaria*, *Fusarium* species, etc.) and bacteria (*Pseudomonas*, *Xanthomonas*, *Erwinia* species, etc.) lead to significant economic losses worldwide in fresh produce crop production (Barth et al., 2009). They can be introduced on the crop via the seeds itself or during crop growth in the field. The impact of phytopathogens on agricultural systems and strategies for plant disease control is an important research subject in plant pathology (Chen & Nan, 2015). Many of these 'bad bugs' from a plant perspective will be considered as 'ugly bugs' by food microbiologists as they will contribute to food spoilage. Visual mold growth or bacterial spots make fruits and vegetables nonacceptable for sale. While bacterial or fungal phytopathogens cause the decomposition of plant tissue (e.g. soft rot initiated by pectinase and cellulase enzymatic activity), this will make bacterial penetration into tissues of fruits and vegetables easier leading to enhanced spoilage. Microbial damage to the leaf cuticle will also inevitably facilitate the entry of human bacterial pathogens such as *Salmonella* or STEC into the subsurface areas of the leaf tissue and impact on food safety aspects (Golberg et al., 2011). However, a diverse community of epiphytes ('commensals in the phyllosphere or rhizosphere') colonizing fruits and vegetables represent significant competitive barriers to intruding spoilage microbes. A decline in epiphytic microflora has been associated with increased growth of spoilage microorganisms, illustrating the role of competition (Alegbeleye et al., 2022). As such, the third group of microorganisms being part of the microbiota can be considered 'good bugs'.

The terms 'the good', 'the bad', and 'the ugly' are arbitrary as microbial species may be beneficial or (human or plant) pathogenic depending on its genetic composition (virulence factors), its abundance, its

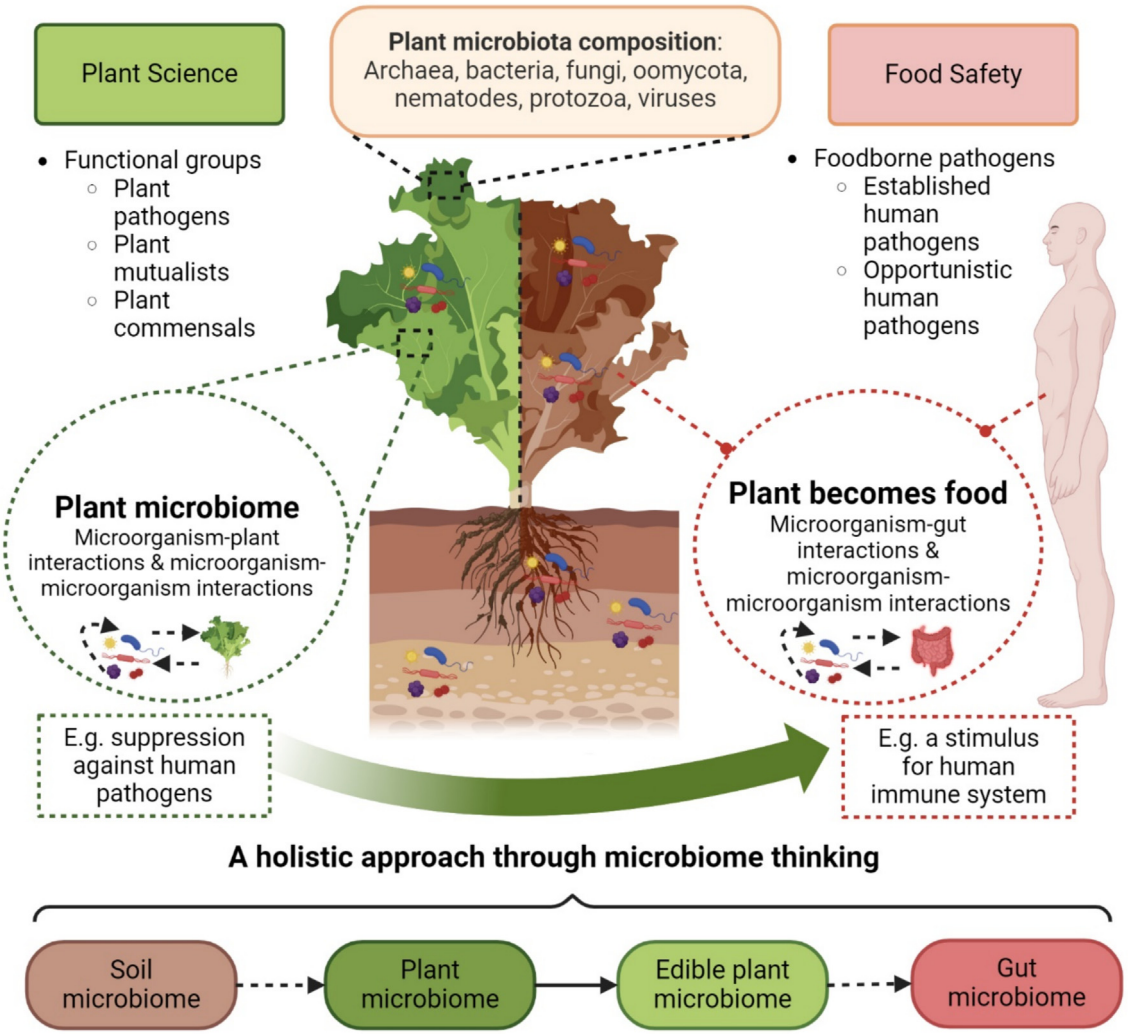

Figure 2. An illustration of the holistic approach through microbiome thinking when plant science meets food safety. Plant microbiologists usually look at plant-associated microorganisms grossly based on three functional groups: plant pathogens, mutualists, and commensals. Food microbiologists usually focus on foodborne human pathogens (established or opportunistic) on edible plants. According to the holistic approach through microbiome thinking, the microorganism-plant interactions, microorganism-microorganism interactions in plants, and microorganism-gut interactions, microorganism-microorganism interactions in humans should be considered. Besides, the impact of soil microbiome on plant microbiome, and the impact of edible plant microbiome on gut microbiome (and further on human health) should also be considered. Created in BioRender. Uyttendaele, M. (2025) https://BioRender.com/0dmxext.

environment, and the presence of a susceptible (human or plant) host. The terminology is merely used to facilitate the understanding of the debate in multidisciplinary research when plant science meets food science. This debate has become particularly obvious in relationship to the use of microorganisms as biopesticides as is exemplified in the following. The second group of microorganisms being part of the microbiota, the 'plant mutualists or plant beneficial microorganisms', is of particular interest in modern agriculture for the development of biocontrol and biostimulant products (Beyari, 2024; Sohrabi et al., 2023). They are considered as 'good bugs' by plant microbiologists (Mendes et al., 2013). The push for sustainable agriculture drives scientists to find eco-friendly alternatives to chemical pesticides or fertilizers. As a result, specific bacterial strains of for example *Bacillus* and *Pseudomonas* species but also some defined fungal strains are employed as biocontrol agents to combat crop diseases and support integrated pest management or agro-ecological fresh produce production. These 'good bugs' help to control the spread of phytopathogens and boost plant growth and resilience to stress. However, the safety of some of these biocontrol agents is debated relating to their ability to cause human infectious disease and thus might represent an 'ugly bug' or even a 'bad bug' from a food safety perspective. Food safety concerns are in particular raised with biopesticides as during application, quite high numbers of a microbial agent are deliberately deposited on crops, without necessarily the mandatory respect of a set preharvest interval (waiting time before harvest) which might result in contamination of fresh produce with elevated levels of this particular microbial strain postharvest. These food safety concerns on biopesticide strains have become particularly emerging for the use of commercial *Bacillus thuringiensis*-based biopesticides in the primary production of fresh produce (De Bock et al., 2021; EFSA, 2016). *B. thuringiensis* is a common soil organism and insect pathogen, genetically closely related to the established human foodborne pathogen *B. cereus*. As mentioned in Lesson#2, the QPS assessment approach was developed in the EU for microorganisms intended for use in food or feed chains (EFSA, 2023b; Leuschner et al., 2010). The QPS pillars are related to (i) taxonomic identity, (ii) body of knowledge (ecological aspects such as natural presence in agricultural soils, use as microbial plant protection product, attachment to edible plant components, prevalence, and concentrations in the food and feed chain, environmental distribution, etc.), and (iii) safety concerns (case reports of human disease/ foodborne outbreaks, particularly foodborne infections or intoxications, presence of virulence factors in the genome sequence of the strains, *in vitro* and *in vivo* safety tests). In a recent update of the QPS list, *B. thuringiensis* has not been recommended for the QPS status due to safety concerns (EFSA, 2025). *B. thuringiensis* strains belong to the *Bacillus cereus sensu lato* group, which is an established foodborne pathogen, have toxigenic/pathogenic potential (diarrheal enterotoxin genes), and there is possible involvement in foodborne outbreaks. Apart from Gram-positive *Bacillus* species, many Gram-negative bacterial genera, including *Aeromonas*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, and *Stenotrophomonas* have bivalent interactions with plant and human hosts. Several members of these genera show plant growth-promoting as well as antagonistic properties against plant or human pathogens. They could therefore be utilized for the development of biostimulants, biopesticides, or protective cultures on fresh produce (Berg et al., 2005; Schuenzel & Harrison, 2002; Uhlig et al., 2021). These genera are dominant residents on edible crops postharvest, which means that large quantities of these bacteria (often $>10^{5-6}$ colony-forming units per gram) are normally ingested during the consumption of fresh produce. These bacterial genera have not (yet) been associated with large foodborne outbreaks and thus are not recognized as established foodborne pathogens. On the other hand, some members of these genera are known to be opportunistic human pathogens with the ability to cause nosocomial infections in immunocompromised or elderly persons in hospital or healthcare settings (Berg et al., 2005; Li et al., 2019; Schroth et al., 2018). As far as there is no (repeated) evidence of foodborne infections in healthy individuals or associations with foodborne outbreaks in the general population, these types of opportunistic human pathogenic bacteria can be considered as 'ugly bugs'. To minimize the risk of foodborne infections in immunocompromised patients caused by opportunistic human pathogenic bacteria, a low-microbial diet (neutropenic diet) is recommended. This diet involves avoiding raw (fresh-cut) vegetables, fruits, or herbs and instead consuming peeled or thoroughly cooked or canned vegetables and fruits (De Bock et al., 2023; Nithya & Babu, 2017). However, for the general population, the likelihood that those bacterial hazards that are intrinsic to the microbiota of plant-based foods are harmful is negligible.

Finally, although in the original plant rhizosphere microbiome perspective by Mendes et al. (2013), the 'true' human pathogens were also considered "ugly bugs" both food and plant microbiologists involved in the EU COST Action HUPLANTcontrol workshops agreed that the established human foodborne bacterial pathogens *Salmonella* spp., STEC, and *L. monocytogenes* are to be considered as 'bad bugs'. They share concerns that these human pathogens are increasingly reported to be responsible for fresh produce-related foodborne diseases (Berger et al., 2010; Carstens et al., 2019; Marshall et al., 2020; Smith et al., 2018a, 2018b) as is exemplified in Table 1. Many of these products are ready-to-eat, with no/minimal processing and thus no interventions are available to inactivate any human pathogens prior to consumption, although good agricultural practices and preventive measures might limit their occurrence (Gil et al., 2015). Notably, the contamination of fresh produce with these established human pathogens occurs infrequently (Barril et al., 2022; Van Pelt et al., 2018). Prevalence data may vary depending on the types of produce sampled as well as other factors like geographical origin, cultivation method, season, time period, etc. Two meta-analyses summarizing prevalence data in vegetables reported values of 2.30 and 3.40% for *L. monocytogenes*, 1.30 and 1.91% for STEC, and 1.60 and 0.86% for *Salmonella* (Barril et al., 2022; Silva et al., 2017). Different individual studies reported variable values of 0.11 to 1.8% for *L. monocytogenes*, 0 to 0.17% for STEC, and 0 to 0.38% for *Salmonella* (Denis et al., 2016; McLauchlin et al., 2022; Wijnands et al., 2014). Moreover, if the human pathogen is present, it is in low numbers (usually the detection methods look for detection per 25 grams) and if enumerated they are rarely found in numbers exceeding 10 per gram. These established pathogens are thus not part of the abundant indigenous microbiota (Ceuppens et al., 2017). However, 'bad bugs' warrant attention and further research, in particular in times of external drivers such as climate change (e.g. impacting on water scarcity), sustainability, and circular economy (EFSA, 2023a; Focker et al., 2022; Uyttendaele et al., 2015). From a food safety perspective, it is currently presumed that these human zoonotic pathogens in plant-based raw foods are introduced from animal sources, where they have their main reservoir and are not indigenous components of plant microbiomes, while from a plant science perspective, some human zoonotic pathogens may persist or adapt to plant niches, and as such can be considered as part of the plant microbiome (Schikora et al., 2020). However, the plant microbiome might effectively suppress human pathogens through antagonistic interactions (Schuenzel & Harrison, 2002) opening the possibility of harnessing agricultural microbiomes for human pathogen control (Brennan et al., 2022) (Fig. 2). This is where divergent perspectives in mitigation approaches between food and plant microbiologists may emerge from e.g. the conflicting approach to keep the microbial loads on produce as low as possible to minimize the introduc-

tion of potential microbial contaminants versus approaches to maximize suppression.

Concluding remarks

When plant science meets food safety, a common language is needed in communication about the diversity and versatility of the plant microbiota, and discussing the opportunities to develop intervention strategies for human pathogens in fresh produce primary production, to ensure safe plant-based raw or minimally processed food from farm to fork. In the debate, there are various views on microorganisms and when they are considered 'good, bad, or ugly bugs'. We all agree that microbes are everywhere, many of them being beneficial organisms that we can exploit to enhance fresh produce production yield (and preventing economic losses) while ensuring food safety (and protecting public health). There are, however, several human pathogens, harmful organisms causing human infectious diseases that are of concern in fresh produce production and further postharvest processing. To differentiate human pathogenic microorganisms from commensal or beneficial microbiota, there is a need for an unambiguous definition of taxonomic identity, pathogenicity, and virulence characteristics of the bacterial strain supported with strong epidemiological/microbiological evidence to establish it as a foodborne pathogen. Furthermore, knowledge of the behavior and the interaction of human pathogens within the plant, plant-based food, or human gut microbiota is essential. In microbiome thinking, the occurrence or prevention of human pathogens on edible crops, either occasionally as established primary pathogens, or as opportunistic pathogens for immunocompromised persons, will need to be balanced against the costs and benefits of available intervention strategies, the overall public health impact and the challenges to comply to the sustainable development goals. Both plant and human health can benefit from the plant microbiome. There is a need for further research (Box 4) to achieve societal acceptable and effective strategies from farm to fork to prevent and control both human and plant pathogen contamination and persistence. Active listening has been shown to be an important skill in dialogue between the various microbiological disciplines involved (food and plant science) in the development of these strategies. Scientists throughout the HUPLANTcontrol EU COST Action have found that they are also flexible organisms that have 'evolved' like a microbe (although a bit slower) and have shown to be adaptative to the multiple perspectives that multidisciplinary collaboration brings in a One Health approach.

Box 4 Outstanding Research Questions

- Can human (bacterial) pathogens become naturalized within the plant/soil microbial community?
- Can human pathogenicity emerge in plant-associated microorganisms from some particular plant primary production practices and what are the mechanisms?
- Can plants act as reservoirs or secondary habitats for human (zoonotic) pathogens and what is the relevance in terms of circular agro-production?
- How do human pathogens interact with the plant microbiome, and to what extent do suppressive interactions occur?
- Can we exploit protective cultures (e.g. biological control agents) to control human pathogens in fresh produce or other plant-based foods?
- Microbes are everywhere, how safe is safe enough?

CRediT authorship contribution statement

Xingchen Zhao: Writing – original draft, Visualization, Conceptualization. **Leo Van Overbeek:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Catherine M. Burgess:** Writing – review & editing. **Nicola Holden:** Writing – review & editing. **Fiona Brennan:** Writing – review & editing. **Gro S. Johannessen:** Writing – review & editing. **Ana Allende:** Writing – review & editing. **Monica Höfte:** Writing – review & editing. **Bart Cottyn:** Writing – review & editing. **Joël F. Pothier:** Writing – review & editing. **Adam Schikora:** Writing – review & editing. **Mieke Uyttendaele:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

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

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