THE EFFECT OF TEMPERATURE ON THE ABILITY TO FORM BIOFILMS BY THE FOOD PATHOGEN *L.MONOCYTOGENES*

Yulia K. Yushina, Elena V. Zaiko*, Anzhelika A. Makhova, Dagmara S. Bataeva

[1] V. M. Gorbatov Federal Research Center for Food Systems, Moscow, Russia

*Corresponding author email: e.zaiko@fncps.ru

I. INTRODUCTION

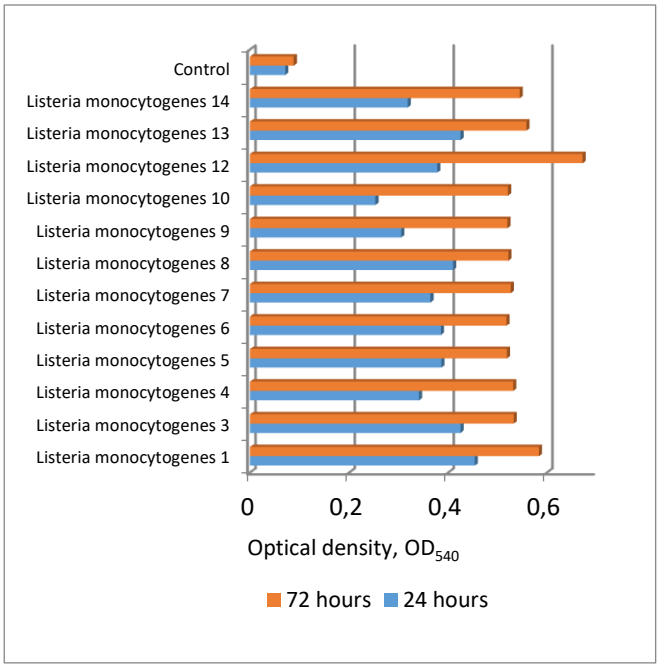
L. monocytogenes is capable of forming biofilms, which allows it to attach to the surfaces of equipment in food processing plants made of stainless steel, high-density polyethylene and glass [1]. Biofilm formation increases the resistance of *L. monocytogenes* to adverse conditions, including disinfectants used in enterprises, which makes it difficult to completely destroy [2]. Most studies focus on the formation of biofilms by pathogens at temperatures optimal for their growth, which are not typical for food enterprises. The purpose of this work was to evaluate the ability of Listeria monocytogenes to form biofilms at various temperatures, including those typical of food processing environments.

II. MATERIALS AND METHODS

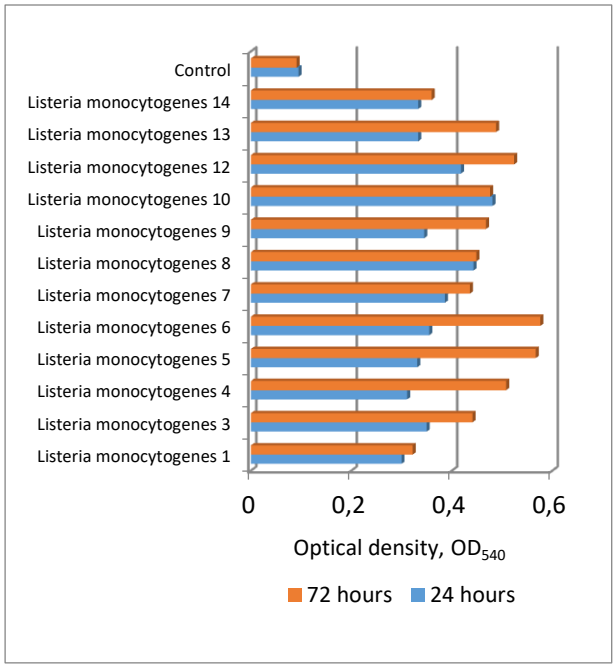
Twelve strains of *Listeria monocytogenes* isolated from various objects of the production environment of meat processing and poultry processing enterprises were selected as research objects. All strains were tested for their ability to form biofilms in monoculture on polystyrene surfaces for 72 hours at 37 °C, the optimal growth temperature of the studied microorganisms, and at 4 °C, the low positive temperature characteristic of premises in food enterprises. The ability to form biofilms was studied in vitro in microtiter plates. A nighttime broth culture of bacteria was diluted 1:100 in LB broth (Becton Dickinson, USA) and 150 µl were added to the wells of a 96-well flat-bottomed polystyrene tablet (Corning, USA). After incubation, the planktonic cells were removed and the biofilms were stained with a solution of crystalline violet (Servicebio, China) with an exposure of 1 hour, followed by the addition of 96% ethanol to extract the dye bound to the biofilms. The optical density of the dye extracted with alcohol was measured on a photometer at a wavelength of 540 nm. Wells filled with sterile broth served as a control. The excess of the optical density of the crystalline violet over the control indicated the formation of biofilms by bacteria. The ability of strains to form biofilms (respectively, and strains as producers of biofilms) was classified using the following scale: no biofilm formation (OD540 = 0) → very weak (0 < OD540 < 0.2) → weak (0.2 < OD540 < 0.4) → strong (0.4 < OD540 < 1.0) → very strong (OD540 > 1.0).

III. RESULTS AND DISCUSSION

After 24 hours, *Listeria monocytogenes* formed persistent biofilms on the polystyrene surface. The ability to form biofilms varied among the strains, with temperature and time being of great importance. It is believed that a low positive temperature (+4°C) is a deterrent to the growth of microorganisms, however, the data obtained indicate that there is no negative impact on their ability to form biofilms. After 24 hours of incubation, 58% (7/12) of the ability to form biofilms at 37 ° C and 4 ° C differed by no more than 0.04 units. optical density at OD_{540} (Figure 1), which indicates the insignificance of the influence of low positive temperature as a deterrent factor for biofilm formation. At the same time, in three strains of *Listeria monocytogenes* 10, *Listeria monocytogenes* 11 and *Listeria monocytogenes* 15, the rate of biofilm formation was higher at 4 °C than at 37 °C during the first 24 hours.



a) 37 °C



b) 4 °C

The intensity of formation of Listeria biofilms at different temperatures

At 37 ° C, an increase in incubation time to 72 hours contributed to the formation of a denser biofilm compared to 24 hours. At 4 °C for 72 hours, the formation of biofilms by *L. monocytogenes* microorganisms occurred less intensively, however, the positive dependence of the intensity of biofilm formation on an increase in incubation time remained in most strains (9/12). In three strains (*Listeria monocytogenes* 10, *Listeria monocytogenes* 8, *Listeria monocytogenes* 1), the duration of incubation did not affect the intensity of biofilm formation. The data obtained indicate a high intraspecific heterogeneity of strains in their ability to form biofilms under the same conditions. Although some authors reported a correlation between lineage and film forming ability [3], other results did not support these findings [4], which is also illustrated in our study.

IV. CONCLUSION

As a result of the conducted studies, the ability to form biofilms of pathogenic L. monocytogenes was shown at both 37 °C and 4 ° C. The low positive temperature (4 °C) was not a limiting factor in the ability to form biofilms. In addition, intraspecific features of the strains were noted for their ability to form biofilms under the same conditions. The results highlight the critical importance of implementing effective biofilm control strategies to ensure food safety.

REFERENCES

1. Doijad, S.P.; Barbuddhe, S.B.; Garg, S.; Poharkar, K.V.; Kalorey, D.R.; Kurkure, N.V.; Rawool, D.B.; Chakraborty, T. (2015) Biofilm-Forming Abilities of Listeria monocytogenes Serotypes Isolated from Different Sources. PLoS ONE 10: e0137046.
2. Rodríguez-López, P.; Saá-Ibusquiza, P.; Mosquera-Fernández, M.; López-Cabo, M. Listeria monocytogenes-carrying consortia in food industry. (2015) Composition, subtyping and numerical characterisation of mono-species biofilm dynamics on stainless steel. Int. J. Food Microbiol. 206: 84–95.
3. Borucki, M.K.; Peppin, J.D.; White, D.; Loge, F.; Call, D.R. (2003) Variation in biofilm formation among strains of *Listeria monocytogenes*. Appl. Environ. Microbiol. 69:7336–7342.
4. Di Bonaventura, G.; Piccolomini, R.; Paludi, D.; D'Orio, V.; Vergara, A.; Conter, M.; Ianieri, A. (2008) Influence of temperature on biofilm formation by *Listeria monocytogenes* on various food-contact surfaces: Relationship with motility and cell surface hydrophobicity. J. Appl. Microbiol. 104:1552–1561.