

STUDY OF THE EFFECT OF NATURAL FACTORS ON THE PRODUCTION AND INHIBITION OF BIOFILMS OF *STAPHYLOCOCCUS AUREUS*

Amina Taha Hussein AL-Salim¹, Tariq Zaid Ibrahim¹

¹Food Science Department, College of Agriculture, University of Mosul,
University street 94GW+J9, 41002 Mosul, Iraq

*e-mail: amina.alsalim1990@gmail.com

Abstract

The importance of the study lies in the possibility of *Staphylococcus aureus* bacteria causing harm to humans through their ability to form biofilms on surfaces and thus their ability to contaminate food processing places and thus contaminate food and produce toxins. Therefore, the study aimed to test the ability of some local *S. aureus* to form biofilms and to know the optimal conditions for it for the production of biofilms.

In this study, use two isolates from *S. aureus* bacteria were obtained from the laboratory of the College of Science, Department of Life Sciences at University of Mosul. We used two mediums: tryptex broth and soybeans. Their acid number was adjusted using HCl and NaOH 0.1N, using the physiological solution added to its ethylene diamine tetraacetic acid (EDTA) rate of 0.2%, to scrape and draw the biofilms that have formed. The isolates used in the study were activated using tubes containing 5 mL of tryptex broth and soybeans and incubated at 37 °C for 24 hours, after which the diagnosis was confirmed using the staphylococcus system analytical profile index (API) 20. The number and calculation of living cells growing in 1mL of pollinated medium were identified for two isolates of *S. aureus* that were returned to inoculate biofilm production using the droplet counting method. Several cells were then taken to study the production of *S. aureus* biofilms using tissue culture plates manufactured by Nuclon (USA). Then the effect of incubation temperature on forming biofilms of *S. aureus* was studied using the droplet counting method and at three temperatures of 8, 25 and 37 °C for 24 and 48 hours. The effect of different pH levels 3, 4, 5, 6, 7, 8 and 9 on forming *S. aureus* biofilms at 37 °C was also studied for 24 hours. Finally, the effect of the medium type on forming the biofilms of *S. aureus* bacterium isolated was grown on a medium consisting of tryptex-soya broth + NaCl in concentrations 0.5 -8 %.

The study result showed that the isolates belong to *S. aureus*, as was indicated from the results of the phenotypic investigation. Its diagnosis was confirmed by the dependence on the forms of colonies in the nutrient medium, microscopic examination after dyeing them with the Gram stain differential, and some biochemical tests that adopted the API (Analytical Profile Index) 20E test for the initial diagnosis of bacteria, which gave accurate results on the shape and composition of biofilms. While the results of the susceptibility of *S. aureus* bacteria to the production of biofilms after 24 and 48 hours of incubation at 37 °C, where it was observed that the two tested isolates showed susceptibility to the production of biofilms at 24 hours 1×10^6 , 3×10^6 cfu, while at 48 hours it was 3×10^6 and 4×10^6 cfu for each of the 1 and 2 isolation respectively. While the results of the effect of incubation temperature on the logarithm showed the number of living cells in the formation of biofilms, the best temperature for the formation of biofilms was 25 °C, the logarithm of the number of living cells of biofilms after 48 hours of incubation in the first and second isolates was to 8.69 and 8.60 cfu, respectively, upon completion of the 48-hour incubation, the growth rate of the living cells of isolates 1 and 2 increased, with the logarithm of the number of living cells of the biofilms reaching 8.87 and 8.77 cfu, respectively. The results of the pH effect in the production of biofilms at a temperature of 37 °C for 24 hours of incubation showed a clear effect on the formation of biofilms, where the optimal pH was 7, where the logarithm of the number of living cells of the biofilms of the first and second isolates was 8.39 and 7.47 cfu, respectively, the inhibition of biofilm formation was observed at pH 3, as the logarithm of the number of live cells of biofilms in the isolates 1 and 2 reached 5.17 cfu, for both. The test of the effect of sodium chloride showed high resistance to bacteria in the formation of biofilms; the form continued even at a concentration of 8% sodium chloride, while at a concentration of 3% sodium chloride, it gave the highest growth.

This study concludes that the isolates of *S. aureus* can stick and form biofilms on various surfaces, which is evidence of its ability to contaminate food processing places. The optimum temperature for the growth of the biofilms was 25 and 37 °C. In contrast, at a temperature of 8 °C, it led to a reduction in the growth rate of the biofilms for 24 hours of incubation in the two isolates, and the optimum pH for the rate of biofilm formation for all *S. aureus* is pH 6 and pH 7. The bacteria also produced biofilms in high salinity media.

Key words: *Biofilms, Staphylococcus aureus, pH, Tryptex broth, Soya Broth.*