Listeria monocytogenes, biofilm formation and fresh cut produce

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The risk of pathogen contamination and growth is one of the main safety concerns associated with fresh-cut produce, as highlighted by the increasing number of produce-linked foodborne outbreaks in recent years. The pathogens of major concern in freshcut produce are *Listeria monocytogenes*, pathogenic *Escherichia coli,* and Salmonella spp. *Listeria monocytogenes* is able to grow and multiply in vegetables packaged and stored in modified atmosphere. Biofilms formed by *Listeria monocytogenes* pose a serious threat to the safety of fresh cut produce as they can persist for long periods of time in the food processing environment and thus represent a source of recurrent contamination. Moreover the occurrence of naturally formed biofilms on fresh produce has been demonstrated. In this article the microbiological safety of fresh-cut produce and factors affecting *Listeria monocytogenes* survival and growth on fresh-cut produce are discussed. Moreover, the structure and physiology of *Listeria monocytogenes* biofilms are reviewed.

Keywords *Listeria monocytogenes*, biofilm formation, fresh cut

1. Introduction

The definition of fresh cut produce is very broad as it includes any fresh cut fruit or vegetable or any combination thereof that has been physically altered (cut, sliced, peeled, diced etc.) but remains in the fresh state. Fresh cut produce has become increasingly popular as consumers perceive it as being healthy, tasty, convenient, and fresh. Fresh-cut products are attractive to consumers because they offer uniform piece size, reduced preparation time and the product is entirely usable. All these factors have led to the rapid growth of this industry. Fresh cut produce may represent a food safety concern because contrary to other food processing techniques such as drying or canning, fresh-cut processing does not preserve the produce as it does not include any effective microbial elimination step. Fresh-cut products are stored at refrigeration temperature because they are even more perishable than the whole products from which they are derived, due to potential contamination during processing and greater nutrients availability. *Listeria monocytogenes* is a pathogen of particular concern for fresh cut produce safety as it may cause severe illness and is able to grow at refrigeration temperature [1]. The genus *Listeria* consists of a group of Gram-positive bacteria of low G+C content closely related to *Bacillus* and *Staphylococcus* [2]. *Listeria monocytogenes* is a rod shaped, non-spore -forming, Gram positive, facultative anaerobic bacterium [3]. Cells are typically 0.4 μm in diameter and 0.5 – 2 μm in length. They are motile by means of peritrichous flagella when cultured below 25 °C [3]. *L. monocytogenes* is ubiquitous although its prevalence in the environment is not high [4]. *L. monocytogenes* has been isolated from soil, water, sewage, a large variety of vegetables, and the feces of humans and animals [5]. *L. monocytogenes* grows across a broad pH range (4.3-9.8) and tolerates low water activities (aw 0.91) while being able to grow at temperatures around 4°C [1]. Moreover, it can grow in aerobic modified atmospheres also with competitive microorganisms [6]. *Listeria monocytogenes* is the causative agent of listeriosis. Pregnant women, neonates, the elderly, and immunosuppressed individuals are particularly susceptible to this infection. During the early stages, human listeriosis often displays non-specific flu-like symptoms and gastroenteritis. However, if not treated, it can develop into septicaemia, meningitis, encephalitis, abortion and, in about 30% of cases, death [7].

2. *Listeria monocytogenes* in fresh cut produce

Listeria monocytogenes has been isolated from a wide variety of raw vegetables including potatoes, cabbage, cucumber, tomato radish and others [8, 9, 10]. It has also been demonstrated that *L. monocytogenes* is able to actively proliferate on several vegetables including celery, lettuce, corn, asparagus and broccoli [11, 12, 13, 14], while other vegetables such as fennel, carrots and tomatoes have been shown to be a poor substrate for growth of this bacterium. It has also been demonstrated that *Listeria monocytogenes* is able to grow and multiply in vegetables packaged and stored in modified atmosphere [11, 15, 16]. These findings, with the additional risk posed by cross-contamination during processing, have raised concern regarding contamination of fresh cut produce. Several studies have been conducted to assess the prevalence of *L. monocytogenes* in fresh cut minimally processed vegetables. Lin and colleagues [17] found that only one out of 63 salad samples purchased in Florida were contaminated by *Listeria monocytogenes*. Little and colleagues [18] isolated *Listeria monocytogenes* from 4.8% of analysed ready-to-eat salads. During a study conducted

in Spain [19]. *L. monocytogenes* was isolated from 4.28% of fresh cut broccoli and spinach packaged under modified atmosphere. Evaluation of prevalence of *L. monocytogenes* in 512 packages of fresh cut vegetables purchased in São Paulo (Brazil) led to 3.1% of positive results [20]. A meta-analysis of studies on unprocessed and minimally processed vegetables revealed an average contamination by *Listeria monocytogenes* around 2.63 cfu/g and a frequency of concentrations greater than 100 cfu/g, which was lower than 1% [21]. Despite data indicate a low prevalence (Table 1) and low concentrations of *L. monocytogenes* in minimally processed vegetables and fresh produce, consumption of these products has been linked to outbreaks of listeriosis in various nations.

Table 1 Prevalence of *Listeria monocytogenes* in fresh cut produce and fresh vegetables [22].

Product	Year	Country	Prevalence (%)
Cabbage	1988	USA	1.1
Lettuce	1995	Costa Rica	3.8
Cabbage	2000	USA	3.0
Lettuce	2000	Norway	0.5
Cabbage and herbs	2003	USA-Mexico	1.0
RTE salad	2003	UK	< 1.0
Leafy salads	2006	Brasil	<1.0
Fres cut lettuce	2006	Spain	3.4

3. Outbreaks of listeriosis linked to consumption of fresh produce

For a long period of time scientists have shown lack of concern about fresh cut produce, and vegetables in general, as a source of listeriosis. This is possibly due to the fact that vegetables grown in industrialized countries had rarely been linked to any kind of bacterial foodborne illness. Things changed during the 1980s. In Table 2, the most important references linked to listeriosis outbreak associated with fresh and minimally processed vegetables are reported. The first listeriosis outbreak which was clearly linked to consumption of fresh cut vegetables occurred in Canada in 1981 [23]. Coleslaw was individuated as the source of infection, and *L. monocytogenes* strain 4b was isolated from the blood of patients. Ho and colleagues highlighted epidemiological association (without microbiological confirmation) of an outbreak of listeriosis involving 23 persons from eight Boston hospitals in 1979 to the consumption of raw celery, tomatoes and lettuce [24]. More recently sporadic cases of listeriosis in England and Wales were associated with the consumption of pre packed mixed salad vegetables and mixed salads [25, 26]. In the USA, cases of hospital-acquired listeriosis have been caused by contaminated diced celery [27] and consumption of cantaloupe melon caused a multistate outbreak resulting in several deaths and one case of miscarriage [28].

Table 2 Listeriosis outbreaks linked to consumption of fresh produce.

Country	Year	Vehicle	Reference
Canada	1981	Coleslaw	[23]
USA	1979	Celery, lettuce	[24]
UK	2010	Mixed salad	[25]
UK	2010	Mixed salad	[26]
USA	2010	Celery	[28]
USA	2011	Cantaloupe melon	[27]

4. Biofilms and fresh cut produce

Fresh cut produce show higher rate of contamination from *Listeria monocytogenes* compared to the whole products from which they are prepared. This finding has been attributed to cross contamination of the pathogen during processing phases [29, 30]. Biofilms formed by *Listeria monocytogenes* pose a serious threat to the safety of fresh cut produce as they can persist for long periods of time in the food processing environment and thus represent a source of recurrent contamination [31]. Moreover the occurrence of naturally formed biofilms on fresh produce has been demonstrated [32, 33]. According to Lindow and Brandl [34] biofilms may account for up to 80% of the total microbial population on plant surfaces. The main aspects of biofilms and strategies employed for elimination of biofilms from food contact surfaces will be reviewed in the following sections.

5. Listeria monocytogenes and biofilm formation

Biofilms have been observed and described in many microbial ecosystems since the invention of microscopy. Despite this, a general theory regarding their importance was not elaborated until 1978 [35]. The definition of biofilm has constantly evolved over the past three decades and several theories have been proposed. Presently, it is widely accepted that biofilms consist in microbial sessile communities characterized by cells that are irreversibly attached to a substratum or interface or to each other, embedded in a matrix of extracellular polymeric substances, mainly polysaccharides, and with an altered phenotype (particularly, growth rate and gene transcription) compared to planktonic cells [36]. *In vitro* biofilm formation can be divided into key steps in which reversible attachment of freely moving bacteria to a surface is followed by irreversible binding to the surface, growth of microcolonies and production of a polymer matrix [37]. The next step is the maturation of the biofilm in a three- dimensional structure often showing water filled channels and tower-like or mushroom-like structures. Finally, some bacteria detach from the biofilm and are dispersed in the environment, thus allowing colonization of other surfaces [38, 39]. It has been suggested that biofilm may represent the default mode of growth for several bacterial species in natural environments as they provide defense from chemical and physical stresses and represent a mechanism to stably colonize a favorable niche, exploiting the benefits of gene transfer and division of metabolic burden [40]. As cited above, cells growing in biofilms show several differences compared to planktonic cell. Biofilms generally show slower growth rate, variation in the transcriptome [36], increased conjugation rate [41] and, most importantly, an enhanced resistance to biocides and antibiotics [42, 43]. Biofilms occur on a wide variety of surfaces, including living tissues [36], industrial equipments and food processing surfaces, such as conveyer belts, plastic an stainless steel equipment [44, 45, 46]. Since bacterial cells can be easily transferred from biofilms to food products, biofilms formed by pathogens, such as *Listeria monocytogenes* are of particular concern for food industries.

It has been demonstrated that *Listeria monocytogenes* can grow and form biofilms on several food processing surfaces including rubber, plastics, glass and stainless steel [44, 45]. Biofilms of listeria protect cells from the action of antimicrobials and sanitizers [47, 48], potentially allowing long term persistence of the microorganism in the food processing environment. This evidence suggests that *Listeria monocytogenes* biofilms represent a threat to food safety, as bacteria can be transferred to food products when they come into contact with biofilms [31, 49]. For this reasons it appears critical to detect and remove *L. monocytogenes* biofilms in food processing environments in order to improve food safety.

6. Structure and physiology of *Listeria monocyogenes* biofilms

Several techniques have been employed to investigate the structure of *L. monocytogenes* biofilms. Among them SEM [50], epifluorescence microscopy [51] and confocal laser scanning microscopy (CLSM) are the most widely used [52]. In static incubation conditions it has been observed that, depending on the strain tested, *L. monocytogenes* biofilms may consists of honey comb-like structures [53], three-dimensional mushroom shaped structures with water filled channels and pores, or more simple structures including monolayers [50, 52, 54]. In contrast, studies conducted in flow cells (dynamic conditions) have highlighted the presence of grossly spherical microcolonies surrounded by a network of chains [55].

Biofilm formation is a highly complex process which requires an extensive variation of gene expression and, consequently, major physiological changes. This process has been shown to be influenced by several environmental factors, including temperature, pH, osmolarity, exposure to bile salts, static versus dynamic growth conditions and nature of the colonized surfaces [45, 46, 55, 56, 57]. In food processing environments, biotic factors may also influence biofilm formation by *L. monocytogenes*. Resident microorganisms and biofilms can have both positive and negative effects on *L. monocytogenes* biofilm formation [58]. The inhibition of biofilms may be based on the secretion of antimicrobial agents [59, 60] or on competition for important nutrients [61]. Conversely the presence of some bacterial species, including *Pseudomonas fragi* and *Staphylococcus aureus*, can enhance biofilm formation by *L. monocytogenes* trough mechanisms that have not been clearly disclosed, but may involve small secreted peptides [62, 63] .

Several molecular determinants are involved in biofilm formation at different stages both in *L. monocytogenes* and other bacterial species. Among them a major role in *L. monocytogenes* biofilm formation would be played by flagella, quorum sensing systems and extracellular DNA.

Flagella are very important for biofilm formation in several bacterial species [39]. In *L. monocytogenes* immobilized flagellum and flagellum minus mutants show biofilm-defective phenotype compared to wild-type bacteria, thus suggesting flagella mediated motility may play an important role in biofilm formation [64]. *PrfA*, an important virulence factor and flagellar biosynthesis regulator has also been shown to be involved in the regulation of biofilm formation [65].

Quorum sensing systems involved in *L. monocytogenes* biofilm formation include the *LuxS* system and the peptide based *agr* system whose components are encoded by operon *agr*. Mutants of *luxS* gene show enhanced biofilm formation compared to parental strains in *L. monocytogenes*. It has been suggested that *luxS* may contribute to inhibition of biofilm formation converting S-ribosyl homocysteine to the autoinducer molecule Ai-2 [66]. Even if agr-dependent

mechanisms allowing biofilm formation have not yet been elucidated, it has been demonstrated that adhesion and early stages of biofilm formation are affected by mutations of *agrA* and *agrD* genes in *L. monocytogenes* [67].

The extracellular matrix in which biofilm forming bacteria are embedded is an extremely complex mixture of several substances which usually includes extracellular DNA (eDNA). As for several other bacterial species, it has recently been shown that eDNA plays an important role in *L. monocytogenes* biofilm formation [68].

Detachment of cells or cellular aggregates is the final stage of biofilm development and allows dispersion of microorganisms and colonization of new surfaces. Mechanisms of detachment from biofilms have not been clearly disclosed but different theories have been proposed. Cells may detach from biofilms as a result of cell growth and division or clusters may be removed due to external forces [36]. Detachment may result from the activity of bacteriophages [69] or the use of type IV pili to climb biofilms [43]. Finally detachment regulating systems based on catabolic repression, production of specific enzymes and rhamnolipids have also been proposed [70, 71, 72].

7. Biofilm formation and food industry: a critical point for the food safety

The food industry should always ensure the microbiological safety of the products. This dogma is especially true in minimally processed foods, such as fresh-cut vegetables, in which the absence of heat treatment does not contribute to inactivate potentially pathogenic microorganisms. Since any occurring microbial contamination during production, processing, packaging and transport can not be reduced by thermal treatment the biofilm formation is a key concern in the rising market of fresh-cut [73]. The ability of some microorganisms, including the pathogenic *L. monocytogenes*, to settle on the surfaces of food processing plants is well known to represent a strategy to survive in an unfriendly environment. Furthermore, it must be considered that some factors typically occurring in the food processing such as moisture loss, deposits of soil or debris on the surfaces, could assist the biofilm formation by microorganisms proceedings from both raw materials and *in situ* contaminations. Undoubtedly, the risk assessment and reduction related to the occurrence of *L. monocytogenes* biofilms may constitute an important expense for the enterprises. Therefore, an optimized management of the food-processing plant should consider the prevention strategies as the most effective approach. In particular, the proper design of food contact equipment is often the first strategy to counteract the biofilm formation. Materials for industrial installations should always be preferred with smooth rather than irregular surfaces. Indeed, roughened materials expose a greater surface area to the microorganisms, being the depressions the more favorable sites for colonization [74, 75, 76]. For the same reason, food contact materials should be resistant to corrosion and damage in order to avoid cracks or scratches. Moreover, the installations should be free of sharp edges and overall its surfaces always easily cleanable, allowing the “clean in place”, a system including jetting and spraying of the surfaces with an increased turbulence and flow velocity without the manual involvement of the operator [77]. A good cleaning process should remove any food residues and other compounds that may promote bacteria proliferation and biofilm formation [78]. In addition, the cleaning flush should be carried out in a way that can break-up or dissolve the exopolysaccharide matrix associated with the biofilms so that disinfectants can gain direct access to the bacteria cells [77]. Gião and Keevil [79] demonstrated that water flow does not have the same efficiency in removing cells from different material surfaces, emphasizing the need to optimize cleaning and sampling procedures by considering the conditions in which cells attach to surfaces and the physico-chemical characteristics of the surfaces.

It is well known that biofilm formation is strongly affected by the hydrophobic or hydrophilic interactions between microbial cell charge and contact surfaces so that great care should be taken in the choice of materials for installations. Materials most commonly used in the food industry can be hydrophilic, such as stainless steel and glass, or hydrophobic, for example polymeric materials [80, 81]. Several authors are presently investigating relationships between biofilms and synthetic surfaces in order to provide important insights that could lead to new strategies to remediate and avoid listerial biofilm formation in the food industry. However, probably depending on the several factors involved in biofilm formation and different methods applied, their conclusions are often divergent. Thus, Rodríguez and co-authors [82], suggested that *Listeria* biofilms may adhere more tightly to hydrophobic surfaces than hydrophilic surfaces when measured at a cellular level. In contrast, Di Bonaventura and co-authors [45], found that biofilm levels of *L. monocytogenes* were significantly higher on glass at 4, 12 and 22°C, compared to polystyrene and stainless steel, while at 37°C, the same strain produced biofilm at significantly higher levels on glass and stainless steel, compared to polystyrene. The same authors reported a positive correlation between hydrophobicity and heat suggesting that the biofilm formation is significantly influenced by temperature, probably due to modification of the cell surface hydrophobicity [45]. Recently, Choi et al. [83] observed that biofilm formation of *L. monocytogenes* on polystyrene surfaces was not significantly affected by the temperature, but the hydrophobicity of *L. monocytogenes* was influenced by addition of glucose and sodium chloride at 37°C. In another study, Xu et al. [84], showed an increased ability to self-aggregation and biofilm development in *L. monocytogenes* when incubated at high NaCl concentrations (4% to 10%), while, at the same experimental conditions, the hydrophobicity of the strain declined.

The most frequently employed chemical agents for sanitation procedures are strong oxidizing agents with a broad antimicrobial spectrum: hypochlorous acid, chlorine, iodine, ozone, hydrogen peroxide, peroxyacetic acid, quaternary ammonium chloride and anionic acids [85, 86, 87]. The effectiveness of these methods on *L. monocytogenes* biofilm eradication was studied and compared at different experimental conditions in order to establish the best treatment for

specific claims [51, 88, 89, 90, 91]. Nonetheless, new formulations have been recently proposed. For example, although the applications of chlorine dioxide (ClO_2) often refers to aqueous solutions, among innovative sanitation procedures, gaseous ClO_2 shows better potential to decontaminating food contact surfaces. Recent research works demonstrated the efficacy of gaseous ClO_2 to reduce the growth of *L. monocytogenes* planktonic cells and biofilms at room temperature on stainless steel coupons on ready-to-eat meat processing equipment [90, 92], suggesting that this approach could be used in the fresh cut vegetable industry as well. In order to investigate new approaches to control the biofilm formation in the food industry, Park et al. [93], reported on the effect of aerosolized sanitizers on the inactivation of different microbial pathogens including *Listeria monocytogenes* biofilms. With the same aim, Chorianopoulos et al. [94], proposed the use of nanostructured titanium dioxide combined with UVA irradiation as an alternative means for *Listeria monocytogenes* biofilm disinfection in food processing. In alternative to conventional chemical-based strategies, physical methods such as irradiation or ultrasound have also been reported as effective techniques against both biofilm and planktonic cells [86].

However, in the last years, the increasing consumers attitude to avoid chemicals and to prefer environment- friendly treatments, is addressing new green emerging strategy. These approaches open always more original and innovative perspectives. For example it is known that surfactants are chemical products usually utilized for cleaning food contact surfaces modifying its hydrophobicity and thus affecting cell adhesion [95]. In the last years, biosurfactants, surface- active compounds of microbial origin, attracted the attention due to their low toxicity and high biodegradability compared to synthetic surfactants [96]. Rhamnolipid and surfactin, respectively a glycolipid produced by *Pseudomonas aeruginosa* and a lipopeptide from *Bacillus subtilis* were investigated to prevent the adhesion of *L. monocytogenes* on different pre-conditioned surfaces such as stainless steel, polypropylene, polystyrene [97, 98]. Recently, Zezzi do Valle Gomes and Nitschke [99] demonstrated that the pre-conditioning with the biosurfactants rhamnolipid and surfactin can delay the adhesion of food pathogenic bacteria even reducing the hydrophobicity of a polystyrene surface. The same biosurfactants also showed an interesting potential as agents to disrupt pre-formed biofilms of *L. monocytogenes,* being surfactin more efficient than rhamnolipids. With a similar approach, Borges and coauthors [100], proposed that isothiocyanates from vegetables origin can be an eco-innovative intervention strategy to prevent and control biofilms, with immediate potential application in the food sector. These authors demonstrated that isothiocyanates are able to inhibit planktonic bacterial growth, cell motility and to change cell surface properties, also affecting *E. coli, L. monocytogenes, P. aeruginosa* and *S. aureus* biofilm formation. Orgaz et al. [101], have recently investigated the effect of chitosan, a polysaccharide industrially derived from partial deacetylation of chitin with recognized antimicrobial properties against planktonic cell growth, on already established biofilms. Their results indicated a 6-log cell reduction after the exposition of *L. monocytogenes* biofilms to 1% native chitosan for 60 min at 20°C, suggesting its usage in solution or as surface coating.

Another emerging approach is the utilization of different essential oils as anti-biofilms agents. Essential oils are volatile compounds that are formed by aromatic plants as secondary metabolites. These molecules have been extensively reported for their antimicrobial activity and potential applications in foods [102]. Nonetheless, essential oils are characterized by a strong aroma which makes their usage in the food industry problematic from a sensorial point of view. However, since these are aromas of vegetable origin, in fresh-cut products they may be considered by the consumer not as foreign odours, but also like an added value. Thus, the effect of various essential oils and their individual constituents on biofilms formed by *L. monocytogenes* has been investigated and several authors suggest them to be good candidates for further development of eco-friendly disinfectants [103,104, 105,106,107].

In the field of the green-based biofilm control strategies a valuable alternative was recently proposed by Woo and Ahn [108] by using a probiotic approach. These authors found that co-culture with *Lactobacillus paracasei* and *Lactobacillus rhamnosus* effectively inhibited by more than 3 log the biofilm formation of *Listeria monocytogenes* through mechanisms of competition, exclusion and displacement, suggesting that probiotic strains can be used as alternative way to successfully reduce the biofilm formation. Similar results were reported by Zhao et al. [109] whom, by using a competitive-exclusion based on co-culturing *Lactococcus lactis* and *Enterococcus durans* in poultry processing floor drains, observed from 2- to 6-log reductions of *L. monocytogenes* biofilm cells.

Finally, another promising approach to control and eradicate biofilms is the use of [110, 111]. Currently, bacteriophage preparations employing phage P100 are approved by the Food and Drug Administration (FDA) as ingredients for “food in general” in order to control *L. monocytogenes* contamination [112]. In a recent study, treatment with phage P100 significantly reduced *L. monocytogenes* cell populations under biofilm conditions on both stainless steel coupons surface and polystyrene microtiter wells [113], regardless of serotype, growth conditions and biofilm levels.

In addition, some authors have suggested that antimicrobial peptides or bacteriocin-producing strains may also contribute to control the initial adhesion and biofilm formation by *L. monocytogenes* on abiotic surfaces [114, 115, 116]. The application of enterocin AS-48 produced by *Enterococcus faecalis* and *Enterococcus faecium* strains to polystyrene microtiter plates synergistically improved the bactericidal effects of biocides against planktonic and sessile *L. monocytogenes*, avoiding the biofilm formation for at least 24 h at a bacteriocin concentration of 25 mg/ml [117]. The elaboration of antibacterial coatings based on natural bactericidal substances produced by living organisms such as antimicrobial peptides, bacteriolytic enzymes and essential oils has been recently suggested to

develop a new generation of biofilm-resistant surfaces [118]. In Table 3 we resumed the main chemical, physical-based, and emerging "green strategies" proposed to prevent and remove *L. monocytogenes* biofilms in the food industry.

Table 3 Some chemical and physical-based approaches and emerging green "strategies" proposed to prevent and remove *L. monocytogenes* biofilms and other pathogenic microorganisms from different contact surfaces.

	Treatment	Contact surface	Microorganisms	Reference
Chemicals and physicals	Ozone Ultrasound Ultrasound + Ozone	Stainless steel	*Listeria monocytogenes*	[119]
	Chlorine dioxide	Stainless steel	*Listeria monocytogenes*	[92]
	Chlorine dioxide gas Chlorine dioxide aqueous Sodium hypochlorite aqueous	Stainless steel	*Listeria monocytogenes*	[90]
	Quaternary ammonium compounds Chlorine Peroxide	Polyvinyl chloride drain pipes	*Listeria monocytogenes*	[120]
	Chlorine Quaternary ammonium compounds Peroxyacetic acid	Stainless steel coupons	*Listeria monocytogenes*	[121]
	Peroxyacetic acid Nisin	Stainless steel coupons	*Listeria monocytogenes*	[114]
	Titanium dioxide and UV irradiation	Stainless steel, glass	*Listeria monocytogenes*	[94]
Emerging green strategy	Yarrow essential oil	Polystyrene, stainless steel, high density polyethylene	*Listeria monocytogenes* *Listeria innocua*	[107]
	Cymbopogon sp. essential oils	Stainless steel	*Listeria monocytogenes*	[105]
	Isothiocyanates	Polystyrene	*Listeria monocytogenes* *Escherichia coli* *Pseudomonas aeruginosa* *Staphylococcus aureus*	[100]
	Chitosan	Teflon	*Listeria monocytogenes* *Bacillus cereus Salmonella enterica* *Staphylococcus aureus* *Pseudomonas fluorescens*	[101]
	Rhamnolipid Surfactin	Polystyrene	*Listeria monocytogenes* *Salmonella enteriditis* *Staphylococcus aureus*	[99]
	Probiotic strains	Polystyrene	*Listeria monocytogenes* *Salmonella typhimurium*	[108]
	Bacteriophage P100	Stainless steel	*Listeria monocytogenes*	[113]
	Enterocin AS-48 Enterocin AS-48 + biocides	Polystyrene	*Listeria monocytogenes*	[117]

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