Investigation of the effect of different temperature and acidic conditions on biofilm structure of *Salmonella enterica* serotype Virchow by FTIR spectroscopy

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Introduction

Salmonella is one of the most common and widely distributed bacterial pathogens worldwide and the creative factor of salmonellosis It is a major public health problem, and every year millions of human cases are reported worldwide. The ability of Salmonella to form biofilms on biotic and abitic surface causes a major problem and biofilm cells are more resistant to cleaning and disinfection processes (1,2 and 4).In this study we aimed to determine the effect of environmental stress conditions (temperature and pH) on biofilm structure of Salmonella enterica serotype Virchow originated from Turkey.

Materials and Methods

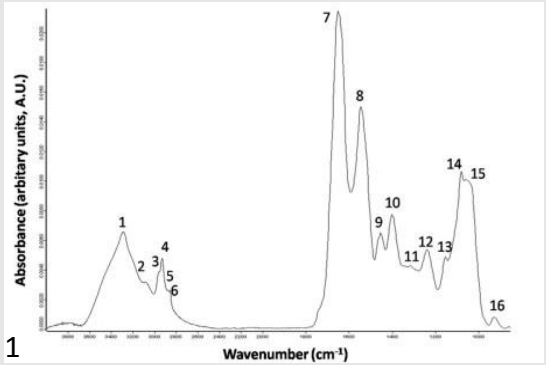
In this study, the influence of environmental factors including temperature (20 °C, 25 °C, 30 °C) and pH (5.2, 5.9, 6.6) were determined on biofilm formation by *S. enterica* serotype Virchow which are isolated from food samples. To observe the pellicle formation of the isolates, Luria Bertani (LB) without salt (Wo/NaCl) was used and the strains were incubated at different temperatures for 10 days (3).

Afterwards, pellicles were analyzed visually and by Fourier transform infrared (FTIR) spectroscopy using attenuated total reflectance (ATR) method. For FTIR analyses, after 10-day-incubation, pellicles were transferred to micro-centrifuge tubes under sterile conditions and then all pellicles were lyophilized by freeze-drying. FTIR spectra were recorded in the mid-IR region using Tensor 27 FTIR (Bruker) and spectral analyses were performed using OPUS 5.5 software (Bruker). Statistical significance was tested by ANOVA.

Results

After incubation at 20, 25 and 30°C temperature, biofilm formation in standing LB broth was visualized after 10 days as a floating pellicle at the air-broth interface that totally blocked the surface of the culture. Pellicles which were prepared at 20 and 25°C were not be dispersed by shaking while they easily disrupted at 30°C. We observed 16 peaks corresponding different biomolecules such as protein, lipid, carbohydrate and nucleic acid in FTIR spectra of samples (Figure 1).

According to our results, the amount of carbohydrate, protein, lipid and nucleic acid was significantly decreased with increasing temperature (especially between 20 and 30 °C) (Figure 2). We did not observe considerable alterations related to pH changes within temperature groups. Our hierarchical cluster analysis supported these results and separated groups according to the temperature (results not given).



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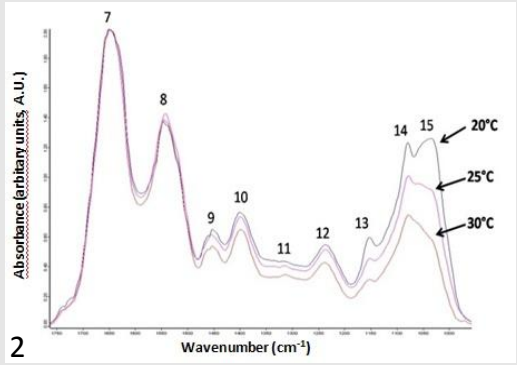

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Conclusions

We observed the most significant alteration in peak 15, decreasing as the temperature increases. In our samples, we assigned this carbohydrate peak at 1046 cm^{-1} as cellulose, which is one of the main components of biofilm structure. Taking into consideration our morphological and molecular results, we concluded that cellulose has an important role in biofilm formation and its stability. Additionally, decrease in protein level may be associated with curli fimbria protein. Alterations in lipids may reflect changes in membrane structure of bacterial cells during biofilm formation. Also our univariate and multivariate analyses showed that temperature has more significant effect than pH on biofilm formation and stability. Our results show that ATR-FTIR spectroscopy can be used as an effectively for rapid monitoring of molecular alterations during the biofilm formation.