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식품공정환경에서의 *Listeria monocytogenes*의 바이오필름

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Listeria monocytogenes Biofilms in Food Processing Environments

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Abstract

Listeria monocytogenes is a major concern in food processing environments because it is ubiquitous and can easily contaminate food during processing. Contaminated food and the surfaces in food facilities can serve as reservoirs of *L. monocytogenes*, which can lead to the serious foodborne illness listeriosis in consumers. *L. monocytogenes* can adhere to materials commonly used in food processing equipment and form biofilms. In the biofilm mode, *L. monocytogenes* is significantly more resistant to disinfection or sanitizers than its planktonic counterparts. Many researchers have studied the effects of surface materials on bacterial adhesion and the formation of biofilms. Recent studies have focused on preventing the establishment of *L. monocytogenes* in niches in the food plant environments.

Keywords : *Listeria monocytogenes*, adhesion, biofilm, control

Introduction

In spite of the many studies aimed at reducing the bacterial contamination of food, *L. monocytogenes* remains a threat to consumer health, indicating that such contamination is not fully under control. In particular, the psychrotrophic nature of *L. monocytogenes* allows its replication in refrigerated, ready-to-eat food products including poultry, milk, coleslaw, soft cheese, and meat products that have been contaminated during processing and packaging (Borucki *et al.*, 2003). Several studies have suggested that *L. monocytogenes* contaminates food from environmental sources and food processing facilities; some persistent or recurrent strains of *L. monocytogenes* have been found in food processing environments for extended periods of time (Kathariou, 2002; Tompkin, 2002). *L. monocytogenes* has persistent or nonpersistent characteristics depending on the strains and some phenotypic properties appear to differ between the persistent and nonpersistent strains. One of the major causes for concern about *L. monocytogenes* in these environments is its ability to attach to many different surfaces. Persistent strains isolated from dairy and nondairy foods show significantly higher adherence to food processing surfaces, such as stainless steel, than nonpersistent strains (Norwood and Gilmour, 1999; Lunden *et al.*, 2000). These bacteria infect food that comes in contact with a contaminated surface (Eginton *et al.*, 1995; Beresford *et al.*, 2001). Therefore, it has been suggested that the persistent strains serve as a reservoir for the re- or cross-contamination of foods during their manufacture and are a potential source of listeriosis outbreaks.

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Characteristics of *L. monocytogenes*

Listeria is a genus of gram-positive bacteria containing six species: *L. monocytogenes*, *L. innocua*, *L. aseeligeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi* (Rocourt, 1999; Vazquez-Boland *et al.*, 2001). *L. monocytogenes* and *L. ivanovii* are pathogenic, the former causing disease in humans and animals, and the latter in animals (Vazquez-Boland *et al.*, 2001).

L. monocytogenens is a facultative intracellular foodborne pathogen that causes listeriosis, a serious illness which includes meningitis, septicemia, and stillbirth, with a mortality rate of up to 30% (Schlech, 2001). Listeriosis generally affects the elderly, neonates, pregnant woman, and immunocompromised individuals. This microorganism has been isolated from a variety of sources including soil, vegetation, silage, fecal material, sewage, water, and the intestines of healthy animals and humans (Farber and Peterkin, 2000; Hof, 2001). Four serotypes (4b, 1/2a, 1/2b, and 1/2c) from the 13 known serotypes have been isolated from a wide range of foods (Farber and Peterkin, 1991). Three of these serotypes (4b, 1/2a, and 1/2b) are associated with the majority of human listeriosis cases (Farber and Peterkin, 1991).

L. monocytogenes are motile as they possess peritrichous flagella; the wild-type bacterium is peritrichous in a temperature-dependent manner – it is flagellated and motile at 20 – 25℃ but nonmotile with very few flagella above 35℃ (Seeliger, 1961; Seeliger *et al.*, 1986). They are catalase-positive and produce acid from glucose and other fermentable sugars. *L. monocytogenes* can grow at a temperature of 1 – 50℃, a pH range of 4.0 – 9.5, and at water activities greater than 0.90. In addition, it can survive under adverse conditions such as low temperatures, low pH, and high salt concentrations, despite its inability to form spores (Ryser and Marth, 1999). Moreover, this bacterium, due to its capacity to survive at low temperatures and grow in food processing plants, occasionally contaminates refrigerated ready-to-eat food, soft cheeses, dairy products, raw foods, and equipment surfaces (Farber and Peterkin, 1991). Its ability to grow in such diverse environment is just one of the many challenges presented by this dangerous bacterium (Dieterich *et al*, 2006).

Adherence and Biofilm Formation

L. monocytogenes adheres to several different materials used in food processing environments, such as stainless steel, rubber, polypropylene, glass, and plastic surfaces (Chae *et al.*, 2006; Mafu *et al.*, 1990). The adhered *L. monocytogenes* cells show an increased resistance to cleaning agents, disinfectants, and heat (Frank and Koffi, 1990; Ronner and Wong, 1993; Aarnisalo *et al.*, 2000), all of which are used in the sanitation of food processing plants. The formation of biofilms of bacteria and their adherence to food contact surfaces have great implications on hygiene because adhered cells and biofilms show an increased resistance against the stress factors commonly used in the disinfection of food contact surfaces (Aarnisalo *et al*, 2000; Norwood and Gilmour, 2000). Cell attachment and biofilm formation by *L. monocytogenes* are influenced by several factors including strain characteristics, the physical and chemical properties of the attachment substrate, the growth phase of the bacteria, temperature, growth media, and the presence of other microorganisms (Chavant *et al.*, 2002; Kalmokoff *et al.*, 2001; Norwood and Gilmour, 2000; Norwood and Gilmour, 2001). Bacterial persistence is suggested to be related to the ability of strains to form biofilms and survive the sanitizing treatments (Borucki *et al.*, 2003; Holah *et al.*, 2002). The mechanism of adhesion has been studied based on the physicochemical properties of the bacteria and the relative hydrophobicity of the surface material (Chavant *et al*, 2002). Silva *et al.* (2008) examined the adhesive abilities of 10 isolates of *L. monocytogenes* to 8 commonly used kitchen materials (strainless steel 304, marble, granite, glass, two types of polypropylene, and two types of silestone) and assessed the viability of the adhered cells. *L. monocytogenes* adhered most tightly to granite and marble, followed by stainless steel 304, glass, silestone, and finally the polypropylene surfaces. However, the viability of the adhered cells was the highest on the polypropylene surfaces (nearly 100%), followed by granite (78.7%), marble (69.5%), and it was the lowest on white silestone (18.5%). They suggested that the adhered viable cells were responsible for the post-processing contamination of food products (Silva *et al.*, 2008). *L. monocytogenes* can adhere to stainless steel, rubber, glass, and polypropylene in as little as 20 min (Mafu *et al.*, 1990). *L. monocytogenes* produces extracellular material within a 1-h period following attachment (Mafu *et al.*, 1990) and form a bilayer biofilm on a glass surface within 24 h (Chae and Schraft, 2000).

L. monocytogenes strains exhibit differences in the number of adhered cells. (Ronner and Wong, 1993; Norwood and Gilmour, 1999; Chae and Schraft, 2000; Kalmokoff *et al.*, 2001). The highest difference in adherence levels between the strains was approximately 100-fold (Norwood and Gilmour, 1999; Chae

and Schraft, 2000). Differences in the formation of biofilms and cell attachment have also been observed (Kalmokoff *et al.*, 2001). *L. monocytogenes* is capable of adhering to food contact surfaces at a temperature of 4－45℃ (Kim and Frank, 1994; Chae and Schraft, 2000). A higher level of adherence to stainless steel was observed at 18℃ than at 4℃ or 30℃ (Norwood and Gilmour, 2001). The authors suggested that the optimum adherence at 18℃ could be due to the production of extracellular polymeric substances at 21℃ but not at 10℃ or 35℃ (Herald and Zottola, 1988) and the presence of numerous flagella at 20℃ but fewer at 37℃ (Peel *et al.*, 1988). However, adherence levels are higher at 45℃ than at 10℃ or 30℃ on stainless steel and Buna-N rubber (Smooth and Pierson, 1998), although this study did not assess the adherence at 18℃.

The adherence of cells, which is required for the formation of biofilms, occurs in five stages (Fig. 1): 1) initial, irreversible attachment, 2) exopolysacchrides (EPS) formation, 3) mirocolony formation, 4) biofilm maturation, and 5) sloughing off cells. Stage 1 and stage 2 both aid the binding of cells to the surface by initially using EPS glycocalyx polymers in an adhesion and capture mechanism and then as a food source for cell division (Costerton *et al.*, 1987). The EPS matrix around the biofilm acts as a reservoir, and it also serves as a layer of protection against adverse environmental conditions and biological and chemical agents (Costerton *et al.*, 1995). EPS consist largely of water (98－99%) and the remainder is comprised of nutrients such as polysaccharides and glycoproteins (Carpentier and Cerf, 1993). The production of EPS allows bacteria to irreversibly attach to a surface. It acts against antibacterial agents

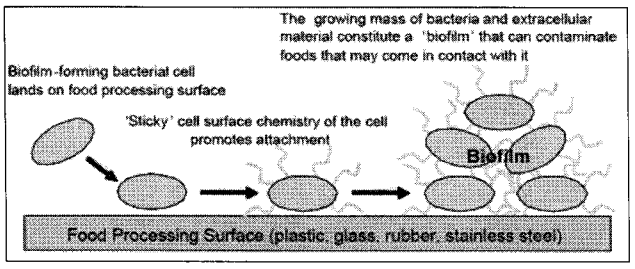

Fig. 1. *Listeria monocytogenes* can form biofilms on a variety of food processing surfaces including glass, plastic, rubber, and stainless steel. An effective sanitation and monitoring program is paramount for the eradication of *Listeria* from food processing environments as well as the use of antimicrobial products and / or other measures to ensure that products are free of such pathogens (Muriana and Kushwaha, FAPC-136).

by trapping the molecules and preventing them from entering into the system (Costerton *et al.*, 1987). This protection gives biofilms the ability to resist antibacterial agents and allows them to survive on surfaces even after cleaning and sanitation (Carpentier and Cerf, 1993).

Biofilms readily form in the food industry environment because of the availability of water, nutrients, and surfaces for attachment (Gibson *et al.*, 1999; Midelet and Carpentier, 2004). This is a problem in the food industry because the hygiene of the surfaces affects the overall quality and safety of the food product (Gibson *et al.*, 1999; Hyde *et al.*, 1997). The transfer of attached bacteria to a food product can lead to food spoilage or the transmission of diseases. Microbial contamination can also lead to mechanical blockage, corrosion of equipment, and the impairment of the intended functions of equipment such as heat transfer (Sharma and Anand, 2002). The improper cleaning and sanitizing of food contact surfaces may lead to the direct bacterial contamination of the product (Gibson *et al.*, 1999). The improper cleaning and sanitizing of environmental surfaces can also lead to the transfer of microorganisms through the air, human contact, and contact with other equipment (Gibson *et al.*, 1999). The major sources of microbial contamination of foodstuffs are the areas used for food handling, storage, or processing (Tompkin *et al.*, 2000). The most effective way of preventing biofilm formation in the food industry is to develop an effective cleaning and sanitizing regimen (Gibson *et al.*, 1999). An effective cleaning program should eliminate any soil and detach microorganisms on the work surfaces; sanitation should kill any bacteria that cleaning did not remove (Carpentier and Cerf, 1993; Gibson *et al.*, 1999). An ineffective sanitation program can lead to problems concerning the quality and safety of the food product (Gibson *et al.*, 1999).

Control of Biofilms

Biofilms are resistant to chemical cleaners and sanitizers and thus survive and proliferate on processing surfaces after cleaning and sanitizing. This leads to the contamination of food products by biofilms, further bacterial proliferation and possible cause illness following ingestion. The resistance of biofilms to chemical agents has made the development of new and more effective methods an important issue in food processing environments.

Good sanitation practices are currently used to control the

growth of *Listeria* in the food industry (Pearson and Marth, 1990). The efficacy of different sanitizers has been researched and it has been concluded that the sanitization of surfaces with chlorine, acid anionics, quaternary ammonium, and iodophors is effective against *Listeria*; however, treated areas need to be devoid of organic material or else the detergents will be neutralized (Pearson and Marth, 1990). For an effective system, sanitation needs to be conducted immediately after the cleaning process (Carpentier and Cerf, 1993). The exposure time, temperature, concentration, pH, soil content, water hardness, and the possibility of bacterial attachment need to be considered when devising an effective sanitation program (Curtis, 2006). Bacterial attachment is a large problem because once the bacteria attach to a surface, they can form biofilms, which are more resistant to sanitizers than planktonic cells (Sharma and Anand, 2002). The ability of *L. monocytogenes* to adhere to surfaces and form biofilms, regardless of temperature, makes it a very serious sanitation problem (Bremer *et al.*, 2002). Various sanitizers are known to inactivate planktonic *Listeria* spp., but their ability to inavtivate *Listeria* biofilms is a current topic of study.

The differential ability of *L. monocytogenes* to attach to the different surfaces used in the food industry, including plastic and stainless steel, has made it evident that surface selection is an important method to help control contamination, but it is not as effective as once believed. Proper surface selection can help to reduce the ability of microorganisms, such as *L. monocytogenes*, to form biofilms. Hypochlorite is more effective at inactivating biofilm cells than iodophors on either plastic or stainless steel. The combination of chlorine and iodophors completely inactivated biofilm cells. Therefore, stainless steel and the combination of the abovementioned sanitizers is the most effective protocol to reduce the formation of *L. monocytogenes* biofilms (Jeyasekaran and Karunasagar, 2000).

After studying the adherence abilities of *L. monocytogenes*, it was deduced that not only does *L. monocytogenes* adhere to surfaces, but that this adhesion can also lead to biofilm formation (Law *et al.*, 1985).

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