The Biological Strategies

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1 Introduction

The use of synthetic chemicals effectively increases the durability and prolongs the shelf life of food products (Jongman et al., 2022). For example, fungicides prochloraz and bavistin have been effectively employed to preserve the freshness of avocados and mushrooms (Shimshoni et al., 2020). However, as synthetic chemicals gained traction in the early to mid-twentieth century, concerns arose regarding antimicrobial resistance (AMR), environmental pollution, and potential adverse health effects associated with their use (Fang et al., 2019; Gaston et al., 2020). Given the risks to human health and the increase in food safety incidents, it is not surprising that a shift from chemical to non-chemical applications, such as biological control, is necessary to ensure product quality and safety.

The term "biocontrol" refers to the use of biological agents or natural substances to prevent, reduce, or eliminate the growth of pathogenic microorganisms or pests in food products at the pre/post-harvest and processing stages (Jongman et al., 2022; Leneveu-Jenvrin et al., 2020). These biological strategies work in harmony with the environment, posing minimal risks to human health and leaving virtually no harmful residues on food. Embracing biocontrol approaches enables the food industry to bolster food safety and quality while reducing reliance on synthetic chemicals, thereby promoting sustainable and eco-friendly practices throughout the food supply

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chain (Oyetunji et al., 2024). Although various biological methods have been developed over the past few decades, their widespread adoption faces challenges due to limited applicability in existing food processing system (Jongman et al., 2022).

This chapter provides an overview of the latest biocontrol applications used at different points along the food supply chain. These applications include bacteriophages (phages), their endolysins and depolymerases, competitive bacterial and fungal species, as well as plant-derived products. We delve into the antimicrobial mechanisms of these biocontrol approaches and discuss their potential applications in the food industry.

2 Bacteriophage-Based Biocontrol Strategies

Phages are viruses that selectively infect and kill bacterial hosts, leaving other cells unaffected. This unique specificity makes them promising candidates for use as biopesticides or biocontrol agents in food production processes (Goodridge & Bisha, 2011). Phages were first described by William Twort in 1915, and further independently discovered by Félix d'Herelle in 1917. d'Herelle is widely considered a pioneer in applied phage science as he introduced the concept of phages in clinical medicine for both preventive and post-operative treatment (Kortright et al., 2019; Wittebole et al., 2014). Recognizing the selectivity and potency of phages, d'Herelle conducted lots of trials administering phages intravenously to combat invasive bacterial infections (Garvey, 2022). However, phage therapy became overlooked due to the challenges in quality control and reproducibility, compounded by the rapid progress in antibiotic discovery and development since the 1930s (Wittebole et al., 2014). On the other hand, the rise of AMR due to the overuse and misuse of antimicrobials has escalated into a global health crisis, leading to increasing treatment failures (Ferri et al., 2017). Therefore, novel strategies such as phages and their enzymes have recently received renewed attention (Garvey, 2022). Moreover, an increasing number of researchers are turning their focus to the application of phages as a promising strategy for pathogen control in food systems.

2.1 Bacteriophage

2.1.1 Antibacterial Mechanisms

As the most abundant life forms on Earth, phages outnumber bacteria by ten times, with an estimated total number of 10^{32} (Verheust et al., 2010). The extensive natural reservoir of phages presents myriad potential benefits in antibacterial contexts. These include broad-spectrum activity against diverse bacterial strains, including those exhibiting AMR, controlled antibacterial profiles preserving the indigenous

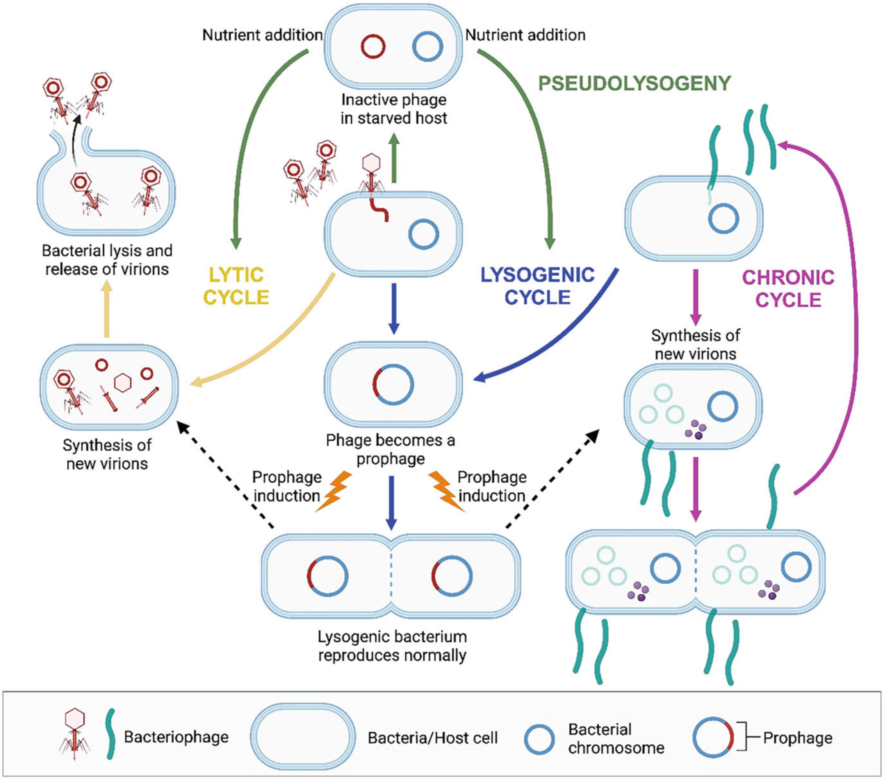

Fig. 1 Different life cycles of bacteriophages

microbiome, potential for minimal adverse effects, and cost-effectiveness (Helmy et al., 2023). As obligate intracellular parasites of bacterial cells, phages exhibit diverse life cycles within the bacterial host, including lysis, lysogeny, pseudolysogeny, and chronic infection (Fig. 1) (Huang et al., 2022; Wittebole et al., 2014). In phage therapy, primary focus is on lytic phages, which predominantly belong to three families within the *Caudovirales* order: *Myoviridae*, *Podoviridae*, and *Siphoviridae*. Additionally, there are reports on the applications of filamentous and cubic phages in bacterial inactivation (Villalpando-Aguilar et al., 2022; Wittebole et al., 2014).

Regardless of the phage life cycle, the initial stage involves attachment to host cells via different host receptors, including proteins, carbohydrates, or lipopolysaccharides, before penetrating the bacteria (Fig. 1) (Dunne et al., 2021). This process significantly influences the spectrum of potential phage-bacteria interactions. For example, phage λ specifically interacts with the LamB receptor of *Escherichia coli*. Spatiotemporal dynamics highlight the critical role of this event in successful bacterial invasion (Chatterjee & Rothenberg, 2012). After attaching to their specific receptor, phages create a pore in the bacterial cell wall and inject their nucleic acid into the cell. Subsequently, phage early genes are expressed, hijacking the bacterial synthetic machinery to replicate viral nucleic acids and proteins, particularly in the case of lytic phages. After new phage particles are assembled and packaged, the bacterial cell lyses to release the phage progeny. The late phage enzymes, such as lysins, holin, and inhibitors of bacterial wall protein synthesis, are then exploited for virion burst in the extracellular environment (Du Toit, 2017; Wittebole et al., 2014). The number of viral particles released, known as the burst size, varies significantly depending on the specific phage, the condition of the bacterial host, and various environmental factors, including surrounding nutrient levels (Weinbauer, 2004).

In the lysogenic cycle, temperate phages integrate their genetic material into the host bacterial chromosome either randomly or at specific sites (Fig. 1). Once integrated, the prophage remains silent for extended periods and replicates with the bacterial chromosome. Therefore, there is no independent replication of the prophage at this stage (Feiner et al., 2015). Prophage DNA is transmitted vertically to its progeny along with the entire bacterial genome until induction occurs, due to various environmental stress factors, such as oxidation, heating, and ultraviolet radiation. This induction results in excision of the viral genome from the host chromosome (Ładziak et al., 2024; Xu et al., 2018). Pseudolysogeny represents another interaction mode between phages and bacteria, initiated under conditions of low nutrient availability, such as under unfavorable growth conditions (Fig. 1). Ripp and Miller (1997) described pseudolysogeny as a phage-host interaction which the phage nucleic acid neither integrates into the host chromosome as a prophage (lysogeny) nor induces a lytic reaction. Instead, it remains inactive within the cell. Lastly, some phages exhibit a chronic infection lifestyle in which host cell is not lysed upon the release of progeny phage particles (Fig. 1). Instead, particles are continuously excreted to the extracellular environment through the membrane. Depending on the phage, its genome may be integrated into the host genome or remain in the cytoplasm (Mäntynen et al., 2021). Therefore, lysogenic, pseudolysogenic, and chronic infection life cycles are less effective at causing immediate death of the host bacteria. On the other hand, phages with a lytic life cycle are superior to temperate phages in terms of therapeutic potential.

2.1.2 Phage Biocontrol in Pre/Post-harvest Applications

The use of phages is gaining recognition as a sustainable biocontrol technology, noted for its remarkable specificity in targeting bacterial pathogens across various environments, covering each stage of the food processing from farm to table. Recently, with Generally Recognized As Safe status for the control of harmful pathogens in the food industry, some phage products have been introduced into the commercial market to address common foodborne pathogens, including *E. coli*, *Listeria monocytogenes*, and various *Salmonella* serotypes (de Melo et al., 2018). This advancement is highly promising, highlighting the industry's confidence in the effectiveness and safety of phage-based formulations. Many commercial phage companies, including FINK TEC GmbH, Phagelux, Intralytix, Passport Food Safety

Solutions, and Micreos Food Safety, have received FDA approval for their food safety products (Vikram et al., 2022).

Phage therapy has emerged as a promising pre-harvest intervention strategy for controlling pathogens in various meat-producing animals, including sheep, cattle, poultry, and swine, before slaughter (Goodridge & Bisha, 2011; Wittebole et al., 2014). To date, extensive studies on phage therapy aimed at controlling foodborne pathogens in poultry have been conducted, demonstrating significant efficacy in pathogen reduction. For instance, in a study conducted by Clavijo et al. (2019), the effectiveness of SalmoFREE®, a newly patented phage preparation designed to target *Salmonella*, was assessed throughout the production cycle of broiler chickens. Cloacal swab results indicated that the phage product successfully decreased *Salmonella* counts to 0% by day 34. These *in vivo* studies investigated the efficacy of employing phages either individually or in combination (cocktail) to combat foodborne pathogens. Generally, using a single phage in certain experiments leads to the development of phage resistance. Conversely, the use of multiple phages in a cocktail reduces the likelihood of resistance emergence (Bach et al., 2003; Raya et al., 2011).

Foods offer a nutrient-rich environment that supports the survival and proliferation of various bacterial pathogens. Numerous studies have documented intervention strategies utilizing phages to reduce pathogens in post-harvest food materials. The literature shows that carefully selected phages can effectively reduce the prevalence of specific harmful pathogens. In a recent study focusing on meat systems, Vikram et al. (2020) conducted a comprehensive investigation of the Intralytix phage cocktail EcoShield PX™, specifically targeting Shiga toxin-producing *E. coli*. Their results showcased the effectiveness of these phages in reducing *E. coli* levels (by 3.0 log CFU/g) across various food products, including ground beef, beef chuck roast, cooked chicken, chicken breast, salmon and cheese. In fruits and vegetables, Wong et al. (2020) explored the efficacy of a five-component phage cocktail in controlling seven *S. enterica* strains across four different serovars, including Enteritidis, Newport, Javiana, and Thompson. The study involved inoculating cantaloupe and romaine lettuce leaves with the phage cocktail 24 hours prior to bacterial inoculation. Results varied significantly across different *Salmonella* targets, highlighting that while the phages demonstrated potential for *Salmonella* biocontrol, their success was influenced by the strain specificity.

2.2 Phage Enzymes

2.2.1 Endolysin

The use of endolysins to control the proliferation of pathogenic microorganisms in food represents a relatively novel concept that has gained increasing interest in recent years. Endolysins are enzymes encoded by all phages, synthesized within the infected host bacterium at the end of the lytic cycle. These enzymes efficiently cleave peptidoglycan, the major component of the bacterial cell wall, resulting in cell lysis and subsequent release of progeny phages (Chaudhary et al., 2024). Gram-positive bacteria, unlike their Gram-negative counterparts, lack an outer membrane structure outside the cell wall. This characteristic allows endolysins to exert bacteriolytic activity with high specificity and efficacy when externally applied, without harming non-target bacteria (Zheng & Zhang, 2024). Nonetheless, significant progress has been made in using endolysins specific to Gram-negative pathogens like *Pseudomonas aeruginosa*, *Campylobacter* spp., *E. coli*, *Neisseria gonorrhoeae*, *Salmonella* spp., *Vibrio* spp., *Klebsiella pneumoniae*, and *Acinetobacter baumannii* (Endersen & Coffey, 2020). In addition, recent advancements in evaluating the bactericidal efficacy of endolysins have been demonstrated in various food-related applications. For example, the endolysin LysP53 has shown the capability to lyse *S.* enteritidis found on fresh romaine lettuce, indicating its potential as a biocontrol agent for reducing bacterial loads in fresh vegetable produce (Khan et al., 2023). Another study investigated the combined action of LysSA97 and carvacrol oil to combat *Staphylococcus aureus* in milk and beef. The results showed that the combined treatment resulted in an average reduction of 4.5 log CFU/mL in *S. aureus* counts across the tested food samples (Chang et al., 2017).

2.2.2 Depolymerase

Depolymerases, categorized as tail spike proteins, can degrade extracellular polysaccharides, including those forming capsules or biofilm matrices. Additionally, they can cleave structural polysaccharides such as lipopolysaccharide or peptidoglycan strands (Fernandes & São-José, 2018). Phages that express depolymerase activity exhibit improved access to host receptors due to their ability to break down capsular polysaccharides. This feature is believed to enhance efficacy against bacterial biofilms (Pires et al., 2016). Therefore, in devising phage or phage cocktail biocontrol strategies, incorporation of phages encoding polysaccharide depolymerases could bolster efficacy, broaden specificity, and facilitate biofilm removal. Additionally, employing depolymerases sourced directly from phages introduces a novel strategy for combating pathogenic bacteria and their biofilms. For example, the depolymerase LKA1gp49, derived from the *Pseudomonas* phage LKA1, effectively attaches to and cleaves the B-band lipopolysaccharide, thereby reducing the virulence of *P. aeruginosa* (Olszak et al., 2017). Furthermore, the depolymerase has shown effectiveness in eliminating *Klebsiella* sp. biofilms from food contact surfaces, achieving an 80% reduction after 4 h (Garvey, 2022). In practice, depolymerases offer greater advantage and flexibility compared to phages, avoiding certain disadvantages like the necessity for purification and endotoxin removal. Beyond endolysins and depolymerases, other phage-related enzymes involved in peptidoglycan degradation, such as virion-associated peptidoglycan hydrolases, have also received attention in recent years (Gutiérrez et al., 2018).

3 Bacteria-Based Biocontrol Strategies

Another biocontrol strategy involves the utilization of bioprotective microorganisms and/or their metabolites, which exhibit antagonistic effects against spoilage and pathogenic microorganisms, to enhance microbial safety and extend the shelf life of food products (Rathod et al., 2022). In addition to demonstrating antagonistic activity, these protective microorganisms must be safe for human consumption and must not negatively affect the nutritional or sensory quality of the product. Moreover, certain antagonistic microorganisms, known as probiotics, provide additional benefits. These include regulating the intestinal microflora balance, alleviating the impacts of allergens, easing lactose intolerance, lowering cholesterol levels, fostering immune response, and enhancing resistance to infections (Hossain et al., 2017; Kaur et al., 2015). Most probiotics are bacteria, with lactic acid bacteria (LAB) being the most prevalent type. Nonetheless, certain molds and yeasts are also utilized as probiotics. Among the frequently employed probiotic species are *Lactobacillus*, *Bifidobacterium*, *Carnobacterium*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, *Bacillus*, *Leuconostoc*, and *Propionibacterium* (Hossain et al., 2017). Of these, *Lactobacillus* and *Bifidobacterium* are the most extensively researched genera.

3.1 Probiotics

3.1.1 Mechanism of Probiotic Action

The mechanisms underlying bacterial antagonism or antimicrobial activity are pivotal for maintaining equilibrium between beneficial and potentially pathogenic microorganisms (Hossain et al., 2017). The inhibitory ability results from multiple mechanisms, including competition for colonization sites, and nutrients. Additionally, probiotic microorganisms produce a variety of antimicrobial metabolites, such as bacteriocins, hydrogen peroxide, organic acids, biosurfactants, and enzymes, that can proficiently inhibit the growth of pathogens (Oliveira et al., 2018). For instance, the probiotic bacterium *L. reuteri* releases antimicrobial reuterin, which exhibits broad-spectrum activity against a range of pathogens, including bacteria, fungi, protozoa, and viruses (Spinler et al., 2008). Probiotic LAB produce organic acids, primarily acetic and lactic acids, to reduce the likelihood of intestinal colonization by pathogens. These organic acids create a favorable environment for the resident microbiota by lowering intestinal pH, thereby establishing an acidic milieu that inhibits the proliferation of pathogens (Servin, 2004). Moreover, some probiotics utilize enzymatic mechanisms, releasing enzymes capable of hydrolyzing bacterial toxins, modifying toxin receptors, and inhibiting toxin-mediated diseases. The LAB strain *Ped. parvulus* can biodegrade the mycotoxin ochratoxin A by hydrolyzing the amide group using specific peptidases, subsequently releasing non-toxic moieties (Ghadaksaz et al., 2022).

By releasing antimicrobial compounds, probiotics also exhibit the capacity to inhibit the formation of biofilms by pathogens, serving as a defense mechanism against infections. For example, the bacteriocin produced by probiotics significantly reduced the biofilm of numerous human pathogens, such as *S. aureus*, *S. pyogenes*, *K. pneumoniae*, *Shigella flexneri*, *Candida albicans*, and *Aspergillus fumigatus*, through structural deformation and disruption of bacterial membrane integrity (Krishnamoorthi et al., 2022). Some probiotic candidates prevent pathogen colonization, competitively inhibiting the pathogen adhesion to host cell surfaces (Hossain et al., 2017). This mechanism relies on specific proteins present on the surface of probiotic agents, such as S-layer (surface-layer) proteins found in *L. helveticus* and *L. crispatus*, which can inhibit the adhesion of pathogenic *E. coli* O157: H7 to T84, HEp-2, and HeLa cells (Johnson-Henry et al., 2007). It's important to note that the action mechanism of each probiotic depends on the specific strain, emphasizing the need to carefully select and scientifically evaluate individual probiotics for their intended use. These mechanisms typically involve the production of bioactive molecules or direct cell-to-cell interactions (Hossain et al., 2017).

3.1.2 Probiotics Applications

Similar to phage products, probiotics can be applied both pre-harvest on farms and post-harvest in foods. In livestock farming, probiotics are used to improve growth performance and control enteric pathogens (Hung et al., 2012). Therefore, probiotics could be a promising alternative to antibiotic feed additives. For example, some studies have highlighted poultry and their products as significant reservoirs of *Campylobacter* spp. Campylobacteriosis, caused by various species such as *C. jejuni*, *C. upsaliensis*, *C. coli*, and *C. lari*, is a prevalent zoonotic disease commonly associated with poultry production (Guyard-Nicodème et al., 2015). Some *in vitro* studies have successfully identified anti-*Campylobacter* probiotics, including strains of *L. salivarius* (Messaoudi et al., 2011). Additionally, *in vivo* trials have also reported the effective role of probiotics in inhibiting or reducing the shedding of *C. jejuni* load. Nishiyama et al. (2014) demonstrated the effectiveness of *L. gasseri* SBT2055 in inhibiting *C. jejuni* adherence and invasion of in vitro, as well as reducing the *C. jejuni* load in chickens in vivo (Table 1). Similarly, Caggia et al. (2015) showed that two probiotic strains, *L. rhamnosus* FS10 and *L. paracasei* PM8, isolated from cheese, exhibited strong antimicrobial activity in vitro against various foodborne pathogens, including *E. coli*, *S. aureus*, *L. monocytogenes*, and *E. faecalis*. The authors suggest these strains as promising candidates for incorporation into functional foods aimed at enhancing food safety.

Additionally, supplementing probiotics in other livestock, including cattle, sheep, and swine, has shown effective control and reduction of pathogen load (Table 1). Peterson et al. (2007) documented that daily administration of a feed supplement containing *L. acidophilus* NP51 to cattle led to a notable decrease in fecal *E. coli*

Table 1 The biocontrol use of probiotics in different pre/postharvest applications

Foods	Probiotics	Target pathogen	Observed effect	References
Chicken	*L. gasseri* SBT2055	*C. jejuni*	1–2 log CFU/well reduction	Nishiyama et al. (2014)
Cattle	*L. acidophilus* NP51	*E. coli* O157:H7	Reduction of counts in fecal sample	Peterson et al. (2007)
Sheep	*L. alimentarius* DDL 48, *L. reuteri* DDL 19, *B. bifidum* DDBA, and *E. faecium* DDE 39	*Shigella* & *Salmonella*	1.21 log and 1.09 log CFU/g reduction, respectively	Apás et al. (2010)
Swine	*L. fermentum I5007*	*Clostridium* spp. & *Escherichia* spp.	Decreased Clostridium spp. & *Escherichia* spp. levels	Liu et al. (2014)
Lettuce	*L. curvatus B.67*, *L. sakei D.7*, *L. plantarum I.60*, *L. mesenteroides J.27*, *L. plantarum M.2*, or *L. plantarum M.21*	*L. monocytogenes*	1.07–1.62 log CFU/cm^2 reduction	Hossain et al. (2020)
Cheese	*L. acidophilus La-5 and B. longum ATCC15707*	*E. coli* O157:H7 and *S. aureus*	1.7–1.88 log and 0.88–3 log cycle reductions, respectively	El-Kholy et al. (2014)
Sausage	*L. rhamnosus* E-97800, *L. rhamnosus* LC-705, *L. rhamnosus* GG, and *P. pentosaceus*	*E. coli* O157:H7	Decreased counts from around 5 to 2 log CFU/g	Erkkilä et al. (2000)
Mung bean sprouts	*B. subtilis LCA1*	EHEC cocktails	About 2.0 log CFU/g EHEC reduction	Shen et al. (2017)

O157:H7 counts. Moreover, the inclusion of *L. fermentum* I5007 in feed inhibited the growth of *Clostridium* spp. and *Escherichia* spp. in piglets (Liu et al., 2014). Apás et al. (2010) demonstrated that the administration of a probiotic mixture, containing *E. faecium*, *B. bifidum*, *L. reuteri*, and *L. alimentarius*, resulted in a significant reduction in *Shigella* and *Salmonella* in sheep feces by 1.21 and 1.09 log CFU/g, respectively. These findings demonstrate the potential antimicrobial activity of probiotics against significant foodborne pathogens, effectively limiting their colonization in animals. As a result, the use of probiotics can enhance animal growth rates and improve the quality and yield of meat, milk, and egg products.

Fresh produce, such as fruits and vegetables, is susceptible to microbial contamination. Table 1 also outlines various biocontrol applications of probiotics in foods. According to Hossain et al. (2020), six LAB strains isolated from kimchi demonstrated promising antimicrobial efficacy against *L. monocytogenes* on lettuce leaves. Vescovo et al. (1996) and Torriani et al. (1997) pioneered the use of *L. casei* to combat pathogens in ready-to-eat vegetables, highlighting its inhibitory properties against pathogens. Torriani et al. (1997) noted that the incorporation of 3% culture permeates of *L. casei* IMPC LC34 in salads led to decreased total mesophilic bacteria counts and inhibited the growth of enterococci, coliforms, and *A. hydrophila*. These probiotics are also widely used in fermented foods such as cheese, yogurt, kimchi, and sausage. For instance, the application of probiotics *B. longum* ATCC15707 and *L. acidophilus* La-5 has shown remarkable effectiveness in preventing the growth of *E. coli* O157:H7 and *S. aureus* during the production and storage of domiati cheese (El-Kholy et al., 2014). Erkkilä et al. (2000) reported that the probiotic *L. rhamnosus* E-97800, *L. rhamnosus* LC-705, *L. rhamnosus* GG, and commercial *P. pentosaceus* effectively suppressed the proliferation of pathogenic *E. coli* O157:H7 in dry sausage.

3.2 Other Antagonistic Bacteria

In addition to probiotics, alternative bacteria have been explored for pathogen control in food. Janisiewicz et al. (1999) successfully applied the antagonistic *P. syringae* L-59-66 to suppress the growth of *E. coli* O157:H7 on wounded apple tissue. The observed antagonistic effect is believed to stem from competition for both space and nutrients. Moreover, Alegre et al. (2013) showcased the antagonistic properties of *P. graminis*, a Gram-negative bacterium isolated from ‘Golden Delicious’ apples, against *E. coli* O157:H7 present in fresh-cut peach and apple. Similarly, *B. subtilis* isolates, collected from mung bean seeds and lettuce stems, were utilized in another study to surpress *E. coli* O157:H7 contamination in mung bean sprouts (Shen et al., 2017). The effectiveness of antagonistic approaches is subject to several factors, such as storage temperature, nutrient availability, inoculation concentration, and biofilm formation. For instance, *P. fluorescens* served as a biocontrol agent to regulate the growth of *E. coli* O157:H7 on spinach. Notably, a reduction in pathogen viability ranged from 0.5 to 2.1 log CFU/g, was recorded. Crucially, the efficacy of biocontrol was notably influenced by storage temperature, with more pronounced inhibitory effects observed at lower temperatures (Olanya et al., 2013). In addition, Kim et al. (2013) reported that the biofilm-forming strain *Paenibacillus polymyxa* T5 displayed potent antimicrobial activity against *E. coli* O157:H7, in comparison to antagonistic cells lacking biofilm formation.

4 Fungi-Based Biocontrol Strategies

A diverse range of fungal species has been employed as biocontrol agents against pathogens in food. The primary factors driving the use of fungal biocontrol are their notably high reproductive rates, both sexually and asexually, along with their short generation times and target-specific properties (Thambugala et al., 2020). Additionally, their efficacy in eradicating host organisms, extensive metabolic diversity facilitating the discovery of suitable isolates for biocontrol, and relative environmental safety due to their role as primary decomposers, all contribute to their expanded usage (Thomas & Read, 2007). Among the numerous fungal species demonstrating potential and applied in biocontrol practices, filamentous fungi (i.e., moulds) and yeasts stand out as the most researched and utilized.

4.1 Mechanisms Underlying the Fungal Biocontrol Activity

Similar to antagonistic bacteria, fungal species exhibit multiple mechanisms to control food and crop pathogens (Fig. 2). These include the release of antifungal metabolites, enzymes, and volatile organic compounds (VOCs), competition for nutrients and space, mycoparasitism, and induction of resistance and growth enhancement (Freimoser et al., 2019). Certain filamentous fungi and yeast species can impede fungal pathogens through competitive mechanisms, resulting in nutrient starvation, a primary cause of microbial mortality and reduced spore generation (Savita & Sharma, 2019). Iron is considered one of the most sought-after nutrients, and competition for it is acknowledged as a significant mechanism of action (Wachowska & Borowska, 2014). When faced with iron deficiency, filamentous fungi produce siderophores, aiding in its iron uptake from the environment (Eisendle

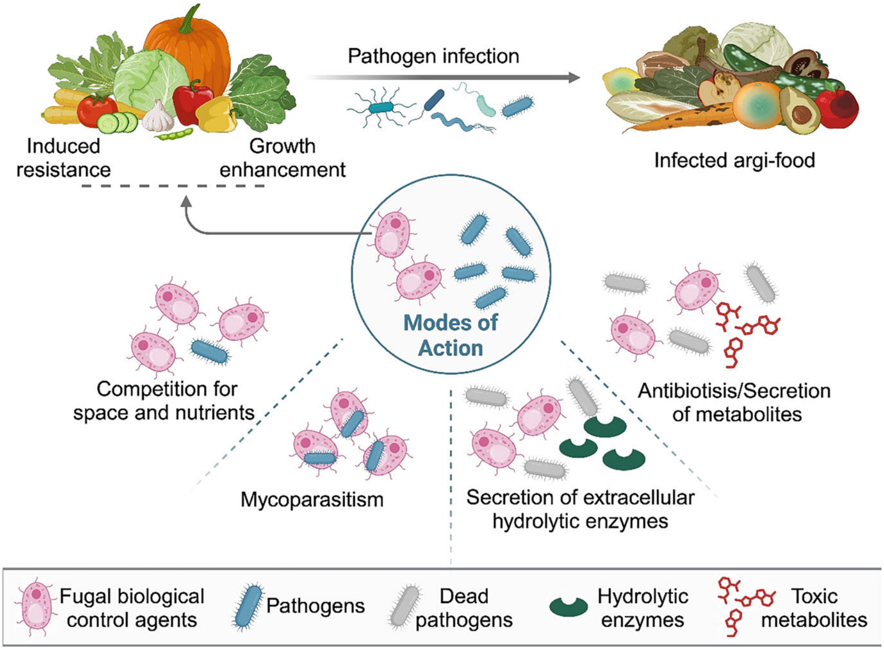

Fig. 2 Multiple action modes of fungi-based biocontrol strategies in agri-foods

et al., 2004). Regarding competition for space, the formation of biofilms stands out as a particularly effective strategy. In fungi, biofilm formation exhibits significant similarities to bacterial counterparts in terms of structure, function, and mechanisms, especially evident in budding yeasts (Bartholomew et al., 2021). The initiation of yeast biofilm formation typically involves individual cell adhesion to a surface, accompanied by cell wall modifications, extracellular matrix secretion, and frequently the development of hyphae or pseudohyphae (Cavalheiro & Teixeira, 2018).

The efficacy of beneficial fungi in biocontrol also relies on the production of secondary metabolites and/or lytic enzymes (Savita & Sharma, 2019). Among the most notable filamentous species exhibiting this trait are *Trichoderma* and *Gliocladium*, known for synthesizing and releasing potent antifungal antibiotics such as gliotoxin, viridin, and gliovirin (Mendoza et al., 2015). The production and release of enzymes that degrade cellular components is a general phenomenon in various interactions between fungi and pathogens. Typically, these enzymes are upregulated under conditions of nutrient scarcity to facilitate the liberation of nutrients from “prey” cells, potentially leading to their demise (Calderón et al., 2019). On the other hand, proteinaceous killer toxins, initially discovered in *S. cerevisiae*, are particularly notable metabolites produced by biocontrol yeasts. These toxins, primarily composed of proteins, play a crucial role in the antagonistic interactions between fungi and other microorganisms, often serving as potent weapons in the competition for resources and ecological dominance. Research on yeast killer toxins has predominantly focused on controlling spoilage yeasts in the food and beverage industry or exploring medical uses (Liu et al., 2015). Recent findings also highlight the significant role of the yeast volatilome in interactions between yeast and pathogens. For instance, the ability of specific food yeasts (e.g., *A. pullulans* and *S. cerevisiae*) to act as biocontrol agents is mainly attributed to their formation of 2-phenylethanol (Parafati et al., 2015).

Moreover, biocontrol fungi are capable of inducing systemic resistance in agricultural products against a wide spectrum of pathogens, a trait thought to greatly enhance their effectiveness in biocontrol. For instance, species like *S. cerevisiae*, *Rhodosporidium paludigenum*, *C. saitoana*, *C. oleophila*, and *Metschnikowia* have been shown to trigger an innate immune response, resulting in resistance against pathogens in the phyllosphere of fruits (Hershkovitz et al., 2012). Additionally, biocontrol yeasts have been employed in conjunction with resistance inducers like rhamnolipids and salicylic acid in certain cases (Yan et al., 2014). However, it’s important to note that in many cases, the mechanisms described have not been fully validated through molecular analyses such as gene deletion and complementation, or heterologous expression. Instead, they have been proposed based on analogies with other biological systems. Nonetheless, with the increasing availability of annotated yeast genomes and diverse transformation techniques, it should become feasible to elucidate various mechanisms and definitively confirm biocontrol activities in future research endeavors (Freimoser et al., 2019).

4.2 Application of Fungal Biocontrol Agents in Argi-foods

4.2.1 Application of Biocontrol Filamentous Fungi

Filamentous fungi have emerged as highly effective biocontrol agents in safeguarding agricultural products. These fungal species include both basal and higher fungi. Among the basal fungi, representatives of the subphylum *Entomophthoromycotina* stand out, with species from genera like *Conidiobolus*, *Erynia*, and *Entomophaga* being particularly notable (Baron et al., 2019). Among higher filamentous fungi, numerous species within the phylum Ascomycota have shown promise, with several already in commercial use and application. These include *Purpureocillium*, *Aureobasidium pullulans*, *Curvularia pallescens*, *Metarhizium*, *Beauveria*, *Fusarium*, *Clonostachys*, *Epicoccum*, and various *Trichoderma* species (Faria et al., 2017). Filamentous fungi have been recognized as sustainable biological control agents suitable for mitigating many post-harvest pathogens affecting fruits, such as guava, mango, strawberry, apple, banana, jujube, citrus, and kiwifruit (Adetunji & Varma, 2020). *A. pullulans* was utilized in an effort to control *C. acutatum* in apples; however, this biocontrol presented limited effectiveness, achieving only a 44% inhibition rate (Di Francesco et al., 2015). In another study, *E. dendrobii* exhibited the ability to penetrate apple fruit through stomatal cells, inhibiting the germination of conidia and the formation of appressoria by *C. gloeosporioides* (Bian et al., 2021). Its antifungal effectiveness was significantly higher when used preventatively compared to post-infection, completely halting pathogen advancement. Compared to bacterial strains such as *Amycolatopsis* sp., *B. subtilis*, and *P. polymyxa*, fungal *E. dendrobii* exhibited superior control efficacy against *C. gloeosporioides* in apples. Filamentous fungi have also been extensively employed to control *C. musae* in bananas. Alvindia and Natsuaki (2008) and Sangeetha et al. (2009) showed the exceptional biocontrol capabilities of *Trichoderma* in managing crown rot in postharvest bananas, a disease caused by several pathogenic fungi, such as *F. verticillioides*, *Lasiodiplodia theobromae*, and *Colletotrichum musae*.

4.2.2 Application of Biocontrol Yeast

Among the biocontrol yeasts valued by the food and industrial sectors, *Saccharomyces* yeasts are particularly noteworthy. Among them, *S. cerevisiae* stands out prominently. This yeast has played a pivotal role in bread, beer, and wine production for centuries and holds the distinction of being the first genetically manipulated eukaryote (Di Canito et al., 2021). In the last two decades, researchers have explored the use of non-*Saccharomyces* as biocontrol solutions in the agri-food industry, recognizing their critical antagonistic activity against undesirable microorganisms (Jolly et al., 2003). This realm may encompass the utilization of killer toxins (zymocins or mycocins), antimicrobial peptides (e.g., Lactoferricin B), or VOCs generated by non-*Saccharomyces* yeasts as natural control strategies to combat contamination (Liu et al., 2015). For example, the increasing interest in minimizing the use of sulfur dioxide in the winemaking industry has spurred scientific investigation into the antimicrobial attributes of non-*Saccharomyces* yeasts as a substitute for conventional chemical additives (Agarbati et al., 2023). Some non-*Saccharomyces* yeasts utilized in winemaking exhibit the ability to manage filamentous fungi as well as spoilage yeasts in the vineyard and during the initial fermentation stage (Sizzano et al., 2023). They can specifically mitigate the presence of spoilage species like *Brettanomyces bruxellensis* and *Zygosaccharomyces rouxii* in bulk wine, through the release of active antimicrobial extracellular molecules (Berbegal et al., 2018).

Research into the antimicrobial characteristics of non-*Saccharomyces* yeasts has expanded beyond winemaking, indicating a broader exploration of their potential for biocontrol purposes across the food industry. Practical studies have showcased their effectiveness in cheesemaking and breadmaking contexts. Additionally, non-*Saccharomyces* yeasts have been widely applied to ensure the safety of pre-/post-harvest fruits and plants. They are used as potential antagonists against phytopathogenic fungi like *Penicillium*, *Aspergillus* genera, as well as *B. cinerea*, which affect table grapes, wine grapes, and raisins (Di Canito et al., 2021). For instance, *R. fluviale* and *R. paludigenum* have been employed to inhibit the growth of *A. japonicas*, *A. uvarum*, and *A. aculeatus* on post-harvest table grapes, through the secretion of lytic enzymes (Tilocca et al., 2019). In recent studies, yeast species from the *Pichia*, *Wickerhamomyces*, *Metschnikowia*, *Dekkera*, and *Rhodotorula* genera have emerged as effective inhibitors. This diversity offers a range of species with potential as antagonists against pathogenic fungi affecting grapes and raisins (Tryfinopoulou et al., 2019). Moreover, Leverentz et al. (2006) clearly indicated the effectiveness of *M. pulcherrima*, isolated from apple surfaces, against various foodborne pathogens, such as *S. enterica* and *L. monocytogenes*, on fresh-cut apple tissue.

5 Plant-Derived Antimicrobial Products

Plants harbor diverse natural compounds endowed with antimicrobial properties. Many studies have demonstrated the antimicrobial properties of plant extracts derived from herbs and spices. Furthermore, phytochemicals in plants, including saponins, tannins, steroids, alkaloids, and flavonoids, significantly contribute to their antimicrobial properties. The structural arrangement of these compounds greatly influences their effectiveness (Gyawali et al., 2015; Quinto et al., 2019). Indeed, the antimicrobial attributes of these natural products are often complemented by their antioxidant capacity. When both properties coexist within a molecule, the compound becomes more potent in combating microorganisms (Pisoschi et al., 2018). Gram-positive bacteria typically exhibit a stronger response to plant extracts than Gram-negative counterparts. The discrepancy primarily results from the presence of lipopolysaccharide cell wall in Gram-negative bacteria, which obstructs the diffusion of hydrophobic compounds (Nakamura & Hatanaka, 2002). Furthermore, the rise in antibiotic resistance in pathogens has prompted research to explore plant extracts as potential alternatives. These extracts hold the promise of solving the challenge of antibiotic resistance and may yield superior results compared to synthetic preservatives. Additionally, research shows that plant extracts can decrease antibiotic resistance through fostering synergistic interactions between natural antibiotics and antimicrobials (Li et al., 2023). This chapter provides an overview of the action mechanisms of plant-derived antimicrobials, their efficacy, and potential usage in mitigating pathogens in foods.

5.1 Plant Derived Compounds

5.1.1 Antimicrobial Mechanisms of Plant Extracts

The exact action mechanisms of plant extracts are often undisclosed or not fully elucidated. Table 2 summarizes some commonly used plant extracts and compounds along with their respective antimicrobial action against target pathogens. It’s worth noting that the type of damage induced may vary among bacteria, even when exposed to the same antimicrobial substance. Additionally, some action modes have been reviewed for different antimicrobial groups, and some potential modes are listed below:

- Essential oils (EOs) cause functional and structural injury to bacterial cell membranes, and the hydrophobicity range plays a crucial role in their toxicity.
- Flavonoids exert their antimicrobial effects primarily by penetrating the bacterial cell membrane, leading to membrane damage and inducing alterations in membrane potential, intracellular pH, and adenosine triphosphate (ATP) synthesis.
- Membrane-disrupting compounds are able to induce cellular content leakage, disrupt active transport or metabolic enzyme function, and dissipate cellular energy metabolism.
- Certain extracts can directly lower the pH of the growth medium or substrate by increasing proton concentration, depressing intracellular pH through ionization of undissociated acid molecules, or disrupting substrate transport.
- Organic acids in plant extracts might inhibit NADH oxidation, thereby disrupting the supply of reducing agents to electron transport systems.
- Organic acids, particularly short-chain organic acids, can also alter membrane permeability and disrupt energy metabolism.
- Antimicrobial compounds from plants target the phospholipid bilayer, disrupt cell enzyme proteins, compromise the bacterial genetic materials, or generate fatty acid hydroperoxides through the oxygenation of unsaturated fatty acids.

The possible action modes mentioned above are commonly cited mechanisms to explain the antimicrobial effects of natural plant-derived products. Nevertheless, it’s

Table 2 Antimicrobial mechanisms of selected plant-derived extracts

Plant extract/ compound	Target microorganism	Action mode	References
Grape seed extract	*L. monocytogenes* and *S. aureus*	Cell membrane permeability and intracellular unbalanced glycolysis and amino acid metabolism; partial disintegration of bacterial cell surfaces resulting in reduced residual cellular content	Al-Habib et al. (2010), Zhao et al. (2020a, b)
Thyme EO	*L. monocytogenes*	Cell wall disruption and increased roughness	Rasooli et al. (2006)
Cinnamon EO and cinnamaldehyde	*S. enteritidis*, *E. coli* O157:H7, *E. coli*, and *L. Monocytogenes*	Lower the content of adenosine triphosphate (ATP) and inhibit the activity of ATPase	Zhang et al. (2022)
Roselle, clove, and rosemary extract	*E. coli* and *S. aureus*	Cell wall disruption and decrease in cytoplasmic pH	Gonelimali et al. (2018)
Lindera glauca EO	*S. flexneri*	Cell membrane damage, metabolic function disruption, and disturbance of redox homeostasis	Chen et al. (2021)
Citrus EO	*Enterococcus* spp.	Morphological alternations	Fisher and Phillips (2008)
Garlic extract	*S.* Hadar	Loss of enzymatic activities, cell wall rupture, and nonhomogeneous distribution of cytoplasmic material	Belguith et al. (2009)
Green, oolong, black, and Fuzhuan tea extract	*S. typhimurium*, *S. aureus*, *E. coli*, and *E. faecalis*	Increased cell membrane permeability and changes in the relative electrical conductivity by catechins	Liu et al. (2022)

important to note that specific plant compounds may exhibit varying effects on different groups of target pathogens (Gyawali et al., 2015).

5.1.2 Applications of Plant Extracts in Foods

While research on plant extracts as antimicrobial agents in the food industry is limited, studies have shown their potential against microbial pathogens. Plant-derived antimicrobial agents are primarily utilized in food systems in the form of biofilms and edible coatings. These specialized films and coatings gradually permeate the food package, thereby prolonging the antimicrobial effect over time. For instance, Ouattara and Mafu (2003) documented an extension of the shelf life of shrimp by 20–21 days through the application of a protein coating containing 0.9% thyme oil and 1.8% trans-cinnamaldehyde, coupled with exposure to 3.0 kGy gamma rays. Moreover, grape seed extract has exhibited antimicrobial properties either independently or in conjunction with other hurdle technologies across various food applications, including cooked shrimp and fish fillets (Zhao et al., 2019, 2021; Zhao, Chen, Zhao, et al., 2020b). However, in the food industry, the use of plant extracts, like EOs, presents some challenges. Because the food matrix interferes with the active compounds in these extracts, their efficacy is often higher in laboratory media than in real food systems. In addition, most EOs derived from cloves, oregano, and thyme have poor water solubility, making it difficult to integrate them into complex food systems, thereby reducing antibacterial activity (Gyawali et al., 2015). Moreover, higher concentrations of these EOs may alter the sensory characteristics of food products. Therefore, it is important to consider the sensory impact and evaluate the appropriate amounts of these natural preservatives are used.

6 Conclusions

As consumer demand for food free of synthetic preservatives grows, the exploration of new alternatives becomes imperative. Biocontrol approaches offer promising avenues for curbing foodborne pathogens and extending the shelf life of foods. Additionally, they have the potential to increase the susceptibility of drug-resistant bacteria to antibiotics. Economically, the quest for natural antimicrobials must be cost-effective, with the integration of multiple approaches like hurdle technology emerging as a viable solution. Combining biocontrol agents from diverse groups or pairing them with other antimicrobial agents can synergistically reduce the required dosage for significant pathogen reduction. However, further research is essential to evaluate the safety and efficacy of these approaches.

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