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Review

Microbial Biocontrol as an Alternative to Synthetic Fungicides: Boundaries between Pre- and Postharvest Applications on Vegetables and Fruits

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Abstract: From a 'farm to fork' perspective, there are several phases in the production chain of fruits and vegetables in which undesired microbial contaminations can attack foodstuff. In managing these diseases, harvest is a crucial point for shifting the intervention criteria. While in preharvest, pest management consists of tailored agricultural practices, in postharvest, the contaminations are treated using specific (bio)technological approaches (physical, chemical, biological). Some issues connect the 'pre' and 'post', aligning some problems and possible solution. The colonisation of undesired microorganisms in preharvest can affect the postharvest quality, influencing crop production, yield and storage. Postharvest practices can 'amplify' the contamination, favouring microbial spread and provoking injures of the product, which can sustain microbial growth. In this context, microbial biocontrol is a biological strategy receiving increasing interest as sustainable innovation. Microbial-based biotools can find application both to control plant diseases and to reduce contaminations on the product, and therefore, can be considered biocontrol solutions in preharvest or in postharvest. Numerous microbial antagonists (fungi, yeasts and bacteria) can be used in the field and during storage, as reported by laboratory and industrial-scale studies. This review aims to examine the main microbial-based tools potentially representing sustainable bioprotective biotechnologies, focusing on the biotools that overtake the boundaries between pre- and postharvest applications protecting quality against microbial decay.

Keywords: microbial antagonists; food; fruit; plant; filamentous fungi; microbial contamination; bioprotection; biocontrol; *Bacillus*; yeast

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1. Introduction

Fruits and vegetables represent a crucial part of the resources contributing to human nutrition, with a relevant impact on human health and well-being and a considerable hedonistic role [1]. These plant-based commodities provide important intakes of water, vitamins, minerals, sugars, fibres and a massive diversity of phytochemicals of high interest for the plethora of functional properties ascribable to these foodstuffs [2]. In a 'from farm to fork' perspective, there are several phases in the production chain of fruits and vegetables in which the plant, in general, and the edible part, in particular, can be infested or attacked by undesired microbial contaminations [3], including phytopathogen that produce mycotoxins (e.g., *Fusarium*) [4]. In managing these diseases/decays, harvest is a crucial phase responsible for a shift in the intervention criteria [5]. While in preharvest, pest management consists of tailored agricultural practices, in postharvest, the contaminations are treated using specific technological approaches [5,6]. The colonisation of undesired microorganisms in preharvest can affect the postharvest quality, influencing crop production, yield and storage [5]. Besides, it is important to underline that postharvest practices can also 'amplify' the contamination, favouring microbial spread and provoking injures of the product, with the consequent spur out of cellular juices, rich in nutrients that sustain microbial growth [7]. Considering eukaryotic microbes, the agent of postharvest diseases are mainly filamentous fungi, such as *Botrytis cinerea*, *Colletotrichum* spp., *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. and *Colletotrichum* spp. are in the first positions of a special classification encompassing the 'world top 10 fungal pathogens' in plant pathology, ranked as a function of their impact on economic losses [8]. As said, filamentous fungi are not only food spoilers, but can also be responsible for safety risks in reason of the production of mycotoxins (secondary metabolites causing disease and death, e.g., nephrotoxic, carcinogenic) and of other metabolites responsible for adverse reactions in humans, but also in animals [4,9,10]. Moreover, prokaryotic organisms can be present on fruits and vegetables; considering these foodstuffs are minimally treated and usually consumed without further processing other than washing, they can be contaminated by food-borne pathogenic bacteria, including *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* [11–13]. These contaminations can reduce the yield, decrease shelf-life, and lower the marketability of the products. This means enormous losses in terms of economic value and wastes that the Food and Agriculture Organization (FAO) estimated in about one-fifth of the global production [14]. Several physical, chemical and biological strategies have been tested to reduce microbial decay on fresh plant-based products [6]. Each approach has peculiar pros and cons in the practical implementation as a function of facets, such as the technological regimen, the characteristic of the matrices, and market rules. In this frame, synthetic chemicals are typically used to protect plants and fruits from the abovementioned multitude of phytopathogenic organisms, even though their adverse effects on human health and on the environment are increasingly concerning [15–17]. This circumstance, therefore, displays the need for sustainable use of agropharmaceuticals, together with the demand for more environment-friendly production systems: These topics in recent years have fuelled a growing public interest in the search for alternative approaches to chemical control in plant disease management [18]. In this context, microbial biocontrol is a biological strategy receiving increasing interest because of the environment-friendly nature of the solution, useful to design sustainable innovation [19,20]. Microbial-based biotools can find application both to control plant diseases and to reduce contaminations on the product [21]. From this perspective, they can be considered biocontrol solutions in preharvest or in postharvest [3,5].

This review aims to examine the main microbial-based tools potentially used as sustainable bioprotective biotechnologies, focusing on the preharvest applications for improving the final quality and on the postharvest application to protect that quality against microbial decay.

2. Microbial Biocontrol and Sustainable Biotechnologies

Climate change is affecting agriculture production through a series of biotic and abiotic stresses. Among them, it can be underlined pathogens, nutrient insufficiencies and weather extremes, and some of those are encouraging the further use of chemicals [22]. In the current scenario, a lot of attention has been paid to the promotion of sustainable agriculture in which the agricultural crop productivities are possible by using their natural capacities, with a slight annoyance of the environment and no compromising the yields [23]. By 2050, agricultural production is expected to increase by at least 70%. At the same time, people are becoming conscious that sustainable agriculture is fundamental to gathering the future world's agricultural stipulates [24]. One way to develop sustainable crop production processes is to enhance the beneficial plant-associated microbiome. This association, known as holobiont, strongly influences the nature of phytomicrobiome [25]. Microorganisms have the potential to increase crop growth by improving nutrient use efficiency, tolerating biotic and abiotic stresses, and diseases resistance. Microbes that exert beneficial roles on plants are called Plant Growth Promoting Microorganisms (PGPM). These microbes may inhabit the rhizosphere, rhizoplane, phyllosphere, endosphere, etc. [25]. The utilisation of PGPM microbial inoculants is an old practice [26], but only recently gained more prominence among researchers. They generally belong to the bacteria (such as *Bacillus* and *Rhizobia*) and fungi (especially *Trichordema*) subgroups [25,27,28]. PGPMs with biocontrol properties have been identified by researchers, conferring benefits to a variety of crop species [22]. Berendsen et al. [29] showed that PGPM isolated from plants exposed to pathogen attack were more effective if used as inoculants, than PGPM isolated from plants with no pathogen attack. In this regard, several companies have started to use individual microorganisms as biocontrol products and develop different valuable strains. The use of these inoculants has demonstrated an increase of 10–20% in crop production [30].

Biocontrol can reduce the utilisation of industrially manufactured chemicals in agricultural production. This would mean a decline in fossil fuels and a reduction in greenhouse gas emissions. Different are the MBCA (microbial biological control agents) modes of action to protect crops from diseases [31]. They may induce resistance against infections by a pathogen in plant tissues without direct antagonistic interaction with the pathogen [32,33]. Other interactions with pathogens are competition for nutrients and space [34]. MBCAs may also interact directly with the pathogen by hyperparasitism or antibiosis. Hyperparasites invade and kill mycelium, spores, and other structures of fungal and bacterial pathogens [35]. Production of antimicrobial with inhibiting effects against pathogens is another direct mode of action [36]. Moreover, risks assessments for MBCAs are relevant if they contain antimicrobial metabolites at an effective concentration in the product [37].

Although the potential at the greenhouse scale of these microbial technologies, results at field trials are still scarce, as the convolution of interactions among microbes, plants, soil and climate is the major bottleneck in the field adoption of the technology [38]. As shown in Table 1, despite the extraordinary body of knowledge produced on the ability of microbial biocontrol agents to protect crops, at the time of the writing, few are the microorganisms registered as active substances in the EU [39]. This is the reason why it is urgent to improve the selection process and application technique and particularly to better understand the interactions between inoculated strains and native microbiomes under field conditions.

Table 1. List of biocontrol microorganisms approved as active substances in the European Union.

Biocontrol Microorganisms	Category
Ampelomyces quisqualis strain AQ10	FU
Bacillus subtilis str. QST 713	BA, FU
Gliocladium catenulatum strain J1446 *	FU
Paecilomyces fumosoroseus Apopka strain 97	FU
Phlebiopsis gigantea (several strains)	FU
Pseudomonas chlororaphis strain MA342	FU
Streptomyces K61 (formerly *S. griseoviridis*)	FU
Trichoderma asperellum (formerly *T. harzianum*) strains ICC012, T25 and TV1	FU
Trichoderma asperellum (strain T34)	FU
Trichoderma atroviride (formerly *T. harzianum*) strains IMI 206040 and T11	FU
Trichoderma atroviride strain I-1237	FU
Trichodermagamsii (formerly *T.viride*) strain ICC080	FU
Trichoderma harzianum strains T-22 and ITEM 908	FU
Trichoderma polysporum strain IMI 206039	FU
Verticillium albo-atrum (formerly *Verticillium dahliae*) strain WCS850	FU
Aureobasidium pullulans	FU, BA
Bacillus amyloliquefaciens subsp. plantarum D747	FU
Bacillus pumilus QST 2808	FU
Candida oleophila strain O	FU
Streptomyces lydicus WYEC 108	FU, BA

FU, fungicides; BA, bacteriocides. Information source: EU Pesticides Database [40]. * Currently named *Clonostachys rosea* strain J1446 [41]. .

In this way, it has been suggested to investigate if the colonisation by inoculated microbial consortia may increase the beneficial effect of native microbiomes. In this regard, microbial consortia technology involves using more than one microbial species in a single inoculant product. The microbes may have the same or different modes of action [42–44], and may be from different phyla, genera, or even groups, for example, a combination of bacterial and fungal strains. Microbial consortia may have an advantage over single strains to synergistically interact and confer benefits to each other [43–45]. Associations with native microbiomes, imitating strongly structured networks in natural rhizosphere soils, may have a better chance to survive and provide benefits to the host, compared with single-strain formulations [46,47]. This mode of delivery can introduce beneficial traits within one generation and has several advantages over conventional application techniques, including better protection against competition from native microflora that significantly increases the colonisation and survival potential of the inoculated strain. Researchers have reported inefficient strains that became efficient in a consortium. For example, Santhanam et al. [44] observed that the inclusion of two bacterial strains with insignificant effects on mortality of sudden wilt pathogens in tobacco, in a consortium with three other bacteria, improved plants' resistance to the same pathogen, in comparison to the consortium of 3 used alone. However, the reverse is true for some PGPM species, as reported by other researchers [43,44].

However, practical considerations render complex the introduction consortia. Validation in silico of the consortia would be complex and need substantial resources. In a commercial setting, development of mass production, down streaming and storage procedures separately for each individual consortium member would need substantially more investments than the production of a single strain. Registration of consortia as plant protection products is also difficult. Regulations in the EU demand the risk assessment of each active ingredient before the product can be registered. In the case of assembled consortia, costs will thus increase substantially [31].

Finally, more research should be done to address issues of inconsistencies observed on crop producers' fields, following the use of microbial inoculants. It is obvious that single strains and consortia are issues that need to be assessed on a case-by-case basis. Therefore, a recommendation would be that more research is done to provide consumers with options that can address their unique needs while being economically practicable [22].

3. The Potential of Preharvest Microbial Applications on the Final Quality of Vegetables and Fruits

Harvesting represents a crucial phase in managing the quality and safety of vegetables and fruits. The infections due to microbial pathogens can occur both in the field and during product storage, leading to undesired spoilage phenomena. In various cases, undesired microbes in preharvest may pursue to impact on fruit/vegetable quality during postharvest (e.g., *Botrytis cinerea*, *Colletotrichum musae*, *Penicillium expansum*, *Alternata alternata*) [48,49]. While it is generally recognised that the preharvest quality strongly influences postharvest outcomes, less attention has been devoted to the effects in postharvest of specific preharvest treatments. The application of physical approaches on the plants is limited, and consequently, little was reported about the postharvest consequences. One example of physical strategies is the preharvest bagging of fruit found to improve postharvest fruit quality [50]. On the opposite, the major part of the scientific literature deals with the study of chemical treatments. The main compounds tested with this kind of management were calcium nitrate, hexanal, ammonium molybdate, gibberellic acid, oxalic acid, chitosan, chitosan oligosaccharide, organic acids, plant oils, forchlorfenuron, methyl salicylate, acetylsalicylic acid, salicylic acid, methyl jasmonate, calcium chloride, and putrescine [51–64]. These investigations include a heterogeneous representation of target products, such as winter guava, mango, apple, mandarin, kiwifruit, strawberry, pepper fruit, red-fleshed pitaya, table grape, pineapple, cherry fruit, papaya, plum [51–64]. In these studies [51–64], the principal parameters assessed in postharvest were quality, resistance, shelf life, reduced spoilage/decay incidence, firmness, total soluble solid, acidity, ascorbic acid, pectin methylesterase activity, respiratory rate, and palatability. A panel of targets is important to design a complete evaluation of preharvest treatments to shape postharvest quality. In the possible applications of microbial-based tools, one further crucial variable is the timing of application, particularly considering that the preharvest encompasses a long period in the plant development (Figure 1) [65]. The application of microbes with different timing led to a significant modification in the count of biocontrol strains at harvest, influencing the possible impacts [66].

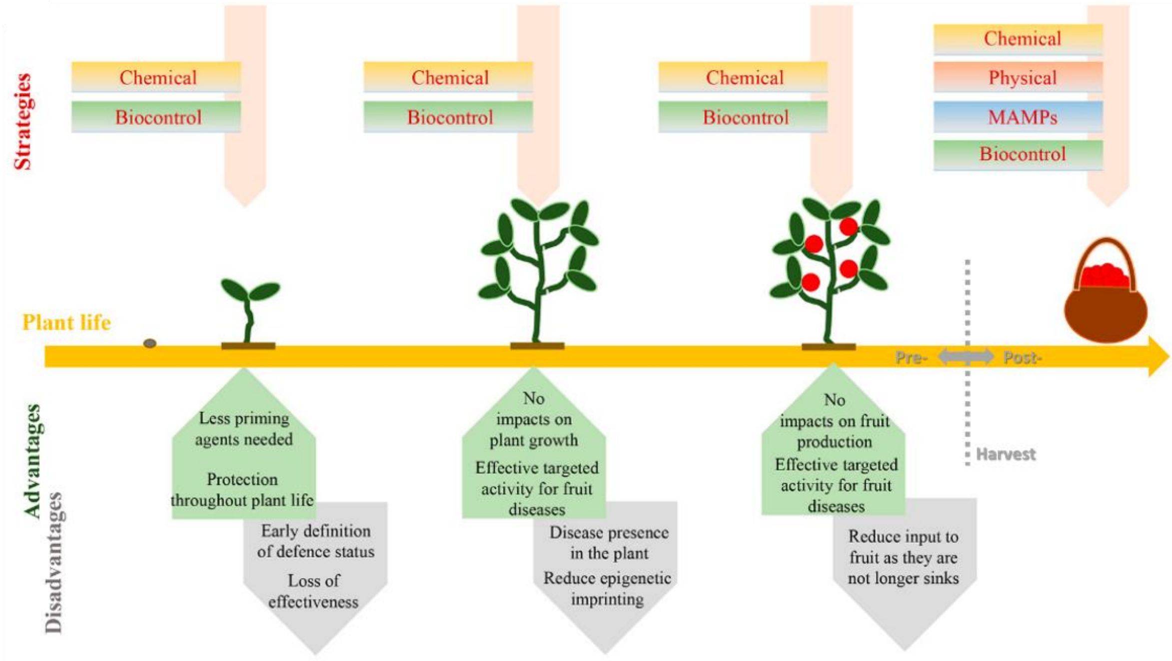

Figure 1. Pros and cons of treatments applied at different stages of plant and fruit development.

Strategies include physical strategies, chemicals, biocontrol and microbe-associated molecular patterns (MAMPs). Reproduce with modification from Pétriacq et al. [65].Even though a huge interest has been deserved to biocontrol both in pre- and postharvest [14], only a few studies delved into the potential protection in the storage of field microbial-based treatments (Table 2). However, the more recent trends seem to indicate rising attention to explore some of the variables that can affect the phenomenon. What appears clear considering the number of crop fruits tested, the variability of microbial agents (species), the different timing/modality of application, and the target monitored to evaluate the postharvest effect (Table 2), it crucial to improve harmonisation in the research activities to steer and promote the innovation in this field.

Table 2. Examples of microorganisms (for biological control) applications in preharvest tested to achieve positive effects (also) in postharvest.

Fruit	Year/Country	Treatments	Effects	Ref.
Strawberry	1997, Italy	Application at flowering and at fruit maturity of *Aureobasidium pullulans* L47 and *Candida oleophila* L66	Antagonists were more active when applied at the flowering stage	[67]
Strawberry	2002, Turkey	Preharvest treatment with *Metschnikowia fructicola* also for the control of postharvest rots	The yeast reduced postharvest incidence of fruit rot significantly better than chemical control	[68]
Mango fruits	2006, India	Application of *P. fluorescens* FP7 plus chitin	Durably effective against anthracnose in postharvest storage.	[69]

Table 2. *Cont.*

Fruit	Year/Country	Treatments	Effects	Ref.
Stone fruits	2017, Spain	Treatments based on *Bacillus amyloliquefaciens* CPA-8 to control brown rot under field conditions	Used at the correct concentration, CPA-8 reduced postharvest brown rot similar to chemical applications	[70]
Apple	2012, USA	*Bacillus megaterium* isolate A3-6, *Bacillus mycoides* isolate A1-1, and *Bacillus cereus* FLS-5	Combined pre- and postharvest application resulted in the greatest suppression of bitter rot	[71]
Table grape	2018, Turkey	*Bacillus subtilis* QST 713 and *Azotobacter chroococum* + *Azotobacter vinelandii* preparations	Postharvest quality retention of table grape cv. 'Antep Karası'.	[72]
Mango fruits	2019, Brazil	Application of a commercial formulation of *Bacillus subtilis* QST 713	Reduced mango fruit decay	[73]
Date fruit	2020, India	Preharvest foliar spray of fungal culture filtrates from *Aspergillus niger* and *Rhizopus oryzae*	Improve plant defence mechanism, with also enhanced quality and shelf life of fruit.	[74]
Stone fruits	2021, Belgium, France, Italy and Spain	Application of two biocontrol agents (BCAs), *Bacillus amyloliquefaciens* CPA-8 or *Penicillium frequentans* 909	With the incidence of brown rot in postharvest < 35%, the efficacy level of the BCA was comparable with chemical application	[75]

In other terms, Table 2 reports some works on a sort of crosstalk between biological control agents in preharvest, that have as a target soil/seed/plant protection, and postharvest biocontrol tools, that aims to protect fruits and vegetables during conservation [49]. In other terms, we are talking of a potential integrative goal of field applications, where the 'pre-' agent has an effect on the 'post-' target [49]. From this point of view, for those pathogens that are the same 'from farm to fork', often quiescent and latent in the field, the management could start in preharvest, taking advantage of the knowledge about the timing of colonisation and the epidemiology of each pathogen [5]. A knowledge essential to design a well-conceived strategy to allow pre-emptive colonisation with a biocontrol agent against target disease [5]. This can be of particular interest when a given plant organ represents a potential target of infection, remembering that the biocontrol agent has to colonise it before the arrival of undesired microbes [76]. On the opposite, for pathogens peculiar to postharvest, a near-harvest application with selected biotools could be tailored to maintain high the antagonistic potential on harvested fruits/vegetables [76]. As a function of this consideration, it changes the interest in the ability of the biocontrol agent to attach, colonise, and survive on the phyllosphere (particular on the carposphere), also after exposure to harsh environmental stressors (e.g., cold, low water potential, low nutrients, UV radiation) and adverse climatic conditions (e.g., wind, rain) [76–78]. From this perspective, it can be remarkable to underline that eukaryotes (i.e., filamentous fungi and/or yeasts) appear more appropriate than prokaryotic for early applications in preharvest, in reason of an improved aptitude to colonise phyllosphere in field and tolerant of harsh environmental conditions, even if in appropriate condition (e.g., high humidity) also bacteria (e.g., *Pseudomonas* spp.; *Bacillus subtilis*) may control necrotrophic pathogens in the field [76]. Particular attention has recently been paid to the ecological relationship between microbes and plants (e.g., endophytes), as one of the facets of interest to elicit positive responses of interest in agriculture [79,80]. These aspects are all part of a holistic perspective allowed by the rising application of high-throughput sequencing-based techniques that contribute to describing fruits and vegetables as holobionts [81], with a microbiota at harvesting that embraces beneficial, pathogenic and spoilage microorganisms [81], and that is the ultimate target of microbial-based solutions in the field.

Indeed, the impact of microbial-based preharvest applications needs to be also evaluated on targets other than microbial decay. For example, Crupi et al. [82] evaluated the addition in preharvest of inactivated yeast on the anthocyanin content, finding that three anthocyanins' content was probably modulated by the treatment. With regard to emerging issues, one aspect is deserved of great attention. On the one side, as said, the persistence of biological control agents after harvesting can appear as a desired phenomenon, enhancing the postharvest performance of the preharvest biological treatment. On the other side, the endurance of the 'biocontrol tool' on the product improves the probability of human ingestion, consequently, doubt about the safety of the strain/species used for this 'extended' biocontrol. With this regard, while Gotor-Vila et al. [70] monitored the survival of the biocontrol strain in postharvest to assure its efficacy, Zhao et al. [83] highlighted, in lab-scale trials, the persistence of high numbers of *B. thuringiensis* spores in leafy greens in both pre- and postharvest stages, suggesting a possible excessive residual dose of *B. thuringiensis* upon consumption.

All these considerations underline the importance to find tailored solutions for each scenario, considering the nature of the crop/production and of the target pathogens, the mode of action and the persistence of the agent in postharvest (including an evaluation of safe consumption for humans), and the climatic conditions.

4. Postharvest Application of Microorganisms as Biocontrol Agents

Although modern food-conservation techniques have prolonged the products' shelf-life, in the postharvest phase of food production, there are still significant product losses caused by spoilage microorganisms, amounting to about 20–25% of the production [14,84].

In this regard, numerous microbial antagonists (fungi, yeasts and bacteria) that can be used on fruits and vegetables in pre- and postharvest have been identified in the laboratory, semi-commercial and commercial studies over years. Many of these antagonists have reached advanced levels of development and marketing, and there are currently several on the market, although their application is mostly targeted to deteriorating microorganisms (mainly fungal pathogens) that cause damage to fruit production when ripening in the field (preharvest). Indeed, the situation in postcollection is more complicated: despite hundreds of reports documenting potential commercially valid antagonists, the widespread use of a single product has not been achieved. Several products reached the market, but were later withdrawn, while others achieved success in niche markets [85]. This has been due to several factors, including inconsistent performance, lack of industry acceptance, cost relative to synthetic fungicides, registration hurdles, and formulation problems [86]. The postcollection process has to be seen as a complex system, which includes conditioning treatments, storage, shipping and all other aspects of the supply chain, managed to address a wide range of problems. There is a large space both for an optimisation of the bioprotection contribution to the process (through the implemented usage of microorganisms already present on the market) and for the selection of new strains [87].

The use of various microbes (yeast, yeast-like fungi, and bacteria) isolated from plant, fruit, and soil, as antagonists (biocontrol agents) to manage postharvest diseases came from the mid-1980s. Numerous papers on this subject have been and continue to be published (recently reviewed in [85]). New antagonists, new ways to use the antagonists, and new ways to integrate their use with other alternative approaches are continually being published. Indeed, a peculiarity of postharvest situation is that a multitude of different physicochemical (and eventually microbiological) techniques are simultaneously applied to preserve or increase shelf-life, spanning from temperature control to washing systems or atmosphere modifications. In this frame, a recent concept has evolved within the search for alternative methods to postharvest disease control: The idea of a multiple decrement approach [86]. In this synergistic strategy, the prevention of disease is brought about by using several methods that each reduces the percentage of decay by a specific amount (e.g., sanitation and or careful harvesting and handling; exposure to a wet/dry heat treatment;

application of a microbial antagonist; use of modified or controlled atmosphere storage and/or packaging or dipping). The various approaches act together additively or synergistically to bring about commercial levels (97–99%) of disease control [86]. The use of microbial antagonists within such a strategy can represent a powerful weapon for decrementing spoiling microorganisms and increasing shelf-life within an overall sustainable method [18]. In the future, this may also lead to a wider application of the abovementioned multiple hurdle approach: From pre- to postharvest phases, in an integrated perspective. Indeed, this strategy can be applied throughout the whole lifespan of fruits and vegetables, if the different actors handling them (both before and after harvest) will better realise to what extent they can take advantage thereof. Indeed, no single intervention can completely eliminate detrimental microbes and consequent decays from a food product [86].

Another advancement, coming from recent findings in this field, is the awareness that complex interactions occur between food-plants and their microbiomes throughout all their production process: As said, in fact, recently, high-throughput sequencing-based techniques revealed fruits and vegetables as holobionts [81]. More information on the composition and function of the host's associated microbiota at harvest will provide the basis for understanding the impact of the host-microbiome interaction on fruit metabolism and disease resistance [88]. Field and postharvest handling of fruits and vegetables was shown to affect the indigenous microbiome, and therefore, substantially impact the storability of fruits and vegetables. The generated knowledge provides profound insights into postharvest microbiome dynamics and sets a new basis for targeted, microbiome-driven, and multi-actor sustainable control (including biocontrol) strategies [81].

Indeed, it is still an open question if a single microorganism-based biofungicide can provide adequate biocontrol of numerous different pathogens on a wide range of harvested commodities, compared to using several microorganisms whose combined function may provide a superior effect. Microbial consortia that inhabit the exterior and interior of organisms have a profound effect on the physiology and health of that organism. Understanding and utilising this interaction in fruit crops and other harvested foodstuffs need to be explored. The use of a synthetic or a natural consortium that could be applied to a harvested commodity for better disease control would represent a novel approach, which will take advantage of new research approaches that were not previously available or utilised [89,90].

On the other hand, microbiome tracking can be implemented as a new tool also to evaluate and assess the existing postharvest bioprocesses and their contribution to fruit and vegetable health. For instance, a very recent study was undertaken to characterise the effect of near-harvest field application of a yeast biocontrol agent (*Metschnikowia fructicola)*, on the strawberry fruit microbiome [91]. High-throughput sequencing revealed significant shifts in the bacterial and fungal community in response to the application of the yeast biocontrol agent at the time of application, after harvest, and after storage and shelf life. This kind of results will provide new insights into the dynamics of the postharvest fruit microbiome that will assist in the development of targeted, microbiome-driven approaches to robust and sustainable disease control strategies, even strengthening the existing products by broadening or fine-tuning their application ranges [81,91].

Both from a legal and 'de facto' point of views, boundaries between pre- and postharvest applications are sometimes fuzzy. Indeed, some products are nowadays proposed for both the applications (see Table 3), also thanks to EFSA recommendations that consider the presence of active cells of preharvest-applied biopesticides remaining after harvest [92]. Moreover, in some sectors, regulations and standards for food processing also apply; therefore, fresh fruit must be handled in compliance with these requirements from harvest. For instance, wine grapes need to be processed only with microorganisms that are also approved for winemaking from harvest onward (transport, prefermenting stages or drying processes). Therefore, non-*Saccharomyces* yeasts that are approved by OIV regulations (Resolution OIV-OENO 576B-2017), are more and more used as bioprotectors [93]. As a consequence, research programs that select and develop dedicated strains with pronounced biocontrol properties are recently coming into view (a non-exhaustive, continuously updating, list of the resulting microorganisms can be found at the end of Table 3).

These aspects would deserve a further in-depth analysis in the near future. Nonetheless, looking at the regulatory aspects at a glance, it is remarkable that 21 out of 39 substances approved in the latest update statement of "Pesticide active substances that do not require a review of the existing maximum residue levels under Article 12 of Regulation (EC) No 396/2005" [94] are microbial-based products, this testifying the food sector orientation towards biopesticides.

Table 3. Biocontrol-based products developed and marketed for postharvest applications.

Product	Active Ingredient	Country/Company	Fruit/Vegetable	Target	Currently Marketed	Ref.
Biofungicides Recommended for Postharvest Applications						
Aspire®	*Candida oleophila*	Ecogen USA	Pome Fruit, Citrus, Strawberry, Stone Fruit	*Botrytis, Penicillium, Monilinia*	No	[86,95]
YieldPlus®	*Cryptococcus albidus*	Lallemand South Africa	Pome Fruit, Citrus	*Botrytis, Penicillium, Mucor*	No	[86,95]
Candifruit™	*Candida sake*	IRTA/Sipcam-Inagra Spain	Pome Fruit	*Penicillium, Botrytis, Rhizopus*	No	[86]
Biosave®	*Pseudomonas syringae*	Jet Harvest Solutions USA	Pome Fruit, Citrus, Strawberry, Cherry, Potato	*Penicillium, Botrytis, Mucor*	Yes	[86]
Avogreen	*Bacillus subtilis*	South Africa	Avocado	*Cercospora, Colletotrichum*	No	[86]
Nexy®	*Candida oleophila*	Lesaffre Belgium	Pome Fruit	*Botrytis, Penicillium*	Yes	[86,95]
BoniProtect® BlossomProtect® Botector®	*Aureobasisium pullulans* (2 strains)	Bio-ferm, Austria	Pome Fruit Grape	*Penicillium, Botrytis, Monilinia*	Yes	[86,95,96]
Pantovital®	*Pantoea agglomerans*	IRTA/Sipcam-Inagra Spain	Citrus, Pome Fruit	*Penicillium, Botrytis, Monilinia*	No	[86]
Noli	*Metschnikowia fructicola*	Koppert The Netherlands	Table Grape, Pome Fruit, Strawberry, Stone Fruit, Sweet Potato	*Botrytis, Penicillium, Rhizopus, Aspergillus*	Yes	[86,95,97]
Biofungicides Developed for Preharvest Applications, Also Recommended in Postharvest						
Serenade® Opti	*Bacillus subtilis*	Bayer	Grape, Berry Fruits, Potato	*Botrytis*, Silver scarf	Yes	[98]
Amylo-x®	*Bacillus amyloliquefaciens*	Biogard, Italy CBC-Europe, Germany	Grape, Apple, Pear, Kiwifruit	*Botrytis, Pseudomonas syringae*	Yes	[99]
Bioprotection Agents Developed for Food Processing, Also Recommended for Postharvest						
Gaia™	*Metschnikowia fructicola*	IOC, France	Harvested grape, withering grape, grape musts	*Botrytis, non-Saccharomyces spoiling yeasts*	Yes	[100]
Nymphea™	*Torulaspora delbrueckii*	ICV/Lallemand, France	Harvested grape, grape musts	*Botrytis, non-Saccharomyces spoiling yeasts*	Yes	[101]

5. Conclusions

Numerous microbial antagonists (fungi, yeasts and bacteria) can be used on fruits in pre- and postharvest, as demonstrated in laboratory, pilot and industrial-scale studies. Many of these biotools have reached advanced levels of development, although their application is mainly targeted towards deteriorating microorganisms (primarily fungal pathogens) during field ripening seasons (preharvest). The situation after harvest is quite dissimilar, due to the postharvest process itself, which also includes technological aspects of the supply chain that constitute a complex system in which microbial biocontrol can play a role. In this review, we summarised the current development of microbial-antagonism based strategies, considering both pre- and postharvest application, also highlighting the prospects of optimisation for both. In particular, the pros and cons of the development and application of microbial consortia were considered, together with the advancements in knowledge about complex interactions between food-plants and their microbiomes throughout all their production processes. Finally, the need for further research is illustrated for providing consumers with more options that can address their unique needs while being economically practicable.

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