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Listeria monocytogenes Biofilms on Food Contact Surfaces Under Different Growth Conditions

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ABSTRACT

Listeria monocytogenes is an opportunistic intracellular pathogen that has become an important cause of foodborne infections worldwide. The organism possesses an ability to form biofilms on food processing surfaces, potentially leading to food product contamination. In the present study, stainless steel (SS) and cutting board fiber (FIB) coupons were used as the substratum for biofilm growth in the presence of sterile distilled water and Tryptone soya broth (TSB) as nutrient medium. The Listeria monocytogenes standard culture (MTCC 1143) belonging to serotype 4b at a concentration of 6 log 10 cfu/ ml was used for creating biofilms on these surfaces. The formation of biofilms was assessed after 24 hours of incubation at 370 C. The formation of biofilms on these surfaces after 24 hrs was observed by scanning electron microscopy (SEM) which revealed the presence of biofilms on both surfaces with more pronounced biofilm formation on fiber as compared to stainless steel in the presence of TSB as nutrient medium. The presence of irreversible biofilms with TSB as nutrient medium after washing the coupons was assessed which revealed the recovery of the organism at the level of 3.77 ± 0.13 log 10 cfu/cm2 and 4.91± 0.33 log 10 cfu/cm2 on SS and FIB surfaces respectively. The presence of sterile distilled water as nutrient medium also revealed the recovery of the organisms but were at the level of 1.82 ± 0.18 log 10 cfu/cm2 and 2.80 ± 0.13 log 10 cfu/cm2 on SS and FIB surfaces respectively. The formation of biofilms even in the presence of sterile distilled water is an area of great concern for the food industry. However the study revealed that the difference in the characteristics of surfaces revealed a difference in the ability of the organism to form biofilm as the biofilm was more pronounced on the fiber as compared to stainless steel. Hence, biofilm growth of L. monocytogenes was sufficient to provide a substantial risk of this pathogen contaminating the food-processing plant environment if wet surfaces are not maintained in a sanitary condition.

KEYWORDS : Listeria monocytogenes, Scanning electron microscopy, biofilms

Introduction

The contamination by *Listeria monocytogenes* is considered one of the major problems in the food industry, because this bacterium is widely distributed in nature, is able to survive under adverse environmental conditions . The organisms attaches to available surfaces in food processing environments and can develop into extensive biofilm. These biofilms consists of a complex consortium of microorganisms enmeshed within an extracellular matrix .The microorganisms in biofilms are usually more resistant to disinfection and sanitization procedures than planktonic cells. Once biofilms are formed over food contact surfaces, it persists there for many years and can act as a continuous source of contamination for foods.

In the present study, stainless steel (SS) and cutting board fiber (FIB) coupons were used as the substratum for biofilm growth in the presence of sterile distilled water and Tryptone soya broth (TSB) as nutrient medium.

Materials and methods:

The abiotic surfaces commonly used in the food industry i.e.,. Stainless steel and cutting board fiber was used to study the amount of biofilm formation on the surfaces under in vitro conditions. Stainless steel coupons of size 3 x 2 x 0.5 cm (l xb x h) and fiber coupons of size 3.7×1.8×0.5 cm was used for formation of biofilms. The coupons were first cleaned with acetone (99 per cent, HiMedia) and then washed using sterile distilled water. The coupons were then immersed in one per cent sodium hydroxide solution and vortexed (Cyclo- Mixer, Remi labworld, Maharashtra) for five minutes at maximum intensity. They were then rinsed with sterile distilled water and cleaned with 70 per cent alcohol and finally were washed with sterilized distilled water and autoclaved at 121 ^{0}C for 15 min. Before addition of inoculum, coupons were dried by keeping at 60 ^{0}C for 1 h.

The formation of biofilm on surfaces was studied at the incubation temperature of 37**°C** after a period of 24hrs. The nutrient medium used in the study was Tryptone soya broth (TSB) and sterile distilled water (DW). An initial inoculums of 10^6 organisms /ml of the standard culture of *Listeria monocytogenes (*MTCC 1143) was used in the study.

The method for attachment and biofilm formation was similar to that described by Vaid *et al.* (2010) with few modifications. One ml of TSB containing the bacterial culture (six $\log_{10}$ cfu/ml) was placed on each coupon kept in a sterile Petri dish. The culture was spread on the top surface of coupon by gentle rotatory motion. Petri dishes with inoculated coupons were incubated at 37 ^{0}C for 24h. After each incubation period, the coupons were aseptically transferred to a sterilized test tube and washed three times with physiological saline solution to remove loosely attached cells. These coupons were the used for enumeration of bacteria.

The enumeration of biofilm cells was according to the method as described by Jeyasekaran *et al.* (2000) with modifications The media used for enumeration of the organism was PALCAM agar (Hi-Media, Mumbai). The biofilm cell mass was determined and microbial counts were expressed as $\log_{10}$ cfu/cm^2 of coupon surface area. The data obtained was subjected to statistical analysis as per the procedure described by Snedecor and Cochran (1994) using statistical software SAS

base 9.2.

The presence of biofilms was further studied by subjecting the coupons to scanning electron microscopy (SEM) as per the procedure of Oliveira *et al* (2010) and was done at RUSKA laboratory, Hyderabad.

Results and Conclusions:
The formation of biofilms in the presence of TSB and distilled water as nutrient medium on the surface of stainless steel and cutting board fiber material is shown in Table-1.

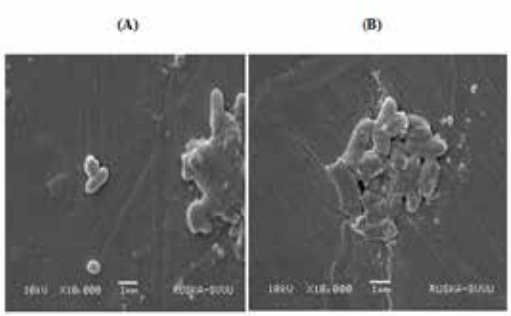
Table 1. Biofilm formation of *L monocytogenes* in presence of two nutrient mediums

S No.	Material	Mean bacterial count log_{10} cfu/cm²± SE	
		Sterile distilled water (SDW)	TSB
1	SS	1.82±0.18[b]	3.77±0.13[d]
2	Fibre	2.80±0.13[c]	4.91±0.33[d]

The presence of irreversible biofilms with TSB as nutrient medium after washing the coupons was assessed which revealed the recovery of the organism at the level of 3.77 ± 0.13 log 10 cfu/cm2 and 4.91± 0.33 log 10 cfu/cm2 on SS and FIB surfaces respectively. The presence of sterile distilled water as nutrient medium also revealed the recovery of the organisms but were at the level of 1.82 ± 0.18 log 10 cfu/cm2 and 2.80 ± 0.13 log 10 cfu/cm2 on SS and FIB surfaces respectively. Statistical analysis of data revealed no significant difference (P≤0.05) between the biofilm cells counts on both surfaces with TSB but with SDW, the FIB surface significantly higher count than SS.

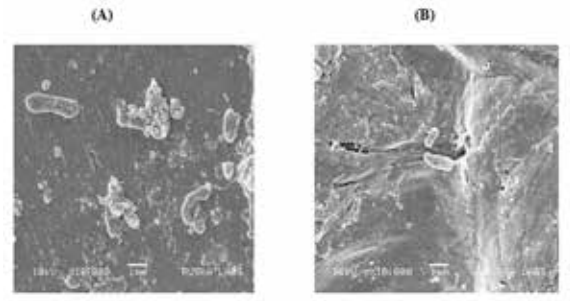
The scanning electron microscopy micrographs of formation of biofilms on stainless steel and fiber in the presence of TSB broth and SDW as nutrient medium are shown in Fig.-1 and Fig.-2. The picture clearly illustrates more attachment of the organism on fiber as compared to stainless steel surface.

Fig.-1 : SEM micrograph of the formation of biofilms on stainless steel surface



A) Biofilms in the presence of SDW
B) Biofilms in the presence of TSB

Fig- 2: Scanning electron microscope micrograph of the formation of biofilms on cutting board fiber surface



A) Biofilms in the presence of SDW
B) Biofilms in the presence of TSB

Hence, the standard strain of *L. monocytogenes* (MTCC 1143) was capable of forming biofilm on tested surfaces, thus suggesting a refined regulatory system enabling the organism to rapidly adapt to changing environmental conditions . The decrease in the number of biofilm cells with reduction in the nutrient content of the medium was in accordance with result of Mai and Conner (2007). The higher number of cells attached to FIB could be attributed to the surface roughness of this material and more hydrophobicity of the surface as compared to stainless steel surface. This result could be linked to the study of Characklis *et al*. (1990) who noted that the extent of microbial colonization appears to increase as the surface roughness increases. This is because shear forces are diminished, and surface area is higher on rougher surfaces. The SEM micrographs revealed the presence of embedded cells with less biofilm matrix on SS surface which was not in accordance with the study of Chavant *et al.* (2002) who observed formation of mats on SS and PTFE surfaces. However biofilm matrix with embedded cells could be seen on cutting boards surfaces. This could be attributed to the difference in strin of L monocytogenes and the type of stainless steel used in the study.

The formation of biofims by the organism in the presence of only sterile distilled water is an area of concern for the food industry as the organism can form biofilms if the cleaning and sanitization procedures are not properly implemented. The growth of biofilms in food processing environments leads to the increased opportunity for microbial contamination of the processed

product. This increases the risk of reduced shelf life and disease transmission. A better understanding of the mechanisms of biofilm development process on food contact materials will help in the eradication of the attached microflora.

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