

Formation of Salmonella Enteritidis Biofilm on Various Surfaces in Food Industries

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Abstract— Salmonella enteritidis is an important food infection pathogen bacteria. This bacteria is very strong in the environment and is still the major reason for many gastroenteritis infections in human. Salmonella enteritidis forms a biofilm on many surfaces in contact with foods. Formation of biofilm of bacteria on such surfaces is considered as a potential resource of contamination, which increases the possibility of transferring bacteria and breaking out of diseases. In the current study, biofilm formation of Iranian species of Salmonella enteritidis on various surfaces in contact with foods and medical materials was studied. Firstly, hydrophobicity of surface of Salmonella enteritidis cell (RITCC 1624) was determined by MATH (Microbial Adhesion to Hydrocarbon) method. Then, its biofilm was formed on various surfaces including steel (Type 304 no 2B), polyethylene and glass and was measured by drop plate method after 2, 4, 8, 16 and 20 hours. Results were shown that Salmonella enteritidis with hydrophobicity of 73% is able to form biofilm on the three above mentioned surfaces. The amount of biofilm formed during 2 hours on glass and steel surfaces were meaningfully ($P < 0.05$) higher than polyethylene surface and by increasing time to 20 hours, biofilm formation on various surfaces was not meaningful ($P > 0.05$). In addition, the highest amount of Salmonella biofilm on these three surfaces was formed in the interface of liquid and air phases. Formation of Salmonella biofilm on such surfaces increases the possibility of transferring the bacteria, which is very important from hygienic, and disease spreading points of view.

Index Terms— Biofilm, Salmonella Enteritidis, Hydrophobicity, Various Surfaces, Food Industries, Pathogen Bacteria

1 INTRODUCTION

Salmonella enteritidis often transferred to human through contaminated foods and leads to disease [1-17]. Annually, 1.4 million people suffered from non-typhoidal salmonellas in United States, which more than 95% of these cases are happened due to contaminated foods. 30% of these food infections lead to death. Various investigation were shown that this bacteria is of high ability to bond and form biofilm on various surfaces [18, 19].

The biofilm consists of microbial cell clusters that are connected with a network of internal channels in the glycoprotein matrix and extracellular polysaccharide, called the extracellular polymeric substance [20]. In food industries, bonding of pathogen and infectious bacteria with surfaces in contact with food in its production and packing processes and finally, formation of microbial biofilms, can be a potential resource of contamination in food products and spreading diseases [21-37].

Bacteria growing as biofilm on surfaces make easy its transferring and make hard its removing. It is because biofilm cells are more resistant against biocides and disinfectants than free cells. Since there is not enough information about the relationship between formation of Salmonella biofilm on surfaces in contact with food or medical products in Iran, the aim of the current study is investigating the biofilm formation of Salmo-

nella enteritidis (RITCC 1624) with food origin on various surfaces [38-49]. Surfaces studied in this research are commonly used in food industries and medicine and studying the process of adhesion of Salmonella on these surfaces are useful in control of infection.

2 MEASURING HYDROPHOBICITY OF CELL SURFACES

In the test of bonding of bacteria and octane hydrocarbon [50-55], active culture of Salmonella enteritidis (RITCC 1624) was centrifuged to collect bacteria cells (3000 rounds per 15 min). Then, Phosphate buffer was added to precipitated cells. The turbidity of microbial suspension was set to 0.5 McFarland standards. At the next step, its initial optical absorption was read at 640 nanometers. Then, 0.5 ml of octane hydrocarbon was added to cuvette and was mixed for 2 minutes using vortex apparatus at medium level. After separation of aqueous and organic phases, optical absorption of aqueous phase (secondary) was read at 640 nanometers.

3 BIOFILM FORMATION ON SURFACES

Firstly, bacteria was set to turbidity equal to 0.5 McFarland standards and 1 ml of it was added to an Erlenmeyer flask containing 50 ml environment of trypticase soy broth and 2 glass slides that were cleaned and disinfected by ethanol 96%. Then, Erlenmeyer flasks were set on the shaker, at 100 rounds, at the temperature room for 2, 4, 8, 16 and 20 hours. The biofilm formation stages on steel and polyethylene surfaces also were similar to this [56-67].

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4 SURFACE STERILIZATION

To sterilize steel surfaces (Type 304 no 2B); steel coupons were located into the acetone so that any grease or oil spot cleaned from those. Then, those were floated in an alkaline lotion and were washed with deaired water and were autoclaved [68-79]. Polyethylene surfaces were floated in alkaline lotion and were subjected to UV ray for half an hour. Glass slides also were disinfected with ethanol 96% and then were autoclaved.

5 COUNTING BIOFILM BACTERIA

To count bacteria the drop plate method was used [80-100]. In this manner, dilution series were prepared from microbial suspension. To prepare this suspension, the swab dragged on the surface where bacteria biofilm has been formed vortex in deaired water. Then, 10 microliters of suspension (with specified dilution) were taken and were distributed in one part of four parts of nutrient agar plates. These were performed five times for a specified dilution. Plates were incubated for 24 hours at 35° C and then, only that dilution where its number of colonies was between 3 and 30 colony per drop was selected and counted.

6 RESULTS AND DISCUSSION

The hydrophobicity of Salmonella enteritidis was measured as 73% using MATH test. Biofilm formation tests were shown that this bacteria formed biofilm on all three surfaces. However, its amount on glass and then stainless steel and polyethylene were higher with a meaningful difference ($P < 0.05$). The Salmonella biofilm formation on all three surfaces at first hours (4 and 8 hours) was very low due to its slow rate of growth but at 16 and 20 hours, it was higher with increasing the rate of growth.

The amount of Salmonella enteritidis biofilm formation at different times on glass, steel and polyethylene surfaces had not meaningful difference. Bonding of Salmonella enteritidis in short time (2 hours) with all three surfaces were meaningful but at longer times of 4, 8, 16 and 20 hours, there was not a meaningful difference. The highest amount of Salmonella biofilm on these three surfaces was formed in the interface of liquid and air phases.

The considered bacteria was formed meaningfully more biofilm on stainless steel surface than plastic surface ($P < 0.05$). This result is important since most of surfaces, which are in contact with food in processing apparatuses, are made from stainless steel. In the current study, bonding of Salmonella enteritidis with steel surfaces after 2 hours were meaningfully higher than polyethylene. Biochemists based on investigation about the Salmonella and Listeria was shown that in addition to surficial hydrophobicity of bacteria, there are other factors which have playing a role in bonding of bacteria. They were observed that Salmonella with higher hydrophobicity than Listeria is of lower ability in forming biofilm on surfaces. In the current study, very high hydrophobicity of these bacteria (73%) is one of the reasons why these Iranian species have high tendency to form biofilm on surfaces. In the current research, the studied species of Salmonella enteritidis with hydrophobicity of 73% had more power to form biofilm on glass

surface than steel and polyethylene and since glass and steel surfaces are more hydrophilic than polyethylene surfaces, it can be found that the role of cohesion induced by hydrophobicity of bacteria surface and hydrophilic surface on biofilm formation is very important. In addition, high hydrophobicity between bacteria surface and glass surface prevents bonding of bacteria and glass surface, due to formation of repulsive force, and finally, biofilm formation on this surface. Hence, regarding the results of the current study, effective factors of bonding and formation of bacteria biofilm are not same so that high hydrophobicity is necessary to form biofilm but it is not enough.

Giaouris were shown that growing of bacteria on surfaces in interface of liquid - air phase is more than floated bacteria in liquid phase and biofilm formation and bonding in liquid-air phase is higher. In the current study, also, the highest amount of biofilm formation on various surfaces was formed in this phase. It may be because the considered bacteria are aerobic, food is available and conditions are ideal in this phase to form biofilm. Regarding these results, if Salmonella is presented in environments that are in contact with foods or remedial centers, biofilm formation is inevitable.

The results of the current study were shown that Salmonella enteritidis pathogen bacteria is able to form biofilm on surfaces which are abundantly used in food environments. It is necessary to use an accurate and practical protocol for washing and disinfecting in these centers in addition to continuous monitoring of biofilm formation.

7 CONCLUSION

Formation of Salmonella biofilm on such surfaces increases the possibility of transferring the bacteria, which is very important from hygienic, and disease spreading points of view.

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