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Dual-species biofilms of *Escherichia coli* and *Pseudomonas* promote temperature-dependent cross-contamination on meat surfaces

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ABSTRACT

Temperature and contact surfaces are critical factors influencing biofilm formation of microbial communities derived from both lab and natural environments. However, there is limited understanding regarding how different temperatures impact transfer dynamics of individual microbial species between meat surfaces in dual-species biofilms. In this study, we evaluated the biofilm-forming capabilities of various meat-derived *Escherichia coli* and *Pseudomonas* strains on pork surfaces at 15 and 25 ℃. The results revealed that lower temperature had a significant negative effect on the growth fitness of *E. coli* compared to *Pseudomonas*. Two robust biofilm-forming strains, *E. coli* C-13 and *P. fluorescens* $S_{1\text{-}2}$ 3, were selected under both temperature conditions to further explore their interactions in dual-species biofilms on meat surfaces. The results showed that *E. coli* exhibited a competitive growth advantage at 25 ℃, while *Pseudomonas* displayed enhanced growth at 15 ℃, indicating temperature-dependent competition patterns. Additionally, three established mathematical models were utilized to simulate the transfer dynamics of the two strains within mono- and dual-species biofilms. As anticipated, the numbers of transferred cells progressively declined with increased imprinting reactions between the meat surfaces. Interestingly, the transfer rates of both strains markedly improved in dual-species *vs.* mono-species biofilms at 15 ℃, highlighting the influential role of inter-species interactions on transfer dynamics of microbes that may cross-contaminate meats. Our findings advance current understanding of mono- and dual-species biofilm development on meat surfaces under different temperature conditions. They also offer scientific evidence to support strategies for controlling microbial cross-contamination during meat processing to ensure product safety.

1. Introduction

Bacteria possess a remarkable ability to adhere to surfaces and establish spatially-organized communities known as biofilms, which are encased within a self-produced matrix composed of extracellular polymeric substances, extracellular DNA and proteins[1]. Biofilms play a crucial role in the bacterial ecosystem, providing microorganisms with protection and stability through the formation of intricate, three-dimensional, and multicellular structures[2]. Biofilms formed in natural environments typically consist of multiple species, contributing to the structural complexity of these communities and fostering diverse inter-species interactions. These microbial interactions are pivotal in shaping the species composition, activity, and functionality of the microbial communities[3-5]. Furthermore, studies have indicated that mixed-species biofilms exhibited enhanced adaptability to various environments and have a greater potential to cause persistent contamination in industrial settings[6-8].

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In recent years, researchers have discovered numerous factors that can influence the formation of biofilms, including bacterial species[9-10], strain types[11-12], culture substrates, and contact carriers[13-14]. These biofilms, formed by food-spoilage microorganisms, can adhere to both abiotic surfaces (such as stainless steel, plastic, and glass) and the surfaces of living organisms[15-16]. While the formation of biofilms on abiotic surfaces has been extensively studied and reported, there has been limited research on the formation of mixed-species biofilms on biological contact surfaces[12,16].

In the context of pork production and processing, microorganisms have the ability to adhere to the surfaces of carcasses and meat pieces during various operations, including cutting and mixing. These biofilms on biotic surfaces are considered to be the key factor contributing to food contamination[17-18]. Therefore, investigating the formation of mixed-species biofilms on meat surfaces, particularly from meat isolates, holds significant practical importance for effectively controlling microbial contamination in meat production.

Cross-contamination during meat processing and production is a significant concern, with biofilms formed during these operations emerging as a key source of contamination[19]. The transfer of biofilms between surfaces is influenced by factors such as the type of contact surface[20-21], bacterial species involved[22-23], and contact time. Various studies have investigated the transfer of biofilms from abiotic surfaces to food, revealing notable differences in transfer rates across different contact surfaces like stainless steel[24], polyethylene[25], and glass[14,20]. Additionally, contact time and pressure have been identified as crucial factors impacting biofilm transfer. Researchers have developed multiple models to predict bacterial transfer patterns between biological and abiotic surfaces[26-27]. For example, Sheen[28] simulated the transfer of *Listeria monocytogenes* biofilms from slicer's round blade to meat and established an exponential model to describe surface cross-contamination during slicing operations. Wang et al.[29] found that a logistic model effectively described the transfer of *Escherichia coli* biofilms from glass coupons to Hami melon after continuous contact. They also reported that exponential models and multiple power models yielded relatively large prediction errors, whereas the logistic model provided a better simulation of the entire transfer process, as assessed by root mean square error (RMSE) and R^2. However, no transfer models have been reported for predicting the transfer of dual-biofilms from meat to meat. Developing mathematical models to study the transfer dynamics of persistent mixed-species biofilms on meat surfaces holds crucial practical significance for risk assessment of cross-contamination, enabling a better reflection of real-world scenarios.

In this study, *E. coli* and *Pseudomonas* strains isolated from meat and slaughtering environments were used to evaluate their biofilm formation abilities on the meat surface. Representative strains were further selected to examine dual-species biofilm development. Mathematical models were also utilized to simulate the transfer dynamics of mono- and dual-biofilms from meat surface to another. Results revealed substantial variations in biofilm-forming capabilities among the tested strains, with temperature exerting significant impacts. Further investigation showed temperature also affected transfer dynamics of mono- and mixed-biofilms.

2. Materials and methods

2.1 Bacterial strains and growth media

Eight strains of *E. coli* were isolated from fresh pork and the slaughtering line, while eight strains of *Pseudomonas* were isolated from spoiled pork. The isolation and taxonomic identification of these strains were conducted in our laboratory following previously described methods[30] with some modifications. To maintain viability, all strains were stored at −80 ℃ in tryptone soy broth (TSB, Beijing Land Bridge Technology Co., Ltd., Beijing, China) supplemented with 25% (*V/V*) sterilized glycerol. Prior to further experiments, each strain was sub-cultured twice in TSB to optimize growth. Subsequently, a specific volume of bacterial suspensions was centrifuged at 8 000 × *g* for 5 min and then subjected to three washes with sterile saline. The cell density of each strain was adjusted to approximately 10^8 CFU/mL, and confirmed by plating on tryptic soy agar (TSA, Beijing Land bridge Technology Co., Ltd., China).

2.2 Preparation of pork samples

Chilled pork tenderloin samples were obtained from a local retail store in order to minimize the potential variability arising from different cuts of meat. The pork samples were precisely cut into pieces (5 cm × 2 cm × 2 cm) and immediately placed into sterile plastic bags for packaging, and frozen at −20 ℃ for 24 h. To ensure complete eradication of any potential microorganism contamination, the frozen samples were treated with a dosage of 9 kGy of γ-ray irradiation. Following irradiation, the samples were once again frozen at −20 ℃ until further use. Three meat samples were randomly selected and allowed to thaw at 4 ℃ for 12 h. After thawing, these samples were subjected to sterility verification by culturing them on TSA plates. Before conducting the biofilm assays, the meat samples were removed from the freezer, thawed at 4 ℃ for 12 h, and then carefully placed, with the surface (5 cm × 2 cm) facing upwards, in sterilized glass boxes. This ensured a controlled and sterile environment for subsequent inoculation and biofilm formation studies.

2.3 Evaluation of colonizing processes

Three strains of *E. coli* and three strains of *Pseudomonas* were randomly selected and mixed in equal volumes, respectively. The resulting 2 mixed inoculum was then diluted with sterile saline to obtain a concentration of 10^6 CFU/mL, respectively. A volume of 100 μL of each inoculum was spot-inoculated at ten different locations on the surface of each pork sample, covering an area of 5 cm × 2 cm. Consequently, the final inoculation density amounted to 4.0 (lg (CFU/cm^2)). After inoculation, each inoculated pork sample was individually sealed in a glass box using parafilm, a laboratory-grade sealing film composed of a blend of waxes and polyolefins, in order to prevent cross-contamination between samples. The sealed glass boxes were then incubated at two distinct temperatures 15 and 25 ℃. To assess the biofilm formation conditions on meat surfaces, pork samples were taken from the glass boxes at predetermined time points: 6, 12, 24, 48, 72, and 120 h post-incubation under both temperature conditions. Three biological replicates (one piece of pork for each time point) were conducted under both temperature conditions respectively.

2.4 Assessing biofilm formation capability of single isolates

A dynamic analysis was conducted to evaluate the biofilm-forming abilities of eight strains of *E. coli* and eight strains of *Pseudomonas* on the surface of meat. The experiment involved incubating the strains at two different temperatures (15 and 25 ℃) for 24 h. To initiate the experiment, a suspension of each strain was prepared by diluting it with sterile saline to achieve a concentration of 10^6 CFU/mL. Subsequently, the prepared suspension was inoculated onto pork surfaces (5 cm × 2 cm), as described in Section 2.3. Each inoculated pork sample was individually sealed in a glass box using parafilm. The samples (one piece of pork for each strain) were then incubated at 15 and 25 ℃ for 24 h. To assess the biofilm formation, the quantity of biofilm formed by both *E. coli* and *Pseudomonas* on the inoculated pork surfaces was determined. Each temperature condition was performed in triplicate for each strain to ensure reliable results.

2.5 Viable cells enumeration

Each incubated pork sample was rinsed four times with sterile saline solution to remove unattached cells, after which it was homogenized in 80 mL of sterile saline. The biofilms present on the meat surfaces were obtained by slapping the samples 8 times per second for 1 min using a stomacher. The resulting homogenized suspension was subjected to serial dilution, and 0.1 mL aliquots were spread in triplicate onto selective counting plates. For the enumeration of *Pseudomonas*, the selective agar used was Cetrimide-Fucidin-Cephaloridine (CFC) supplemented with cetrimide, fusidic acid and cephalosporin (at concentrations of 10, 50 and 10 mg/L, respectively, obtained from Land bridge Technology Co., Ltd. Beijing, China). The plates were then incubated at 28 ℃ for 48 h. For the enumeration of *E. coli*, TSA agar supplemented with rifampicin (150 mg/L, obtained from Ryon Co., Shanghai, China) was used, and the plates were incubated at 37 ℃ for 24 h. Three biological replicates were performed for all samples.

2.6 Profiling of interactions between two representative strains co-cultured on meat surfaces

For the formation of dual-species biofilms on the meat surface, *E. coli* C-13 and *Pseudomonas fluorescens* $S_{1\text{-}2}$ 3 were selected. To initiate the biofilm formation, suspensions of *E. coli* C-13 and *P. fluorescens* $S_{1\text{-}2}$ 3 were mixed in equal volumes and then diluted with sterile saline to a concentration of 10^6 CFU/mL. An inoculum of 100 μL of the mixed suspension, as well as separate inocula of the two mono-strain suspensions, were each applied to the meat surface (5 cm × 2 cm), following the procedure described in Section 2.3. The final cellular density for both *E. coli* and *Pseudomonas* was adjusted to 4.0 (lg (CFU/cm^2)). After inoculation, the glass box containing the meat samples was sealed with para-film and incubated at 15 and 25 ℃ for 24 h. The biofilm quantification of *E. coli* and *Pseudomonas* was determined according to the method described in Section 2.5.

2.7 Simulation of transfer dynamics of dual-species biofilms between meat surfaces

The continuous cross-contamination performance of mono- and dual-species biofilms of *E. coli* C-13 and *P. fluorescens* $S_{1\text{-}2}$ 3 was evaluated using the blot succession method[31]. More specifically, pork samples containing biofilms were rinsed to remove any loosely attached cells and were then used as donors for transfer. In the transfer process, a second sterile pork sample was placed on top of the donor pork sample, ensuring stable contact between the surface (5 cm × 2 cm) for 4 min. During this contact period, the biofilm was transferred from the donor to the recipient pork sample. This transfer procedure was repeated 15 times, with the same donor sample loaded with biofilm being sequentially contacted with different sterile meat samples. After each transfer, viable biofilm cells on the transferred meat sample were enumerated through a cellular enumeration test. Finally, to compare the different experimental conditions, the data obtained was adjusted using three different mathematical models. The specific mathematical models used for the adjustment were as follows:

1) Logistic function (model I)[31]:

$$Y=\frac{P}{1+C\times e^{-rX}} \tag{1}$$

Where Y is the number of bacteria transferred (lg (CFU/cm^2)) at succession number of X; X is the sequential contact and transfer events between the donor and recipient pork samples; P is the number of remaining bacterial cells after a certain number of consecutive imprinting reactions; C is a dimensionless constant that determines the initial transfer efficiency of bacteria; r represents abscission coefficient, serving as a measure of the extent to which bacteria adhere to the meat surface and reflects their ability to remain attached during transfer process.

2) Multiple square function (model II)[32]:

$$Y=A\times X^{B} \tag{2}$$

Where Y is the number of bacteria transferred (lg (CFU/cm^2)) at succession number of X; X is the sequential contact and transfer events between the donor and recipient pork samples; A and B are constants derived from the regression analysis. Parameter B indirectly reflects the strength of biofilm.

3) Exponential function (model III)[33]:

$$Y=k\times e^{-\frac{X}{D}} \tag{3}$$

Where Y is the number of bacteria transferred (lg (CFU/cm^2)) at succession number of X; X is the number of consecutive transfers; k and D are constants derived from the regression analysis; D represents the reciprocal of the abscission index, and $1/D$ reflects the strength of biofilm. The performance of the models was evaluated using mean squared error (MSE), the coefficient of determination (R^2) was provided by Curve Expert software, and the bias factor (B_f) and accuracy factor (A_f) were calculated using the following equations:

$$A_f=10^{\Sigma\lg\left|\frac{\text{Predictive value}}{\text{Measured value}}\right|/n} \tag{4}$$

$$B_f=10^{\Sigma\lg\frac{\text{Predictive value}}{\text{Measured value}}/n} \tag{5}$$

The performance of the three models was assessed using the aforementioned parameters. To validate each model, the predicted transfer amounts of biofilm at 2, 8, and 12 imprints were compared against the corresponding observed values. This validation process allowed for an evaluation of the accuracy and reliability of the models in estimating the biofilm transfer under different imprinting conditions. By analyzing the agreement between the predicted and observed values at these specific imprint numbers, the performance and effectiveness of each model can be further assessed and validated.

2.8 Statistical analysis

The data obtained from the study was processed and visualized using GraphPad Prism software version 8.0.2. Statistical analysis was performed using the Statistical Product and Service Solutions software (SPSS) version 17.0. To determine the significance of differences in CFU counts between treatment groups, student's *t*-test was employed. The *P*-values were calculated to assess the statistical significance of the observed differences. The results were reported as the mean ± standard error. Significant differences between treatment groups were indicated at a 95% confidence level, denoted by a significance threshold of $P < 0.05$.

3. Results

3.1 Dynamic analysis of biofilm formation on meat surfaces

The dynamic changes in biofilms formed by iso-mixed *E. coli* and *Pseudomonas* at temperatures of 15 and 25 ℃ were depicted in Fig. 1. Over time, the biofilm amounts of both *E. coli* and *Pseudomonas* gradually increased at both temperatures. The initial growth rate of the biofilms was rapid within the first 24 h, followed by a slower increase after 48 h. At 15 ℃, 24th hour, the population of *E. coli* reached 5.38 (lg (CFU/cm^2)) (Fig. 1A), while *Pseudomonas* reached 6.28 (lg (CFU/cm^2)) (Fig. 1B). At 25 ℃, 24th hour, the population of *E. coli* increased to 7.98 (lg (CFU/cm^2)) (Fig. 1A), while *Pseudomonas* reached 8.25 (lg (CFU/cm^2)) (Fig. 1B). These results indicated that higher temperature promoted higher CFU counts for both *E. coli* and *Pseudomonas*, with significantly higher biofilm formation observed at 25 ℃ compared to 15 ℃ (two-sample *t*-test, $P < 0.05$). Overall, the findings demonstrated that both *E. coli* and *Pseudomonas* had the capacity to form substantial biofilms when cultured at both 15 and 25 ℃ for 24 h.

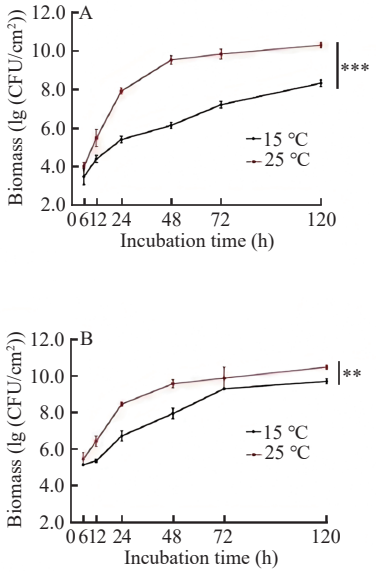

Fig. 1 Time course of biofilm formation by iso-mixed *E. coli* (A) and *Pseudomonas* (B) cultures on meat surfaces at 15 and 25 °C. Area under the curve (AUC) is calculated for each growth condition and statistical significance between 15 and 25 °C is determined by Student's *t*-test. Asterisks indicate statistically significant differences in AUC between 15 and 25 °C. $^{**}P < 0.01$, $^{***}P < 0.001$.

3.2 Temperature served as a critical factor influencing biofilm formation of Pseudomonas and E. coli on meat surfaces

The biofilm-forming abilities of 8 *E. coli* strains and 8 *Pseudomonas* strains were investigated at temperature of 15 and 25 ℃ for 24 h. As illustrated in Fig. 2, *Pseudomonas* exhibited stronger biofilm formation capabilities at 15 ℃ as compared to *E. coli* (two-sample *t*-test, $P < 0.05$). Furthermore, significant differences in biofilm-forming ability were observed between the two temperatures. Notably, the biofilm-forming abilities of both *E. coli* and *Pseudomonas* strains were significantly higher at 25 ℃ compared to 15 ℃ (two-sample *t*-test, $P < 0.05$). Among all the tested strains, *E. coli* C-13 and *P. fluorescens* $S_{1\text{-}2}$ 3 that exhibited strong biofilm-forming abilities at both 15 and 25 ℃ were selected as representative strains for further experiments.

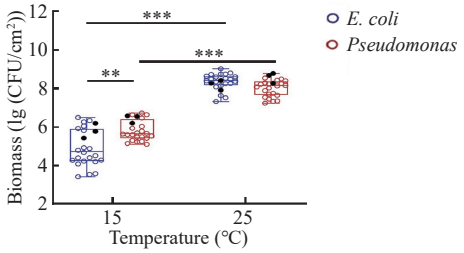

Fig. 2 Biofilm formation by *E. coli* and *Pseudomonas* isolates on meat at 15 and 25 ℃. Biofilm formation is assessed for 8 *E. coli* and 8 *Pseudomonas* strains ($n = 3$ biological replicates per strain) on meat surfaces during 24 h incubation. Black filled circles represent biofilm formation by *E. coli* C-13 and *P. fluorescens* $S_{1\text{-}2}$ 3. Asterisks indicate statistically significant differences in biofilm formation between strains as determined by Student's *t*-test. $^{*}P < 0.05$, $^{***}P < 0.001$.

3.3 Temperature-dependent competition patterns were observed between E. coli and P. fluorescens

The CFU counts of *E. coli* C-13 and *P. fluorescens* $S_{1\text{-}2}$ 3 in mono- and dual-species biofilms grown on meat at temperatures of 15 and 25 ℃ for 24 h were shown in Fig. 3. It was clearly shown that these two species exhibited distinct growth fitness in response to different temperatures. At 15 ℃, the growth fitness of *P. fluorescens* $S_{1\text{-}2}$ 3 and *E. coli* C-13 in dual-species biofilms was significantly enhanced and reduced as compared to those when cultured alone, respectively (Fig. 3A; two-sample *t*-test, $P < 0.05$). These two strains presented opposite tendencies in growth fitness when co-cultured at 25 ℃ (Fig. 3B). The results showed that these two strains presented varied capabilities for surface colonization in the presence of their counterparts at different temperatures, highlighting the temperature-dependent competition patterns between *E. coli* and *P. fluorescens* as dual-species biofilms progressed on meat surfaces.

3.4 Transfer model of dual-species biofilm of E. coli and Pseudomonas from meat to meat

Three models (Logistic mode, Multiple square model, and Exponential model) were employed to analyze the obtained results concerning biofilm transfer. The transfer curves of Logistic model for the biofilms formed by *E. coli* C-13 and *P. fluorescens* $S_{1\text{-}2}$ 3 were depicted in Fig. 4, and the transfer curves of other two models were

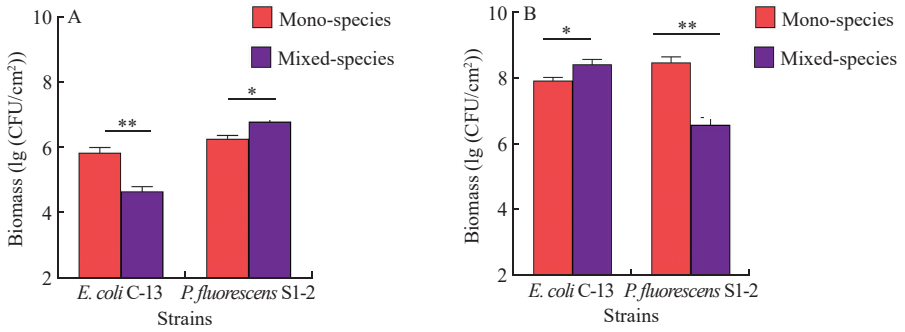

Fig. 3 Interaction between *E. coli* C-13 and *P. fluorescens* S1-2 in mixed biofilms formed at 15 °C (A) and 25 °C (B) for 24 h. Data is presented as mean ± standard error (n = 3). Bacterial counts in mono- and mixed-biofilms at the same temperature are compared using Student's t-test. Asterisks indicate statistically significant differences in bacterial counts between mono- and mixed-biofilms under the same temperature condition. *P < 0.05, **P < 0.01.

depicted in Figs. S1 and S2, respectively. All three models demonstrated similar fitting results, which revealed that at 15 °C, the transfer amount of mono-species biofilms was significantly lower compared to that of dual-species biofilms (two-sample t-test, P < 0.05). However, at 25 °C, both species in dual-species biofilms were not observed significantly increased transfer amount of cell numbers as compared to those in mono-species biofilms. This indicated that the transfer of biofilm cells was significantly influenced by temperature for both mono- and dual-species strains. The corresponding parameters of Logistic model were provided in Table 1, and two others were depicted in Tables S1 and S2, respectively. Notably, a significant difference in the coefficient of "r" (Logistic model), B-value (multiple square model), D-value (exponential model) was observed between mono- and dual-species biofilms. This finding suggested that the attachment strength between *E. coli* and *Pseudomonas* biofilms at 15 and 25 °C differed. The coefficient of determination, R^2, is instrumental in assessing the accuracy and reliability of our transfer model. As R^2 approaches 1, it signifies a higher degree of model fitting. In our study, the R^2 values obtained in F-statistics demonstrated that all three models effectively described the successive transfer of mono- and dual-species biofilm cells from one pork sample to another. Notably, the R^2 of the Logistic model was closest to 1, indicating the highest degree of fit among the models. The A_f value serves as a measure to assess the degree of deviation between the measured and predicted values. In our models, the A_f values ranged from 0.99 to 1.1, suggesting a high degree of fit. Generally, an A_f value within the range of 1.0 to 1.1 is considered ideal, as it implies that actual values are within 10% of the predicted

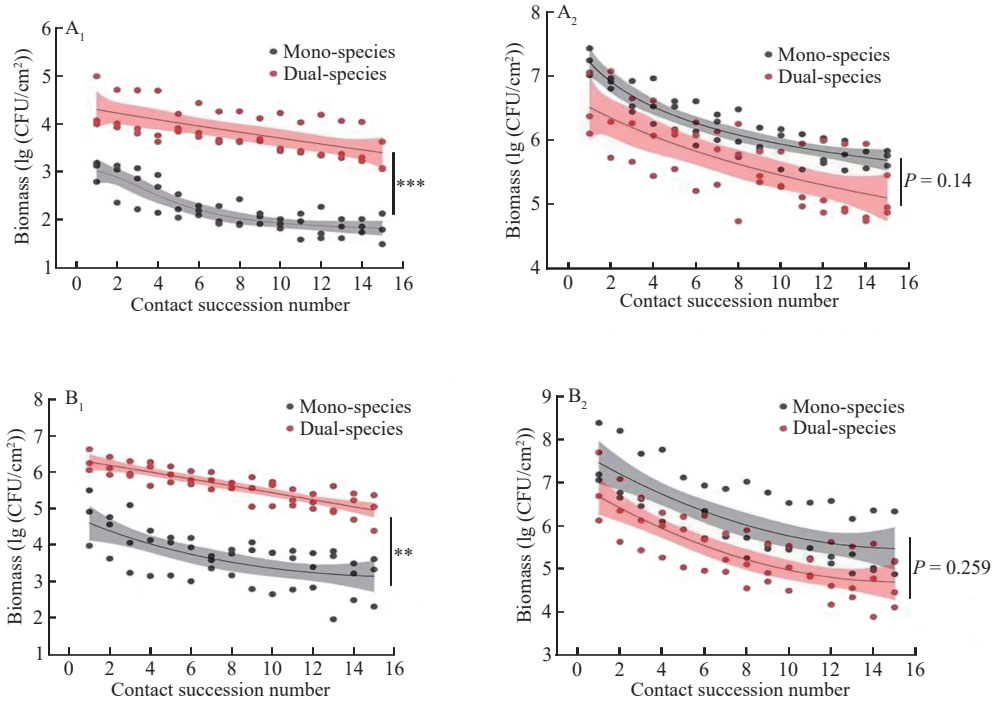

Fig. 4 Fitting curves of the logistic models for biofilms formed by *E. coli* C-13 (A) and *P. fluorescens* $S_{1\text{-}2}$ 3 (B) at 15 °C (A_1, B_1) and 25 °C (A_2, B_2). The curves represent the modeled growth dynamics of mono-species biofilms and dual-species biofilms based on CFU counts. The smooth lines depict the average trends of the data, while the shaded regions indicate the standard errors. Statistically significant differences in the AUC between mono- and dual-species biofilms are denoted by asterisks, **P < 0.01, ***P < 0.001.

Table 1
Parameters of Logistic function model of biofilms formed by *E. coli* C-13 and *P. fluorescens* $S_{1\text{-}2}$ 3.

Strains	Parameters	15 ℃		25 ℃	
		Mono-biofilm	Dual-biofilm	Mono-biofilm	Dual-biofilm
E. coli	P	1.672 ± 0.067^{aA}	0.229 ± 2.299^{aA}	5.506 ± 0.059^{aB}	3.836 ± 0.210^{aB}
	C	-0.516 ± 0.017^{aA}	-0.948 ± 0.569^{aA}	-0.264 ± 0.005^{aA}	-0.425 ± 0.030^{aA}
	r	0.129 ± 0.021^{aA}	0.001 ± 0.011^{bA}	0.131 ± 0.014^{aA}	0.036 ± 0.006^{bB}
	A_f	0.994	0.998	1.000	1.000
	B_f	0.994	0.998	1.000	1.000
	MSE	0.006	0.004	0.002	0.001
	R^2	0.979	0.944	0.996	0.998
	F-test	< 0.000 1	< 0.000 1	< 0.000 1	< 0.000 1
P. fluorescens	P	3.000 ± 0.138^{aA}	0.320 ± 1.751^{bA}	4.871 ± 0.123^{aB}	4.164 ± 0.164^{aB}
	C	-0.413 ± 0.023^{aA}	-0.950 ± 0.296^{bA}	-0.387 ± 0.013^{aA}	-0.420 ± 0.018^{aB}
	r	0.133 ± 0.031^{aA}	0.001 ± 0.005^{bA}	0.089 ± 0.010^{aB}	0.090 ± 0.016^{aB}
	A_f	0.997	1.001	1.000	1.001
	B_f	0.997	1.001	1.000	1.001
	MSE	0.009	0.003	0.002	0.004
	R^2	0.949	0.982	0.995	0.991
	F-test	< 0.000 1	< 0.000 1	< 0.000 1	< 0.000 1

Note: a, b denoted significant differences between mono- and dual-biofilm at the same temperature ($P < 0.05$); A, B denoted significant differences between the 2 temperatures in the same biofilm consortia ($P < 0.05$).

values and, therefore, more accurate. However, it was important to note that, compared to the Logistic model, the other two models exhibited notable deviations between predicted and observed values, particularly during the initial periods of the fitting process. This indicated that their fitting effects were not as precise as those of the Logistic function model. Similarly, the parameter B_f, which is a deviation factor, yielded conclusions akin to those of the A_f values, further reinforcing the superior accuracy of the Logistic model in our analysis.

3.5 Validation of transfer models

The validation results of the transfer curve for *E. coli* and *Pseudomonas* biofilms were presented in Tables S3 and S4, which demonstrated the accuracy of each model. Three points were selected with imprinting times of 2, 8, and 12 to assess the performance of the models. The relative errors of the biofilm transfer varied for *E. coli* and *Pseudomonas* at different temperatures. For the transfer model of mono-biofilm of *E. coli* at 15 ℃, model I exhibited the smallest relative error. However, in the transfer model of dual-species biofilm of *E. coli*, both models I and III displayed relatively low relative errors. At 25 ℃, the relative errors of all three models for the transfer of mono- and dual-species biofilms of *E. coli* were small, indicating their accuracy in these scenarios. In the case of *Pseudomonas* biofilm, at 15 ℃, model II exhibited a small relative error for the transfer of mono-species biofilm. Conversely, at 25 ℃, both model I and III displayed small relative errors for the transfer of mono- and dual-species biofilms of *Pseudomonas*. Based on these findings, we concluded that at both 15 and 25 ℃, model I showed relatively smaller relative errors, suggesting its higher accuracy in predicting the transfer of mono- and dual-species biofilms.

4. Discussion

During meat slaughter and production processing, various spoilage microorganisms have the potential to develop biofilms on both abiotic and biotic contact surfaces such as stainless steel, pork[17], lettuce, and beef[34]. Previous studies have indicated that biofilm formation is a dynamic process consisting of stages such as initial attachment, irreversible attachment, micro-colony formation, proliferation, and biofilm dispersal[35]. In this study, we observed that the formation of mono-species biofilms of *E. coli* and *Pseudomonas* exhibited a gradual increase. However, these changes did not completely align with the dynamic process of mono-species biofilm formation of *E. coli* and *Pseudomonas* on stainless steel surfaces, as reported in our previous study[36]. It was evident that the bacteria utilized culture media for growth and adhesion to abiotic contact surfaces under liquid culture conditions. Conversely, on the meat surface, the bacteria relied on meat nutrients for growth, proliferation, and adhesion. It is important to note that the liquid culture condition may significantly contribute to the variations observed in the biofilm formation process, in addition to differences in the nutritional composition of the culture media. Furthermore, our investigations revealed that *Pseudomonas* demonstrated a stronger ability to form biofilms compared to *E. coli* during the early stages of biofilm development. This finding suggested that *Pseudomonas* exhibited superior initial adhesion capabilities compared to *E. coli*.

Prior investigations into biofilm formation on diverse abiotic surfaces, including stainless steel, glass, polyethylene, have demonstrated variations in the biofilm-forming ability among different strains of the same species[14,37]. In this study, we examined the biofilm-forming capability of *E. coli* and *Pseudomonas* isolates derived from meat, specifically on the meat surface. Our findings aligned with previous studies, as we observed that the biofilm-forming ability was strain-specific for both organisms[36].

We investigated the impact of temperature on the formation of mono-species biofilms using meat-derived *E. coli* and *Pseudomonas* isolates. We observed dynamic changes in biofilm formation and evaluated the biofilm-forming ability of these bacteria on meat at different temperatures. Our findings revealed that the viable cellular number of biofilms formed by *E. coli* and *Pseudomonas* at 25 ℃ was higher compared to that at 15 ℃. These results are consistent with our previous study[36], where we examined biofilm formation of *E. coli* and *Pseudomonas* on stainless steel surfaces at 15 and 25 ℃. In that study, we also observed that biofilm formation by *E. coli* was more pronounced at 25 ℃ compared to 15 ℃. However, we observed an opposing trend for *Pseudomonas*, whereby the amount of biofilm formed was greater at 15 than at 25 ℃. This observation aligns with the findings of Sterniša et al.[14], who demonstrated stronger biofilm-forming ability of *Pseudomonas* at 15 ℃. We propose that the phenomenon observed could be attributed to alterations in the composition of bacterial cell-wall elements and the affinity of cells for water at lower temperatures. These factors likely influence auto-aggregation and contribute to the adaptive capabilities of *Pseudomonas* biofilms at lower temperatures.

Interactions among different species residing in biofilms play a crucial role in shaping their fitness, either through cooperative[14,38] or competitive[39-40] activities. The specific interaction patterns of *E. coli* with other bacteria in biofilm formation have been reported to vary in previous studies. Several studies have indicated that when co-cultured with bacteria such as *Salmonella*, *L. monocytogenes*, and *Enterococcus faecalis*, biofilm formation of *E. coli* was typically inhibited[41-42]. Conversely, some researcher have shown that the extracellular substances secreted by *Acinetobacter* can significantly promote *E. coli* biofilm formation[43]. Similarly, interaction of *Pseudomonas* with other bacteria has demonstrated diverse outcomes. For instance, it has been reported that *Staphylococcus aureus* predominantly promotes *Pseudomonas* biofilm formation[44]. Conversely, in dual-species biofilms, the presence of *Lactococcus lactis* has been found to inhibit *Pseudomonas* biofilm formation[45]. Competition, which frequently occurs between *E. coli* contaminants and *Pseudomonas,* has been identified as an interaction pattern[46-47]. In our previous research, we explored the interplay between *E. coli* and *Pseudomonas* in biofilm formation on stainless steel surfaces, revealing that *Pseudomonas* predominantly inhibited the biofilm formation of *E. coli* at both 15 and 25 ℃[36]. Considering the distinct biofilm-forming abilities of *E. coli* and *Pseudomonas*, we selected representative strains to investigate their interplay on the surface of meat. We observed that at 15 ℃, *Pseudomonas* inhibited the biofilm formation of *E. coli*, while at 25 ℃, it significantly promoted *E. coli* biofilm formation. Conversely, *Pseudomonas* was promoted by *E. coli* at 15 ℃, but significantly inhibited by *E. coli* at 25 ℃. Our study demonstrated that the interaction during dual-species biofilm formation is influenced by both the contact surface and temperature, in addition to the variation in strain isolates. These findings highlight the intricate nature of species interactions and emphasize the importance of considering environmental factors in understanding biofilm formation dynamics.

Biofilms have been identified as significant centers for cross-contamination transmission. Numerous studies have investigated various factors, such as contact surface[24,48-50], contact pressure and time[51-52], that influence the transfer of biofilms. Data has shown that bacteria within biofilms can readily transfer from one surface to another, even during short contact time, resulting in substantial contamination levels[14]. Mathematical models have been applied to understand the transfer dynamics of persistent biofilms, which is crucial for assessing the risk of cross-contamination. Wang et al.[29] conducted simulations on the continuous contact of *L. monocytogenes* biofilms from glass coupons to the surface of Hami melon. They explored the cross-contamination scenarios and found that the logistic model effectively described the transfer patterns of biofilm formation at different stages. Sheen[28] developed surface transfer models for *L. monocytogenes* during slicing operations and established an exponential model to describe surface cross-contamination. They concluded that a minimal number of parameters were required for a well-fitting transfer model, with an R^2 value greater than 0.7. In our research, we selected the logistic model, exponential model, and multiple square model as predictive models for biofilm transfer. We investigated the transfer patterns of mono- and mixed-biofilms of *E. coli* and *Pseudomonas* on meat surfaces using these models. The first two models demonstrated a statistically significant fit to the transfer curve of bacterial biofilms. However, when the multiple square model was utilized to predict the transfer count, the results could not well explain the experimental data. This discrepancy was particularly noticeable in the later stages of bacterial biofilm transfer, suggesting that the multiple square model was not suitable for accurately describing the transfer dynamics of biofilms.

During the model fitting process for *E. coli* and *Pseudomonas,* we discovered that temperature exerted a significant influence on the adhesion strength and stability of mono- and dual-species biofilms on meat surfaces, as well as the transfer amount of mono- and mixed-biofilms. In our study, we observed that higher temperatures (25 ℃) increased biofilm formation by both *E. coli* and *P. fluorescens*, regardless of whether they were in mono- or dual-species biofilms, in comparison to the biofilms formed at the lower temperature (15 ℃). This finding implied that at 25 ℃, microbial species within mono-species biofilms likely underwent intensified intraspecies competition compared to those at 15 ℃. Furthermore, our results indicated that in dual-species biofilms, there was a notable increase in interspecies competition at 25 ℃. We hypothesized that the similar transfer rates observed in mono- and dual-species biofilms at 25 ℃ can be attributed to the robust intra- and interspecies competition, respectively, potentially leading to unstable biofilm structures. Conversely, at the lower temperature of 15 ℃, the strains formed less biomass when grown individually, suggesting less intraspecies competition and consequently lower transfer rates. However, in dual-species biofilms at this temperature, we observed significant interspecies competition. Thus, we speculate that the increased transfer rate in dual-species biofilms at 15 ℃ may be due to the strong interspecies competition fostering unstable biofilm structures. This highlights the importance of considering the contamination risk posed by biofilms on biological surfaces under low temperature conditions in the prevention and control of microbial hazards in meat production.

5. Conclusions

This study revealed notable variations in biofilm formation across the tested strains, underscoring the importance of strain-dependent differences. Temperature was found to be a critical factor, significantly influencing both the quantity and characteristics of the biofilms. Furthermore, we employed three mathematical models to

characterize the transfer process of mono- and mixed-biofilms from one meat surface to another. The results from these models suggest their potential as effective tools for predicting and understanding the spread of biofilms in meat processing and production environments. Our investigation further highlighted that lower temperature notably facilitates the transfer dynamics of tested microbial species when grown as mixed-species biofilms.

Overall, this research enhances our understanding of biofilm formation, transfer dynamics, and the mathematical modeling of mono- and mixed-species biofilms on meat surfaces. The insights gained from this study are crucial for developing strategies to control contamination and maintain meat quality in the food industry.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250261.

References

[1] H.C. Flemming, J. Wingender, U. Szewzyk, et al., Biofilms: an emergent form of bacterial life, Nat. Rev. Microbiol. 14 (2016) 563-575. http://doi.org/10.1038/nrmicro.2016.94.

[2] M.F.R. Mizan, I.K. Jahid, S.D. Ha, Microbial biofilms in seafood: a food-hygiene challenge, Food Microbiol. 49 (2015) 41-55. http://doi.org/10.1016/j.fm.2015.01.009.

[3] A. Culotti, A.I. Packman, *Pseudomonas aeruginosa* promotes *Escherichia coli* biofilm formation in nutrient-limited medium, PLoS ONE 9 (2014) e107186. http://doi.org/10.1371/journal.pone.0107186.

[4] S. Elias, E. Banin, Multi-species biofilms: living with friendly neighbors, FEMS Microbiol Rev. 36 (2012) 990-1004. http://doi.org/10.1111/j.1574-6976.2012.00325.x.

[5] L. Laganenka, V. Sourjik, Autoinducer 2-dependent *Escherichia coli* biofilm formation is enhanced in a dual-species, Appl. Environ. 84 (2018) e02638. http://doi.org/10.1128/aem.02638-17.

[6] L. Ghaderi, A. Aliahmadi, S.N. Ebrahimi, et al., Effective inhibition and eradication of *Pseudomonas aeruginosa* biofilms by *Satureja khuzistanica* essential oil nanoemulsion, J. Drug. Deliv. Sci. Technol. 61 (2021) 102260. http://doi.org/10.1016/j.jddst.2020.102260.

[7] G. Orazi, K.L. Ruoff, G.A. O'Toole, *Pseudomonas aeruginosa* increases the sensitivity of biofilm-grown *Staphylococcus aureus* to membrane-targeting antiseptics and antibiotics, Microbiol. 10 (2019) e01501-19. http://doi.org/10.1128/mBio.01501-19.

[8] A.T. Nguyen, A.G. Oglesby-Sherrouse, Interactions between *Pseudomonas aeruginosa* and *Staphylococcus aureus* during co-cultivations and polymicrobial infections, Appl. Microbiol. Biotechnol. 100 (2016) 6141-6148. http://doi.org/10.1007/s00253-016-7596-3.

[9] S. Manoharadas, M. Altaf, A.F. Alrefaei, et al., Microscopic analysis of the inhibition of staphylococcal biofilm formation by *Escherichia coli* and the disruption of preformed staphylococcal biofilm by bacteriophage, Microsc. Res. Techniq. 84 (2021) 1513-1521. http://doi.org/10.1002/jemt.23707.

[10] J.Q. Li, J.S. Feng, L.N. Ma, et al., Effects of meat juice on biofilm formation of *Campylobacter* and *Salmonella*, Int. J. Food Microbiol. 253 (2017) 20-28. http://doi.org/10.1016/j.ijfoodmicro.2017.04.013.

[11] M. Diez-Garcia, R. Capita, C. Alonso-Calleja, Influence of serotype on the growth kinetics and the ability to form biofilms of *Salmonella* isolates from poultry, Food Microbiol. 31 (2012) 173-180. http://doi.org/10.1016/j.fm.2012.03.012.

[12] X.J. Wang, C. Dong, J.L. Yu, et al., Effect of acid stress on biofilm formation of *Escherichia coli* O157:H7, Food Sci. 40 (2019) 1-7. http://doi.org/10.1128/mBio.01501-19.

[13] G.A. Uhlich, C.Y. Chen, B.J. Cottrell, et al., Growth media and emperature effects on biofilm formation by serotype O157: H7 and non-O157 Shiga toxin-producing *Escherichia coli*, FEMS Microbiol. Let. 354 (2014) 133-141. http://doi.org/10.1111/1574-6968.12439.

[14] M. Sterniša, A. Klancnik, S.S. Mozina, Spoilage *Pseudomonas* biofilm with *Escherichia coli* protection in fish meat at 5 ℃, J. Sci. Food Agric. 99 (2019) 4635-4641. http://doi.org/10.1002/jsfa.9703.

[15] S.H. Flint, J.D. Brooks, P.J. Bremer, Properties of the stainless steel substrate, influencing the adhesion of thermo-resistant streptococci, J. Food Eng. 43 (2000) 235-242. http://doi.org/10.1016/s0260-8774(99)00157-0.

[16] T.T. Gu, A. Meesrisom, Y.G. Luo, et al., *Listeria monocytogenes* biofilm formation as affected by stainless steel surface topography and coating composition, Food Control 130 (2021) 108275. http://doi.org/10.1016/j.foodcont.2021.108275.

[17] D. Park, P. Jong-Hyun, Isolation of bacteriophages having depolymerase and control of pathogenic *E. coli* O103 in biofilm on lettuce, Korean J. Food Sci. Technol. 51 (2019) 604-609. http://doi.org/10.9721/kjfst.2019.51.6.604.

[18] K.L. Vorst, E.C.D. Todd, E.T. Ryser, Transfer of *Listeria monocytogenes* during mechanical slicing of turkey breast, bologna, and salami, J. Food Protect. 69 (2006a) 619-626. http://doi.org/10.4315/0362-028x-69.3.619.

[19] P.T. Sekoai, S.Q. Feng, W.W. Zhou, et al., Insights into the mcrobiological safety of wooden cutting boards used for meat processing in Hong Kong's Wet Markets: a focus on food-contact surfaces, cross-contamination and the efficacy of traditional hygiene practices, Microorganisms. 8 (2020) 8040579. http://doi.org/10.3390/microorganisms 8040579.

[20] N.J. Amaeze, A.B. Akinbobola, R. Kean, et al., Transfer of micro-organisms from dry surface biofilms and the influence of long survival under conditions of poor nutrition and moisture on the virulence of *Staphylococcus aureus*, J. Hosp. Infect. 150 (2024) 34-39. https://doi.org/10.1016/j.jhin.2024.03.023.

[21] J. Verran, A. Packer, P.J. Kelly, et al., Use of the atomic force microscope to determine the strength of bacterial attachment to grooved surface features, J. Adhes. Sci. Technol. 24 (2010) 2271-2285. http://doi.org/10.1163/016942410x508019.

[22] S.A. Sattar, S. Springthorpe, S. Mani, et al., Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using *Staphylococcus aureus* as a model, J. Appl. Microbiol. 90 (2001) 962-970. http://doi.org/10.1046/j.1365-2672.2001.01347.x.

[23] S.C.C. Foong, J.S. Dickson, Attachment of *Listeria monocytogenes* on ready-to-eat meats, J. Food Protect. 67 (2004) 456-462. http://doi.org/10.4315/0362-028x-67.3.456.

[24] E. Gkana, N. Chorianopoulos, A. Grounta, et al., Effect of inoculum size, bacterial species, type of surfaces and contact time to the transfer of foodborne pathogens from inoculated to non-inoculated beef fillets via food processing surfaces, Food Microbiol. 62 (2017) 51-57. http://doi.org/10.1016/j.fm.2016.09.015.

[25] O.S. Papadopoulou, N.G. Chorianopoulos, E.N. Gkana, et al., Transfer of foodborne pathogenic bacteria to non-inoculated beef fillets through meat mincing machine, Meat Sci. 90 (2012) 865-869. http://doi.org/10.1016/j.meatsci.2011.11.008.

[26] K.L. Vorst, E.C.D. Todd, E.T. Ryser, Transfer of *Listeria monocytogenes* during slicing of turkey breast, Bologna, and salami with simulated kitchen knives, J. Food Protect. 69 (2006b) 2939-2946. http://doi.org/10.4315/0362-028x-69.12.2939.

[27] P. Dawson, I. Han, M. Cox, et al., Residence time and food contact time effects on transfer of *Salmonella Typhimurium* from tile, wood and carpet: testing the five-second rule, J. Appl. Microbiol. 102 (2007) 945-953. http://doi.org/10.1111/j.1365-2672.2006.03171.x.

[28] S. Sheen, Modeling surface transfer of *Listeria monocytogenes* on salami during slicing, J. Food Sci. 73 (2008) E304-E311. http://doi.org/10.1111/j.1750-3841.2008.00833.x.

[29] Z. Wang, Y. Ma, Z.S. Li, et al., Characterization of *Listeria monocytogenes* biofilm formation kinetics and biofilm transfer to cantaloupe surfaces, Food Res. Int. 161 (2022) 111839. http://doi.org/10.1016/j.foodres.2022.111839.

[30] X.Q. Yang, F. Tran, M.K. Youssef, et al., Determination of sources of *Escherichia coli* on beef by multiple-Locus variable-number tandem repeat analysis, J. Food Protect. 78 (2015) 1296-1302. http://doi.org/10.4315/0362-028x.Jfp-15-014.

[31] H.H. Wang, X.X. Zhang, Q.Q. Zhang, et al., Comparison of microbial transfer rates from *Salmonella* spp. biofilm growth on stainless steel to selected processed and raw meat, Food Control 50 (2015) 574-580. http://doi.org/10.1016/j.foodcont.2014.09.049.

[32] S. Sheen, C.A. Hwang, Mathematical modeling the cross-contamination of *Escherichia coli* O157:H7 on the surface of ready-to-eat meat product while slicing, Food Microbiol. 27 (2010) 37-43. http://doi.org/10.1016/j.fm.2009.07.016.

[33] I. Reigada, P. San-Martin-Galindo, S. Gilbert-Girard, et al., Surfaceome and exoproteome dynamics in dual-species *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms, Frontiers Microbiol. 12 (2021) 672975. http://doi.org/10.3389/fmicb.2021.672975.

[34] E.U. Turhan, S. Polat, Z. Erginkaya, et al., Investigation of synergistic antibacterial effect of organic acids and ultrasound against pathogen biofilms on lettuce, Food Biosci. 47 (2022) 101643. http://doi.org/10.1016/j.fbio.2022.101643.

[35] P.K. Nyanasegran, S. Nathan, M. Firdaus-Raih, et al., Biofilm signaling, composition and regulation in *Burkholderia pseudomallei*, J. Microbiol. Biotechnol. 33 (2023) 15-27. http://doi.org/10.4014/jmb.2207.07032.

[36] W.D. Zhang, X.Y. Mi, C. Zhang, et al., Meat-derived *Escherichia coli* and *Pseudomonas fragi* manage to co-exist in dual-species biofilms by adjusting gene-regulated competitive strength, Food Microbiol. 109 (2023) 104122. http://doi.org/10.1016/j.fm.2022.104122.

[37] S. Kasetty, D.L. Mould, D.A. Hogan, et al., Both *Pseudomonas aeruginosa* and *Candida albicans* accumulate greater biomass in dual-species biofilms under flow, Msphere. 6 (2021) e00416-21. http://doi.org/10.1128/mSphere.00416-21.

[38] C. Zhang, F. Zhu, A.N. Jatt, et al., Characterization of co-culture of *Aeromonas* and *Pseudomonas* bacterial biofilm and spoilage potential on refrigerated grass carp (*Ctenopharyngodon idellus*), Lett. Appl. Microbiol. 71 (2020) 337-344. http://doi.org/10.1111/lam.13341.

[39] P. Di Ciccio, A. Vergara, A.R. Festino, et al., Biofilm formation by *Staphylococcus aureus* on food contact surfaces: relationship with temperature and cell surface hydrophobicity, Food Control 50 (2015) 930-936. http://doi.org/10.1016/j.foodcont.2014.10.048.

[40] M.V. Kuznetsova, I.L. Maslennikova, T.I. Karpunina, et al., Interactions of *Pseudomonas aeruginosa* in predominant biofilm or planktonic forms of existence in mixed culture with *Escherichia coli in vitro*, J. Microbiol. 59 (2013) 604-610. http://doi.org/10.1139/cjm-2013-0168.

[41] O.A. Alshanta, K. Albashaireh, E. McKloud, et al., *Candida albicans* and *Enterococcus faecalis* biofilm frenemies: When the relationship sours, Biofilm. 4 (2022) 100072. http://doi.org/10.1016/j.bioflm.2022.100072.

[42] J.B. Frozi, L.M.R. Esper, R.M. Franco, Single- and multi-species biofilms by *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. isolated from raw fish and a fish processing unit, Ciencia Rural. 47 (2017) 150234. http://doi.org/10.1590/0103-8478cr20170011.

[43] X.Y. Wang, J. Xie, Quorum sensing system-regulated proteins affect the spoilage potential of co-cultured *Acinetobacter johnsonii* and *Pseudomonas fluorescens* from spoiled bigeye tuna (*Thunnus obesus*) as determined by proteomic, Front. Microbiol. 11 (2020) 940. http://doi.org/10.3389/fmicb.2020.00940.

[44] A. Rieu, J.P. Lemaitre, J. Guzzo, et al., Interactions in dual species biofilms between *Listeria monocytogenes* EGD-e and several strains of *Staphylococcus aureus*, Food Microbiol. 126 (2008) 76-82. http://doi.org/10.1016/j.ijfoodmicro.2008.05.006.

[45] J. Kives, D. Guadarrama, B. Orgaz, et al., Interactions in biofilms of *Lactococcus lactis* ssp. *cremoris* and *Pseudomonas fluorescens* cultured in cold UHT milk, J. Dairy Sci. 88 (2005) 4165-4171. http://doi.org/10.3168/jds.S0022-0302(05)73102-7.

[46] Y. Cheng, S. Zhang, C. Zhang, et al., *Escherichia coli* O157: H7 is challenged by the presence of *Pseudomonas*, but successfully co-existed in dual-species microbial communities, Food Microbiol. 106 (2022) 104034. http://doi.org/10.1016/j.fm.2022.104034.

[47] M. Burmolle, W. Rend, T. Bjarnshlot, et al., Interactions in multispecies biofilms: do they actually matter? Trends Microbiol. 22 (2014) 84-91. http://doi.org/10.1016/j.ijfoodmicro.2008.05.006.

[48] A. Rodriguez, L.A. McLandsborough, Evaluation of the transfer of *Listeria monocytogenes* from stainless steel and high-density polyethylene to Bologna and American cheese, J. Food Protect. 70 (2007) 600-606. http://doi.org/10.4315/0362-028x-70.3.600.

[49] Y.C. Nan, A. Rodas-Gonzalez, K. Stanford, et al., Formation and transfer of multi-species biofilms containing *E. coli* O103:H2 on food contact surfaces to beef, Front. Microbiol. 13 (2022) 863778. https://doi.org/10.3389/fmicb.2022.863778.

[50] X. Pang, H.G. Yuk, Effects of the colonization sequence of *Listeria monocytogenes* and *Pseudomonas fluorescens* on survival of biofilm cells under food-related stresses and transfer to salmon, Food Microbiol. 82 (2019) 142-150. http://doi.org/10.1016/j.fm.2019.02.002.

[51] G. Moore, I.S. Blair, D.A. Mcdowell, Recovery and transfer of *Salmonella Typhimurium* from four different domestic food contact surfaces, J. Food Protect. 70 (2007) 73-80. http://doi.org/10.4315/0362-028x-70.3.600.

[52] B.M. Villegas, N.O. Hall, E.T. Ryser, et al., Influence of physical variables on the transfer of *Salmonella Typhimurium* LT2 between potato (*Solanum tuberosum*) and stainless steel via static and dynamic contact, Food Microbiol. 92 (2020) 103607. http://doi.org/10.1016/j.fm.2020.103607.