

QUANTIFICATION OF BIOFILM PRODUCTION ON POLYSTYRENE BY *LISTERIA*, *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* ISOLATED FROM A POULTRY SLAUGHTERHOUSE

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ABSTRACT

This study assessed biofilm formation on polystyrene by *Staphylococcus aureus*, *Listeria monocytogenes*, *L. welshimeri* and *Escherichia coli*, isolated from a slaughtering plant, grown on tryptic soy broth (TSB) using different glucose concentrations. The tested bacteria produced biofilm in at least one of the concentrations used, and some of them were strong biofilm producers.

Key words: *Listeria*, *Escherichia coli*, *Staphylococcus aureus*, biofilm, polystyrene

INTRODUCTION

A biofilm is a population of microbial cells growing on a surface and enclosed in an amorphous extracellular matrix. Biofilm formed in food processing environments is of special importance as it has the potential to act as a chronic source of microbial contamination that may lead to food spoilage or transmission of diseases, and bacteria in biofilms exhibit enhanced resistance to cleaning and sanitation (12).

Formation and presence of biofilm have been investigated by different quantification methods, among which the microtiter plate system is widely used (1, 12, 14). Microtiter plate systems for quantifying biofilm formation have been investigated using many different organisms and stains (1, 2, 13).

Therefore, this study assessed biofilm formation on polystyrene plates by *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *L. welshimeri* isolated from the

cutting room of a slaughtering plant and grown on tryptic soy broth (TSB) using different glucose concentrations.

This study was conducted in the cutting room of a poultry slaughterhouse in southern Brazil with capacity for 20,000 animals per hour. Samples were collected at the end of the cutting phase, during preoperational cleaning: before the surfaces were washed; after washing with hot water and after washing with 2% sodium hydroxide detergent (Power Foam[®], Johnson Diversey), rinsing and disinfection with 0.5% peracetic acid (Divosan Forte[®], Johnson Diversey), 2% quaternary ammonium (Divosan Divoquat Forte[®], Johnson Diversey), and 1% biguanide (Divosan Divosept 350[®], Johnson Diversey). The following methods were used: Evancho *et al.* (3), Hitchins (6), and Ryser and Donnelly (11) for investigation of *Listeria sp.*; Evancho *et al.* (3) and Lancette and Bennett (8) for counting *Staphylococcus aureus*, and Evancho *et al.* (3), Swanson *et al.* (15) and Kornacki and Johnson (7) for *Escherichia coli*.

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The method for assessment of biofilm formation on polystyrene microtiter plates was based on the techniques described by Stepanovic *et al.* (13, 14), adapted for the analysis of *Listeria sp* (6 strains), *Escherichia coli* (9 strains) and *Staphylococcus aureus* (11 strains). The standard strains used were *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The strains were grown on tryptic soy agar (TSA) without dextrose (Difco) using 1.5% of agar, without glucose (0%), and on TSA supplemented with 0.5; 1; 1.5; 2; 2.5; 3; 3.5 and 4% of glucose, incubated at 36°C for 24 hours and transferred to tryptic soy broth (TSB) without dextrose (Difco), with 0.5% of sodium chloride, using the same glucose concentrations and incubation at 36°C for 24 hours. Thereafter, aliquots of the cultures were added to the TSB using the same glucose concentration until MacFarland scale 1 was obtained.

Later, 200 µL of each bacterial suspension were inoculated, in triplicate, onto 96-well, flat-bottomed sterile polystyrene microtiter plates (Cral). Non-inoculated TSB wells, in triplicate, at each glucose concentration, were used as negative controls. The plates were incubated at 36°C for 24

hours. The bacterial suspension was aspirated and each well was washed three times with 250 µL of sterile physiological saline at 0.9%. After that, the biofilm was fixed with 200 µL of methanol for 15 minutes, and later removed. The plates were dried at ambient temperature, stained with 200 µL of Hucker's crystal violet solution at 2% for 5 minutes, washed in running water and dried at ambient temperature. Afterwards, absorbance was read using an ELISA plate reader (Rosys Anthos 2010) at 550 nm.

The optical density (OD_s) of each strain was obtained by the arithmetic mean of the absorbance of three wells and this value was compared with the mean absorbance of negative controls (OD_{nc}). The following classification was used for the determination of biofilm formation: no biofilm production ($OD_s \leq OD_{nc}$), weak biofilm production ($OD_{nc} < OD_s \leq 2 \cdot OD_{nc}$), moderate biofilm production ($2 \cdot OD_{nc} < OD_s \leq 4 \cdot OD_{nc}$) and strong biofilm production ($4 \cdot OD_{nc} < OD_s$).

All *Staphylococcus aureus* strains analyzed produced biofilm on polystyrene at least at one of the concentrations used (Table 1).

Table 1. Results for biofilm production on microtiter plates for *Staphylococcus aureus*, *Listeria* and *Escherichia coli* in TSB at different glucose concentrations.

Tested media	No biofilm producers			Weak biofilm producers			Moderate biofilm producers			Strong biofilm producers		
	<i>S.a.</i>	<i>L.</i>	<i>E.c.</i>	<i>S.a.</i>	<i>L.</i>	<i>E.c.</i>	<i>S.a.</i>	<i>L.</i>	<i>E.c.</i>	<i>S.a.</i>	<i>L.</i>	<i>E.c.</i>
TSB w/o G	4	1	0	8	3	3	0	3	4	0	0	3
TSB + 0.5% G	11	4	0	1	1	2	0	2	8	0	0	0
TSB + 1% G	11	1	1	1	5	2	0	1	7	0	0	0
TSB + 1.5% G	1	3	0	11	4	7	0	0	3	0	0	0
TSB + 2% G	3	2	0	7	1	3	1	4	7	1	0	0
TSB + 2.5% G	5	0	0	7	3	2	0	4	8	0	0	0
TSB + 3% G	8	1	0	3	4	5	0	2	5	1	0	0
TSB + 3.5% G	8	0	2	1	2	1	0	4	6	3	1	1
TSB + 4% G	2	0	4	4	3	0	1	4	5	5	0	1

TSB: - Tryptic Soy Broth without dextrose – Difco; G: glucose.
S.a.: *Staphylococcus aureus*; *L.*: *Listeria*; *E.c.*: *Escherichia coli*

By using the polystyrene microtiter plate method, it was possible to find out that all strains of *Listeria*, *Staphylococcus aureus* and *Escherichia coli* had the capacity to produce biofilm, even if they are weak producers, in at least one of the growth media tested.

Although hygiene conditions for food production have been continuously improved in food industries, outbreaks of listeriosis caused by the consumption of contaminated products may occur, and therefore, providing an effective control of *Listeria* spp. has been a challenge (5). The strains of *L. monocytogenes* and *L. welshimeri* analyzed were obtained from stainless steel tables and from polyurethane conveyors, and showed weak and moderate biofilm formation in TSB with different glucose concentrations, but especially in media with 2 to 4% of glucose. An *L. monocytogenes* strain isolated from the polyurethane conveyor was a strong biofilm producer in TSB with 3.5% of glucose. These results were consistent with those obtained by Stepanovic *et al.* (12), who concluded that *L. monocytogenes* produces more biofilm in nutrient-rich media.

The data obtained in this study are very important because they show that *Listeria*, *Staphylococcus aureus* and *Escherichia coli* isolated from surfaces in contact with foods in the cutting room of a poultry slaughterhouse can produce biofilm, which may cause the persistence of these microorganisms during food processing and consequently lead to greater risks of food contamination, jeopardizing consumers' health. Further studies are still needed to determine on which surfaces and under which environmental conditions these microorganisms produce more biofilm.

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