



## Review article

## Controlling foodborne pathogens with natural antimicrobials by biological control and antivirulence strategies

Ahmed G. Abdelhamid<sup>a,b,\*</sup>, Noha K. El-DougDoug<sup>b</sup><sup>a</sup> Department of Food Science and Technology, The Ohio State University, Columbus, OH, USA<sup>b</sup> Botany and Microbiology Department, Faculty of Science, Benha University, Benha, 13511, Egypt

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## ABSTRACT

Foodborne diseases represent a global health threat besides the great economic losses encountered by the food industry. These hazards necessitate the implementation of food preservation methods to control foodborne pathogens, the causal agents of human illnesses. Until now, most control methods rely on inhibiting the microbial growth or eliminating the pathogens by applying lethal treatments. Natural antimicrobials, which inhibit microbial growth, include traditional chemicals, naturally occurring antimicrobials, or biological preservation (e.g. beneficial microbes, bacteriocins, or bacteriophages). Although having great antimicrobial effectiveness, challenges due to the adaptation of foodborne pathogens to such control methods are becoming apparent. Such adaptation enables the survival of the pathogens in foods or food-contact environments. This imperative concern inspires contemporary research and food industry sector to develop technologies which do not target microbial growth but disarming microbial virulence factors. These technologies, referred to as "antivirulence", render the microbe non-capable of causing the disease with very limited or no opportunities for the pathogenic microorganisms to develop resistance. For the sake of safer and fresh-like foods, with no effect on the sensory properties of foods, a combination of two or more natural antimicrobials or with other stressors, is now widespread, to preserve foods. This review introduces and critically describes the traditional versus the emerging uses of natural antimicrobials for controlling foodborne pathogens in foods.

Development of biological control strategies using natural antimicrobials proved to be effective in inhibiting microbial growth in foods and allowing improved food safety. In the meanwhile, discovery of new antivirulence agents could be a transformative strategy in food preservation in the far future.

## 1. Introduction

Food is a persistent need, nutritionally, for human life. However, undesirable microorganisms may contaminate food products, food processing facilities or manufacturing environments leading to risk of human diseases. Foodborne pathogens are those microorganisms which cause human diseases via virulence machinery, even sometimes at their low infectious dose (Yousef and Abdelhamid, 2019). Control of such microorganisms has evolved through the human history to allow for extension of the food shelf-life, and production of safer and nutritious foods. Microbial control methods aim mainly to inhibit the growth of undesirable microorganisms via the application of physical, or natural antimicrobials-based strategies. In this review, natural antimicrobials which comprise chemical and biological-based technologies will be discussed.

Combination of two or more natural antimicrobials or with physical methods, also known as "hurdle approach", gained the interest of food industry because of their minimal impact on the nutritive value and sensory properties of foods (Singh and Shalini, 2016). Physical methods of food preservation aim to inhibit or kill undesirable microorganisms from foods or food processing environments. Research continues regarding developing thermal processing which is still used for preservation of many foods until today. While thermal processing, in which foods are heated up to a certain temperature between 50 to 150 °C, is effective to kill pathogenic microorganisms, it causes changes in sensory and organoleptic characteristics of foods (Kong et al., 2007). On the other hand, non-thermal physical technologies such as high pressure processing, irradiation, and pulsed electric field (Amit et al., 2017) have been developed, and gained high consumer acceptance because it is less disruptive to food quality as compared to traditional thermal processing.

\* Corresponding author.

E-mail address: [ahmed.abdelhamid@fsc.bu.edu.eg](mailto:ahmed.abdelhamid@fsc.bu.edu.eg) (A.G. Abdelhamid).

Combination of natural antimicrobials with, particularly, non-thermal technologies will be such an asset because of the high lethality, they could achieve, with maintaining the sensory properties of foods.

Emerging technologies targeting factors by which microorganisms cause disease, also known as antivirulence technologies, have received attention in the last decade as a new line of food safety research.

In this review, we aim to critically describe the significance of natural antimicrobials, and their underlying mechanisms, in controlling food-borne pathogens. Here, natural antimicrobials are categorized into those which target; i) Microbial growth, and ii) Microbial virulence factors.

## 2. Control of microbial growth

The optimal intrinsic and extrinsic factors essential for microbial growth in foods are targeted, so they cannot support microbial growth, or their survival. Intrinsic factors are those related to the food itself, e.g. pH value, water activity, the nutritional composition, existing antimicrobials or redox potential while extrinsic factors include those related to the environment surrounding the food such as storage temperature, and relative humidity surrounding the food (Hamad, 2012). Natural antimicrobials control microbial growth via disruption of cell structure and function. In the current review, such antimicrobials were classified based on their origin as shown in Figure 1, and were divided into 1) Chemical antimicrobials or "preservatives"; and 2) Biologically-based preservatives.

### 2.1. Chemical antimicrobials

Chemical antimicrobials sometimes are known as preservatives. However, "preservative" is a broader term than "antimicrobial" when used alone. Antimicrobials inhibit microbial growth, but do not eliminate the microbe. They act on specific microbial metabolic targets such as cell wall, cell membrane, genetic determinants or enzymes (Pisoschi et al., 2018). Different antimicrobials have different mechanisms to stop microbial growth and could be combined to provide a synergistic effect rather than the effect of individual agents.

Chemical antimicrobials could be classified into two classes, "traditional" and "naturally occurring" (Raybaudi-Massilia et al., 2009; Davidson et al., 2013). Traditional chemical antimicrobials have been used since many decades and approved by many countries. Traditional chemicals used in food preservation include but not limited to sulfites, and nitrites. Organic acids such as benzoic, sorbic, acetic, lactic and propionic acids are commonly used as traditional food preservatives. Organic acids, in their undissociated form, pass through the cytoplasmic membrane lipid bilayer into the cytoplasm where they dissociate into anions and protons (Stratford and Eklund, 2003). Protons decrease the intracellular pH, and consequently inhibit glycolysis and active transport.

Bacteria tend to exclude protons outside the cell on the expense of cellular energy (Raybaudi-Massilia et al., 2009). Thus, the antimicrobial effect of organic acids is likely attributed to such cascade of cellular events (Figure 2). Traditional antimicrobials face some considerations because some could have risk to human health (e.g. nitrites link to cancer in young children), impact important nutrients for the consumer or affect food flavor. For example, sulphite causes degradation of thiamine, an essential vitamin (Garcia-Fuentes et al., 2015).

On the other hand, naturally occurring food preservatives are mostly organic, and extracted from natural sources. Naturally-occurring antimicrobials include lysozymes, spices, essential oils, isothiocyanate, avidin, lactoferrin, phenolic compounds, and garlic oil (Raybaudi-Massilia et al., 2009; Davidson et al., 2013). These chemical preservatives could extend shelf-life of food products, but a few have some shortcomings (Davidson et al., 2013). Of these, certain naturally occurring antimicrobials are present in very low amounts and if higher levels are used, they could affect the taste and smell of foods (e.g. spices).

Chemical antimicrobials may play a vital role in controlling microbial toxins produced in foods. Microbial toxins in foods mostly include bacterial or mold toxins. Bacterial toxins, produced by pathogens growing in food, could be heat labile or stable (Al-Mamun et al., 2018). Studies illustrated different methods, e.g. the use of plant-derived compounds, to reduce bacterial toxins production via down-regulation of the toxin production genes in microorganisms (De Souza et al., 2010; Upadhyay et al., 2015). Many species of molds produce secondary metabolites, grouped as mycotoxins (e.g. aflatoxins produced by *Aspergillus flavus*), which are toxic for humans, animals and birds (Upadhyay et al., 2015). Mycotoxins such as patulin, ochratoxin A, zearalenone, aflatoxins, and fumonisins, commonly contaminate human food products (e.g. cereals) leading to several human diseases (Upadhyay et al., 2015). Several approaches to prevent mycotoxicosis, a disease caused by mycotoxins, include reducing the load of molds or their spores. Furthermore, some chemicals such as hydrogen peroxide, have been found effective to inactivate mycotoxins such as aflatoxins (Gardner et al., 1971).

The use of antimicrobials in foods still lacks the presence of approved methods for determining their antimicrobial efficacy in foods. This means when a company has an interest in an ingredient, it should develop its own industrial validation method (Davidson et al., 2013). More importantly, the consumer perception and need for fresh-like foods may lead to increasing scientific efforts and industrial shifts to natural antimicrobials.

### 2.2. Biologically-based antimicrobials

#### 2.2.1. Lactic acid bacteria

Antimicrobials of biological origin (mainly microbial) mostly use lactic acid bacteria (LAB), their metabolites or both to prevent the growth

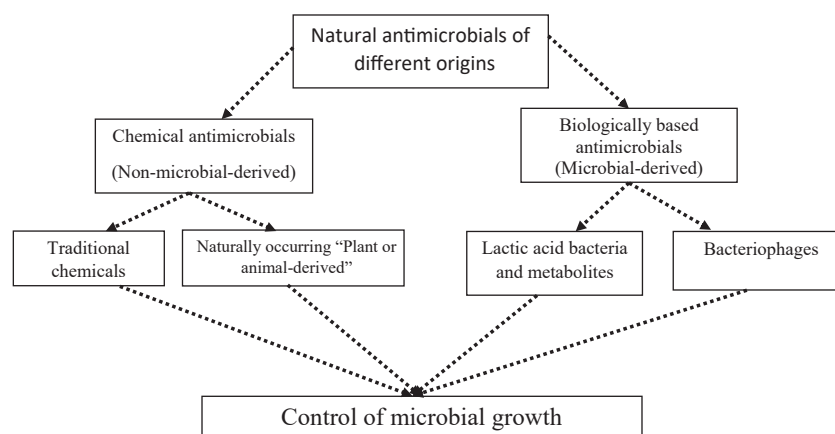


Figure 1. Schematic representation of classifying natural antimicrobials which control microbial growth as presented in the current study.

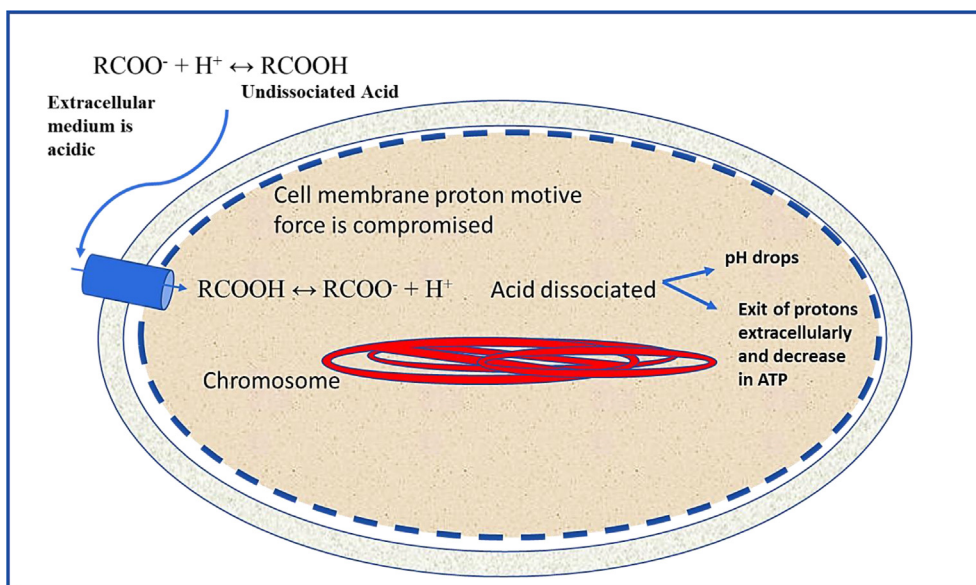


Figure 2. Mechanisms of the antimicrobial effect of “weak” organic acids against foodborne pathogenic bacteria.

of undesirable microorganisms and improve the safety and quality of foods. LAB are considered a form of biopreservation through food fermentation. They are accepted by the consumers as natural biopreservatives and health promoting microbes. In this aspect, LAB are added to the food to produce lactic acid which results in biopreservation by controlled acidification. The efficacy of lactic acid production, by LAB, depends on several factors such as food's fermentable carbohydrates, initial pH, and growth rate of LAB strains (Gobbetti and Di Cagno, 2017). Other LAB metabolites such as diacetyl, hydrogen peroxide and most importantly, bacteriocins could inhibit growth of foodborne pathogens (O'Bryan et al., 2015). In the past two decades, small molecules such as bioactive peptides, produced by LAB, were prepared and exploited in food applications which target suppression of microbial virulence. Details about these emerging applications against microbial virulence are discussed later in this review.

Biologically-based antimicrobials, of microbial origin, showed potential to mitigate mycotoxins in foods. For example, the biological control of aflatoxins in corn grains based on the fact that competitive exclusion of toxigenic strains could be achieved by the use of non-toxic strains, showed some success (Abbas et al., 2006). Several

strains of *Lactobacillus* spp. were effective in the management and control of aflatoxins (Sangsila et al., 2016; Palumbo et al., 2006). Contemporarily, high-throughput approaches such as the application of genetic engineering tools are exploited to mitigate aflatoxins (Kumar et al., 2017).

Bacteriocins are antimicrobial proteins that are produced by different members of LAB (Jack et al., 1995). They do not kill producer bacteria and are ribosomally synthesized. Their mode of action is thought to affect Gram-positive bacteria only, but some may antagonize Gram-negatives. Bacteriocins, in most of the cases, cause cell membrane damage in target bacteria and their use is expanding in food safety. Bacteriocins cause conformational changes in the cytoplasmic membrane with pore creation, resulting in increased permeability and consequently leakage of ions and molecules from within the cell (Raybaudi-Massilia et al., 2009) as demonstrated in Figure 3. Nisin is the well-known bacteriocin, approved in approximately 50 countries, and used as a preservative of processed vegetables, canned foods, and fresh cheese (De Vuyst and Vandamme, 1994). Nisin has broad spectrum inhibitory activity against Gram-positive bacteria such as staphylococci and prevents spore germination in *Clostridium* and *Bacillus* (Biswaro et al., 2018; Gut et al., 2011).

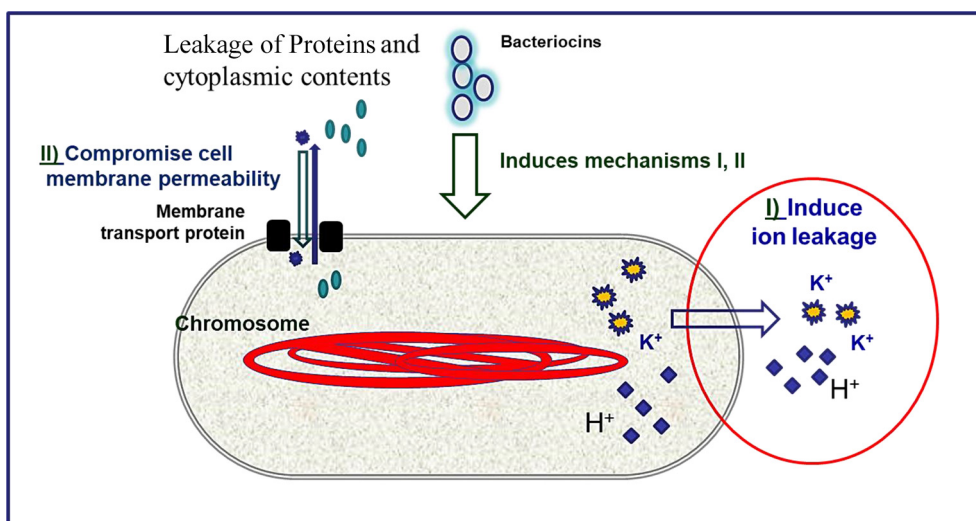


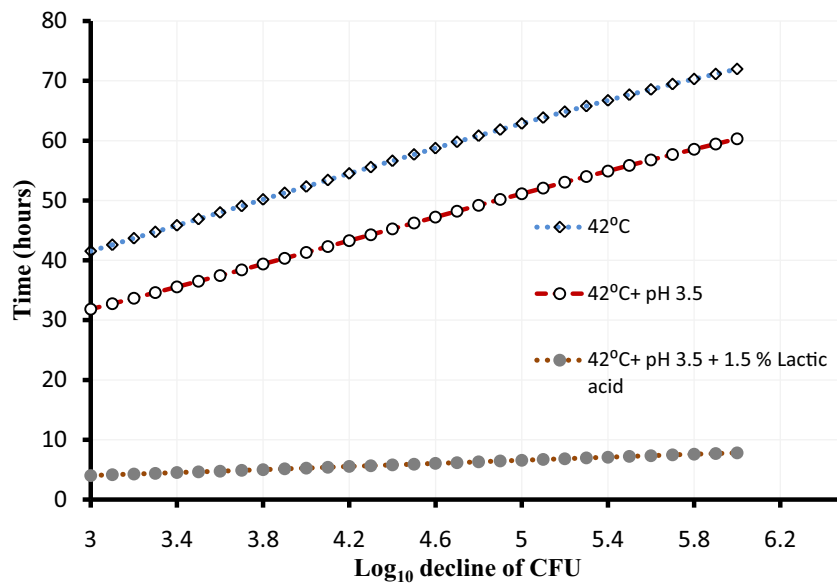
Figure 3. Mode of action of bacteriocins against foodborne pathogenic bacteria.

**Table 1.** Applications of bacteriophages in controlling foodborne pathogens.

Foodborne pathogen	Bacteriophage (phage)	Food/process application	Application outcomes	Reference
<i>E. coli</i> O157:H7	Phage DT1 and DT6	In milk during milk fermentation	100% reduction in CFU/ml within an hour	(Tomat et al., 2013)
	Phage cocktail	Spinach blades	Spraying of phage cocktail resulted in a 4.5 log CFU reduction after 2 h	(Patel et al., 2011)
<i>Campylobacter</i>	Phage CP220	Administration into <i>Campylobacter</i> -colonized chicken	2 log reduction in CFU/g after 48 h in cecal <i>Campylobacter</i>	(El-Shibiny et al., 2009)
	Phage Cj6	On top of raw and cooked beef	largest reductions were recorded at high host cell densities	(Bigwood et al., 2009)
<i>P. syringae</i>	Phage φ6	Canker of kiwifruit	Reduction of 3.9 log CFU/mL using MOI** 1	(Pinheiro et al., 2019)
<i>S. aureus</i>	Phage cocktail	During cheese manufacturing	Undetectable levels of <i>S. aureus</i> after 6 h in fresh cheese	(Bueno et al., 2012)
<i>Cronobacter sakazakii</i>	Phage CR5	Infant formula milk	Complete inhibition of <i>Cronobacter</i> using MOI 10 <sup>5</sup> .	(Lee et al., 2016)
<i>L. monocytogenes</i>	<i>Listeria</i> phage A511	Soft ripened white mold and red-smear cheeses	6 logs reduction of bacterial growth with MOI 10 <sup>6</sup>	(Guenther and Loessner, 2011)
	Phage P100	Catfish and salmon fillet	Reduction by 1.6–2.3 CFU/g at 22 °C on upon surface application	(Soni et al., 2010)
	Phage P100	Cooked ham surface	100-fold reduction after 14–28 days of storage on cooked ham surface	(Holck and Berg, 2009)
	Phage P100	Red smear soft cheese	3.5 logs to complete eradication following phage application during rind washing	(Carlton et al., 2005)
	Phage cocktail	Melon pieces	<i>Listeria</i> population reduction by 6.8 log units after 7 days of storage	(Leverentz et al., 2004)
<i>Salmonella</i> Newport	Phage cocktail	Biocontrol of <i>S. Newport</i> on cherry tomato	Using MOI 5 leads to about 4.4 log reductions	(El-Dougoug et al., 2019)
<i>Salmonella</i> Typhimurium	Phage F01-E2	Turkey deli meats, chocolate milk and hot dogs	5-log reduction of CFU on turkey deli meats, chocolate milk and a 3-log reduction when applied to hot dogs	(Guenther et al., 2012)
<i>Salmonella</i> Typhimurium	Phage cocktail PC1	Meat skin	More than 99 % reduction in CFU at MOI 10 or above	(Hooton et al., 2011)
<i>Salmonella</i> Enteritidis	LPSE1	Milk	Reduced recoverable <i>Salmonella</i> by approximately 1.44 log CFU/mL at MOI 1 and 2.37 log CFU/mL at MOI 100	(Huang et al., 2018)
		Sausage	At MOI 1 in sausage, <i>Salmonella</i> count decreased 0.52 log CFU/mL of sausage homogenate at 28 °C. At MOI 100, the count decreased 0.49 log CFU/ml at 4 °C.	
		Lettuce	Reduction by 2.02, 1.71, and 1.45 log CFU/mL of lettuce homogenate, MOI 1, 10, and 100, respectively	

\* CFU denotes for colony forming unit.

\*\* A multiplicity of infection (MOI) which denotes for the ration of phage titre to the bacterial concentration.



**Figure 4.** Inactivation of *L. monocytogenes* in broth using hurdle technology. Data represent a plot of time (h) required for 3–6 log CFU reduction of *L. monocytogenes* exposed to heat treatment (42 °C) combined with pH 3.5 and 1.5 % lactic acid (solid spheres) compared to combination of heat treatment (42 °C) and pH 3.5 (open spheres) or heat treatment only (open diamond). Inactivation of *L. monocytogenes* profiles was predicted by USDA Pathogen Modeling program [version 8] [<https://www.ars.usda.gov/northeast-area/wyndmoor-pa/easter-n-regional-research-center/residue-chemistry-and-predictive-microbiology-research/docs/pathogen-modeling-program/>].

For example, addition of *L. lactis* to a cheese model resulted in prevention of spore outgrowth in *C. beijerinckii* INIA 63 (Garde et al., 2011). Furthermore, nisin delayed growth of *Listeria monocytogenes*, inoculated onto meat, for more than 2 weeks when stored at 5 °C and did not allow *Staphylococcus aureus* to grow at the same storage conditions (Chung et al., 1989). Pediocin, a bacteriocin produced by *Pediococcus* spp., is also another example of bacteriocins with antagonistic potential against Gram-positive and some Gram-negative foodborne pathogens (Ghosh et al., 2019). Discovery of novel bacteriocins, similar to or more effective than nisin or pediocin, is a growing field in food safety research (Yang et al., 2016; Wu et al., 2019).

### 2.2.2. Bacteriophages

Bacteriophages are viruses that infect bacteria, replicate within the host and those which are lytic, cause cell lysis (Labrie et al., 2010). The application of bacteriophages in the biocontrol of foodborne pathogens has gained increasing interest throughout recent years while their use as preservatives is relatively new. Their specificity, effective mode of action (Spricigo et al., 2013), reduced effect on organoleptic properties of foods (Sharma et al., 2005) and ubiquity (Hughes et al., 1998) are the main advantages of using lytic bacteriophages to eradicate foodborne pathogens. A growing body of evidence about the efficacy of bacteriophages to control spoilage, pathogenic and biofilm forming microorganisms is promising. Recent food applications of bacteriophages against some well-known foodborne pathogens are presented in Table 1. Large numbers of bacteriophages were isolated and have been used in different food matrices to control Gram-positive (e.g. *S. aureus* and *L. monocytogenes*) and Gram-negative (e.g. *Salmonella* spp., *E. coli* O157:H7, and *Pseudomonas syringae*) bacterial pathogens and their effect was food-type and phage-concentration dependent. In terms of food type, bacteriophage DT1 and DT6 caused complete reduction of *E. coli* O157:H7 in milk (Tomat et al., 2013) whereas bacteriophage cocktail sprayed on spinach blades resulted in 4.5 log colony forming unit (CFU)/blade reduction (Patel et al., 2011). This suggests effectiveness of bacteriophages is higher in liquid foods than solid foods. Although not a constant rule of thumb, the higher titers (usually referred to as plaque forming unit (PFU)) of bacteriophages used in food application, the larger inactivation rates of foodborne pathogens. Commercial bacteriophage preparations have been approved for use in food such as ListShield™, SalmoFresh™, and EcoShield™ (Intralytics, Columbia, MD, USA) that target *L. monocytogenes*, *Salmonella* spp. and *E. coli*, respectively, in food and food processing environments. With all these

remarkable findings, future research is needed to maximize antimicrobial action of bacteriophages in food matrices and reduce their host-contact time needed for attachment to, and lysis of the foodborne pathogen (Sillankorva et al., 2012).

### 2.3. Developments for enhancing biological control using natural antimicrobials

Since efficacy of natural antimicrobials can be challenged by several physical or chemical factors in the food environment, strategies pertaining to improve their activity gained a mounting interest. These strategies can be grouped into different categories as described below.

#### 2.3.1. Increasing efficacy of antimicrobials through synergies

To improve the antimicrobial activity of natural antimicrobials, synergism between two or more antimicrobials or between antimicrobials and food-related stressors was feasible. Such synergistic interaction, also known as hurdle approach, aims to get maximum lethality against foodborne pathogens (Leistner, 2000). For example, sequential application of the lipopeptide paenibacterin and desiccation stress resulted in significant population reduction (1.5–1.9 log CFU/ml) of *S. enterica* serovars compared to desiccation stress alone (<0.1 log CFU/ml) (Abdelhamid and Yousef, 2019). Another example was that three antimicrobials, nisin A, lactoperoxidase, and reuterin, synergized to control *L. monocytogenes* and *S. aureus* in a dairy product with 4-log reduction after incubation at 10 °C for 12 days, compared to that reduction by any of the two antimicrobials (Arqués et al., 2008). Successful synergism between antimicrobials, against foodborne pathogens, included the combined use of nisin A and cinnamaldehyde (Shi et al., 2017), Pediocin PA-1 and sodium diacetate (Schlyter et al., 1993), or nisin A and lactoferrin (Murdock et al., 2007). A modeled example of combining the antimicrobial lactic acid with other stressors to inactivate *L. monocytogenes* is illustrated in Figure 4. Figure 4 shows that the time (7.8 h) required for 6-log reduction of *L. monocytogenes* in broth culture using combination of heat, acidic pH, and lactic acid is shorter, than that time (60.3 h) required by heat and acidic pH combination or heat treatment alone (71.9 h).

#### 2.3.2. Improving efficacy of antimicrobials by controlled delivery

The inhibition of target foodborne pathogens can be enhanced by gradual release of antimicrobials into the food environment. For example, a bimodal approach of nisin delivery from its carrier was

**Table 2.** Examples of antivirulence activities against foodborne pathogens.

Antivirulent Agent*	Foodborne pathogen	Activity	Reference
<i>Probiotics</i>			
<i>Bifidobacterium lactis</i> Bb12/ <i>Lactobacillus rhamnosus</i> LGG	<i>Salmonella</i> Typhimurium Enteropathogenic <i>E. coli</i>	Anti-adhesive	(Bernet et al., 1994)
<i>L. acidophilus</i> A4	<i>E. coli</i> O157:H7	Anti-adhesive	(Kim et al., 2008)
<i>E. coli</i> Nissle	<i>S. Typhimurium</i>	Anti-invasive	(Altenhoefer et al., 2004)
<i>Bifidobacteria</i>	Shiga toxin-producing <i>E. coli</i>	Antitoxin effect	(Asahara et al., 2004)
<i>Bifidobacteria</i> and <i>lactobacilli</i>	<i>Clostridium difficile</i>	Antitoxin effect	(Valdés-Varela et al., 2016)
<i>L. acidophilus</i> A4	<i>E. coli</i> O157:H7	Antibiofilm	(Kim et al., 2009)
<i>L. acidophilus</i> La-5	<i>E. coli</i> O157:H7	Anti-quorum sensing	(Medellin-Pena et al., 2007)
Probiotic <i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	Decolonization	(Piewngam et al., 2018)
<i>Chemical or biological molecules</i>			
T315 compound	<i>S. Typhimurium</i>	Antibiofilm	(Moshiri et al., 2018)
Chalcone	<i>S. aureus</i>	Anti-toxin Anti-QS Antibiofilm	(Zhang et al., 2017)
Carvacrol, thymol, trans-cinnamaldehyde	<i>E. coli</i> O157:H7	Antibiofilm Reduced expression of virulence genes	(Yuan and Yuk, 2019)
Mucin glycans	<i>Pseudomonas aeruginosa</i>	Downregulation of virulence gene expression Anti-QS	(Wheeler et al., 2019)
Surface-layer protein extract	<i>E. coli</i> O157:H7	Anti-adhesive	(Johnson-Henry et al., 2007)
Cell-free preparations from <i>Lactobacillus</i> and <i>Bifidobacterium</i> sp.	<i>Campylobacter jejuni</i>	Anti-QS Reduced expression of virulence genes	(Mundi et al., 2013)
Small molecules F12 and F19	<i>S. aureus</i>	Anti-toxin Block of the transcription factor AgrA	(Greenberg et al., 2018)
Methylthioadenosine	<i>S. Typhimurium</i>	Reduced motility Anti-invasive	(Bourgeois et al., 2018)
Resveratrol	<i>S. aureus</i> <i>L. monocytogenes</i> <i>E. coli</i> O157:H7 <i>Campylobacter jejuni</i>	Antibiofilm	(Ma et al., 2018)
Green pepper essential oil	<i>Pseudomonas aeruginosa</i> KM01	Anti-enzymatic	(Myszka et al., 2019)
Sodium citrate and cinnamic aldehyde	<i>E. coli</i> O157:H7 <i>S. aureus</i>	Antibiofilm	(Liu et al., 2019)

\* Antivirulence agents were divided into circumstances where whole cells of probiotics were used or those circumstances where pure chemicals/biological-derived molecules were subject to the cited study.

designed where initial quick rate of release occurred, and as time progressed, nisin release becomes slower (Balasubramanian et al., 2011). Such approach of controlled delivery was more effective in inhibiting *Micrococcus luteus*, a model microorganism, than instant addition of nisin. On the other hand, combination of instant addition of nisin and slowly released nisin showed effectiveness to reduce counts of *L. monocytogenes* in broth (Chi-Zhang et al., 2004). Thus, the controlled delivery of antimicrobials aims to sustain the inhibition of target microorganisms over long time of storage. However, more research is needed to prove the efficacy of delivery approaches in real foods to streamline their applications.

### 2.3.3. Antimicrobial packaging systems

The use of natural antimicrobials as coatings or their incorporation into packaging materials tends to reduce counts of foodborne pathogens and extending the self-life of food products. For example, chitosan films containing 60% lysozyme were efficient to reduce *E. coli* by 2.7 log units (Park et al., 2004). Polypropylene films with ethylene-vinyl alcohol copolymer containing citral and oregano showed profound antimicrobial activity against *L. monocytogenes*, *E. coli* and *S. enterica* in packaged salad (Murriel-Galet et al., 2013, 2012). Chitosan films containing nisin and peptide P34 showed enhanced antimicrobial activity against a group of foodborne pathogens (Cé et al., 2012). Soy protein films incorporated with grape seed extract, Ethylenediaminetetraacetic acid, and nisin resulted in significant reduction by 3, 2, and 1 log CFU/ml of *L. monocytogenes*, *E. coli* O157:H7, and *S. Typhimurium* populations, respectively (Sivaroban et al., 2008). Hence, packaging materials could

be efficient delivery system for antimicrobials in foods to control foodborne pathogens; however, it is advantageous that further research can optimize the best combination of antimicrobials/their concentration and type of the packaging material used.

### 3. Control of microbial virulence

Antivirulence strategies have now become an emerging area of controlling pathogenic bacteria in foods. Virulence factors are bacterial products by which they, adhere, colonize, invade, circumvent host immune system or damage the host itself (Defoirdt, 2018). The production of virulence factors is controlled by various regulatory mechanisms. Of these, quorum sensing (QS), is known to regulate the production of several virulence factors when the pathogen reaches high cell density, and thus has been a pivotal target for anti-virulence agents (Schütz and Empting, 2018). Moreover, biofilm forming ability is a strategy used by pathogens to evade the host immune responses (Watters et al., 2016). In addition, proper folding of virulence factors through the bacterial machinery is important for their biological functions. Therefore, interference of virulence factor's functions (e.g. toxin neutralization) is used for anti-virulence strategies (Rudkin et al., 2017).

Antivirulence approaches intend to disrupt any of the virulence mechanisms/their regulatory machinery or interfere with the virulence factors with the goal to prevent or treat infections. Research emphasized the success of using beneficial bacterial cells (e.g. *Lactobacillus* and *Bifidobacterium*), chemical (e.g. chalcone), or biological molecules (e.g. mucin) to inhibit the virulence of Gram-positive and Gram-negative

pathogenic bacteria. Examples of the most effective antivirulence agents, their mode of action and the target pathogen such as *Salmonella* Typhimurium, *E. coli* O157:H7, *C. difficile* or *S. aureus* are summarized in Table 2. In this regard, probiotics which are defined as “live microorganisms when administered in adequate amounts confer a health benefit on the host” (Abdelhamid et al., 2019; Hill et al., 2014), are showing great promise. Small molecules, peptide in nature, secreted by probiotics were able to reduce virulence of *E. coli* O157:H7, *Salmonella* Typhimurium and *C. jejuni* (Bayoumi and Griffiths, 2012; Ding et al., 2005; Medellin-Peña and Griffiths, 2009). Virulent *C. difficile* is known to cause severe *C. difficile* infections (CDIs) in which disease progression is driven by two exotoxins, namely, TcdA and TcdB (Di Bella et al., 2016). Downregulation of expression of virulence genes encoding TcdA and TcdB in *C. difficile* has been successful by using cell free preparations of probiotics (Yun et al., 2014).

The rise of antibiotic resistance and the evolving understanding of virulence factors from a wide array of pathogens spurred the growing interest in antivirulence approaches. The encouraging promises of antivirulence agents are attributed to their rapid target inactivation, little impact on commensal microbiota, and less pressure imposed on the target pathogen which decreases opportunities for developing resistance (Fleitas Martínez et al., 2019; Langdon et al., 2016; Vale et al., 2016). Of the highly emerging antivirulence strategies is QS inhibitors. QS is a communication system that coordinates the behavior of bacteria (e.g. orchestrate gene expression of virulence genes) in a cell-density dependent manner, and is achieved by signal molecules known as “auto-inducers” (Papenfort and Bassler, 2016). Anti-QS agents aim to destroy the autoinducers or interfere with their binding to the host leading to decreased expression of virulence genes (Mundí et al., 2013). QS auto-inducers are also involved in the ability of microbial pathogens to form biofilms. Biofilm is a multicellular phenotype of bacterial cells when they aggregate, attach to surfaces and surrounded by a polymeric matrix. Biofilm is critical for pathogenesis in some microbial infections (Wu et al., 2015). Examples of anti-QS and anti-biofilm activities exerted by chemical or natural agents are listed in Table 2.

Encouraging applications of antivirulence approach could extend to prevention of polymicrobial infections (Puga et al., 2018). This can be accomplished by antivirulence compounds which target commonly shared virulence factors among different pathogens (Maura et al., 2016). Additional biotechnological paths of such compounds include protection of unvaccinated individuals or immune-compromised people. However, there are some challenges that face antivirulence agents such as requirement of their use in a combined form to act on various virulence factors produced by the same pathogenic strain to ensure effectiveness of virulence disruption (Fleitas Martínez et al., 2019). Additionally, rapid pathogen identification is necessary to determine its underlying virulence factors, which will act as targets for antivirulence agents. Assessment of bacterial persistence after antivirulence agent's withdrawal, and the need for developing narrow spectrum antivirulence agents against certain forms of microbial diseases are further considerations to be met (Dickey et al., 2017). Yet, antivirulence agents are mostly needed for targeting antibiotic-resistant bacterial pathogens, microbial diseases without available vaccination or toxin-related diseases. For example, FDA has approved immunoglobulins purified from donor plasma for the treatment of botulism, a disease caused by a foodborne pathogen *C. botulinum*, through neutralizing botulinum neurotoxins (Dickey et al., 2017). Further research is still needed to allow identification of antivirulence agents, expanding knowledge about their mode of action, and developing methods for their appropriate use in foods. This paves the pathway to broaden the use of antivirulence approaches in real-world applications to improve safety of foods and promote human health.

#### 4. Conclusion

Natural antimicrobials have a great a potential to suppress growth of foodborne pathogens in foods by targeting microbial cellular structures,

or microbial cell homeostasis. The antimicrobial activity of these natural agents in food decreases due to the physical and chemical components of the food environment. Therefore, strategies are continuously developed to maximize the antimicrobial efficacy via synergism, controlling the delivery of antimicrobials in foods, or their incorporation into packaging materials. The proper selection of the antimicrobial agent, and the method of use ultimately boost the pathogen control in foods. On the other hand, antivirulence strategies, which is target-specific, present a promising strategy to design either narrow spectrum or broad spectrum agents to interfere with/halt the function of pathogen's virulence factors. Appropriate choice of antivirulence agents and their target virulence mechanism increases the competitiveness of such agents for use in microbial decontamination of foods.

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