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Research Article

ISOLATION AND IDENTIFICATION OF BIOFILM-FORMING BACTERIA IN FOODS SUPPLIED FOR CONSUMPTION IN OPEN-AIR MARKET STALLS: A CASE STUDY IN AYDIN PROVINCE

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Abstract: In this study, the isolation and identification of bacteria that form biofilms on various food products sold on open-air market stalls in Aydın Province were aimed. Bacteria were isolated from food surfaces in aseptic conditions and isolated after incubation in the Tryptic Soy Agar medium at 37°C for 24-48 hours. DNA isolations of the isolated bacteria were performed and the PCR products obtained were used for sequencing. The Congo Red Agar method was used to qualitatively analyze biofilm formation. According to this method, bacteria forming black colonies were evaluated as biofilm-positive and were subjected to quantitative analysis using the Microplate method. A total of 67 bacteria were isolated from the sampled food items, of which 7 were strong and 2 were moderate biofilm producers, showing that more importance should be given to food hygiene.

Özet: Bu çalışmada Aydın ilinde açıkta satış tezgahlarında satışa sunulan farklı gıda ürünlerinde biyofilm oluşturan bakterilerin izolasyonu ve tanımlanması amaçlanmıştır. Bakteriler, gıda yüzeylerinden aseptik koşullara uygun olarak alınmış ve Triptik Soy Agar besiortamında 37ºC de 24-48 saat inkübasyon ile izole edilmiştir. İzole edilen bakterilerin DNA izolasyonları gerçekleştirilmiş ve PCR ürünleri elde edilmiş ve sekans analizi yapılmıştır. Biyofilm oluşumunu analiz etmek için tanımlanan bakterilerin Kongo Red metodu ile kalitatif tayini yapılmıştır. Bu metoda göre siyah koloni oluşturan bakteriler biyofilm pozitif olarak değerlendirilmiştir. Pozitif olarak değerlendirilen bakterilere Mikroplate metodu uygulanarak kantitatif analiz yapılmıştır. Farklı gıda örneklerinden 7 tanesi güçlü, 2 tanesi ise orta düzeyde biyofilm oluşturan toplam 67 bakteri izole edilmiştir ki bu durum gıda hijyenine daha fazla önem verilmesi gerektiğini göstermektedir.

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Introduction

Food safety is a global public health issue, and in particular, the lack of hygiene and increased consumption in unsanitary environments can increase the hazard for food safety (Madenci *et al*. 2019). At a global scale, HACCP and ISO 22000 "Food Safety Management System" are two important regulations currently used on a global scale to ensure food safety. However, despite the strict regulations, inadequate hygiene practices, emerging as one of the fundamental problems particularly in undeveloped and developing countries, can lead to spread of foodborne diseases (Noronha *et al*. 2006, Abdalla *et al*. 2008,). The presence of bacteria, fungi and viruses as microbiological agents and heavy metals and pesticides as chemical agents and various physical factors, such as dirt, are considered as important risk factors for food related health cases in terms of diseases occurring following human consumption (Özkaya & Cömert 2008, Urazel *et al*. 2014). Biofilm is known as a community of microorganisms that irreversibly adhere to each other and usually to a solid surface. These adherent cells are embedded within an extracellular matrix of mucus-like structures made up of extracellular polymeric substances. The cells within the biofilm typically produce extracellular polymeric components consisting of polysaccharides, proteins, lipids, and DNA that form a polymeric mass. Biofilms can be formed on living or non-living surfaces and can be widespread in natural, industrial and hospital environments (Thomas & Nakaishi 2006, Jayaraman & Wood 2008, Altun & Sener 2008).

Many microorganisms, especially pathogenic ones, form biofilm on food and food contact surfaces when conditions are suitable (Fysun *et al*. 2019). Biofilm formation is affected by various internal (i.e. water activity, antimicrobial substance content, pH value,

nutrient availability, oxygen demand and electrical variability) and external (i.e. surface material, surface area, surface smoothness, fluid flow rate and limited nutrient) factors (Douglas 2003). Biofilms pose major problems for the food industry as they allow bacteria to attach to a variety of surfaces (Hall-Stoodley *et al*. 2004). The presence of biofilms not only reduces the microbiological quality of foods but also causes various diseases. Additionally, the exopolysaccharide matrix of biofilms contains enzymes, heterogeneity, cellular persistence, metabolic activity, genetic adaptation, quorum sensing, stress responses, outer membrane structure, and efflux pumps that provide resistance mechanisms against antimicrobials (Araujo *et al*. 2011). This study was aimed to isolate and identify bacteria growing in various food products offered for sale in sales counters in Aydın province and to observe biofilm formation using qualitative and quantitative methods. Food items in the selected study area are foods preferred and consumed by the public. The proliferation of bacteria in these foods due to lack of control and hygiene and their ability to form biofilms pose a life-threatening threat to human health. Therefore, the study results draw attention to the fact that people should be more conscious in the consumption of open-air sold foods.

Materials and Methods

The food samples used for bacterial isolations were obtained from an open public bazaar/from an open market/from various sources operating as open-air market style in October 2018. The study was carried out as a small-scale sampling. Meat-chicken doner, mussels, chicken, fish, raw meatballs, olives, cheese, desserts, simit and toast were sampled throughout the study.

The samples were collected from the listed food items and the sales counters in contact with these foods, in compliance with aseptic conditions (Fig. 1). The samples were collected using a sterile swab stick under aseptic conditions in sterile glass tubes. The tubes were then placed in a cooler and transported to the Microbiology Laboratory of the Department of Biology, Aydın Adnan Menderes University within 4-6 hours.

Fig. 1. A view of a cheese stall in a public bazaar.

Isolation and identification of bacteria

Nine food samples (chicken doner, chicken, inner surface of mussel shell, outer surface of mussel shell, tulum cheese, olive, tulumba dessert, şambali dessert, tiriliçe dessert) and thirteen stall (chicken metal stall, chicken doner stall, meat doner stall, fish stall, mussel stall, simit stall, cheese stall, metal spoon in olive bowl, plastic olive bowl, wooden stall in toaster, plastic stall in toaster, ceramic tile in steak tartar a la turca, wood cutting place of kokoreç) were examined. The samples brought to the laboratory were incubated at 37°C for 24 hours in a sterile 2 mL Tryptic Soy Broth (TSB) medium (Merck). After incubation, 10^{-1} to 10^{-6} dilutions of the medium were made. From each dilution, spread cultivation onto sterile petri dishes containing Tryptic Soy Agar (TSA) medium (Merck) were obtained, and the dishes were incubated at 37°C for 24 hours. Different colonies grown on petri dishes were selected and purified. The isolated strains were stored at -20°C in 20% skim milk (Çoban *et al*. 2018, Tekin & Çoban 2021, Çoban & Yaman 2023). The morphological, cultural, and biochemical characteristics of the isolates were initially studied according to classical taxonomy outlined in Bergey's Manual of Systematic Bacteriology (Brenner *et al*. 2004) and then molecular identification was performed. The Gram properties and cell shapes of the bacterial isolates were determined by Gram staining. The colony sizes, shapes, structures, edge features, and pigmentation were determined. Biochemical tests including catalase, H_2S production, urease test, and IMVIC tests were performed. Total genomic DNA isolation was performed according to the method of De Boer & Ward (1995). 16S rDNA amplification products were obtained using universal primers (27F-1492R) (A.B.T.) for PCR. The master mix used for PCR contains Taq DNA polymerase, reaction buffer, dNTPs and $MgCl_2$ (Genmark). The PCR reaction steps were as follows: initial denaturation at 94°C for 5 min for one cycle, denaturation at 94°C for 30 sec for 35 cycles, annealing at 55°C for 30 sec, extension at 72°C for 45 sec, and final extension at 72°C for 10 min for one cycle. A pipette was used to add 6X loading dye (1 µL) onto the PCR product (4 µL). The PCR products were loaded onto a 1% agarose gel for electrophoresis with a 100 bp DNA marker, and then visualized. The PCR products were subsequently sent to GATC BioTech, Germany for sequencing.

Analysis of Biofilm Formation

Qualitative analysis

The identified bacterial species based on sequence results were inoculated onto Congo Red Agar medium, and incubated at 37 °C for 24 hours. After incubation, black colonies on the plates were considered positive for biofilm formation, while red colonies were considered negative (Melo *et al*. 2013, Çoban & Yaman 2023).

Quantitative analysis

Bacteria identified as biofilm-positive were further subjected to quantitative analysis. For this analysis, sterile 96-well microdilution plates were used. The biofilm-

positive species were incubated overnight at 37°C in Tryptic Soy Broth medium. Then, 150 µL of each bacterial suspension was pipetted into each well of the sterile microplates, and the plates were incubated for 3 days at 37°C. After the incubation period, the bacterial suspensions in the plates were discarded, and the wells were washed three times with sterile distilled water and dried. Once the wells were dried, 150 µL of Crystal Violet dye was added to each well and allowed to stand for 45 minutes. The wells were then washed and dried. Next, 200µL of a mixture of ethanol/acetic acid (v/v) was added to each well and allowed to stand for 10 minutes. The mixture in the wells was then transferred to a different sterile plate, and the absorbance was measured at 540 nm using a spectrophotometer (Mathur *et al*. 2006, Stepanovic *et al*. 2007, Melo *et al*. 2013, Çoban & Yaman 2023). *Staphylococcus aureus* ATCC 25923 was used as the positive control and Tryptic Soy Broth was used as the negative control. Strains with optical density values ≥ 0.240 were considered strong biofilm producers, strains with optical density values 0.120-0.240 were considered moderate biofilm producers, and strains ≤ 0.120 were considered weak biofilm producers (Mathur *et al*. 2006, Demir & İnanç 2015, Çoban & Yaman 2023).

Results

Bacteria isolated and identified

In the study, a total of 67 isolates were obtained from food and materials as chicken doner (5 isolates), chicken metal stall (9 isolates), chicken (5 isolates), chicken doner stall (1 isolate), meat doner stall (5 isolates), fish stall (1 isolate), mussel stall (2 isolates), inner surface of mussel shell (1 isolate), outer surface of mussel shell (4 isolates), simit stall (5 isolates), cheese stall (8 isolates), tulum cheese (4 isolates), metal spoon in olive bowl (2 isolates), plastic olive bowl (3 isolates), olive (2 isolates), tulumba dessert (1 isolate), şambali dessert (1 isolate), tiriliçe dessert (1 isolate), wooden stall in toaster (2 isolates), ceramic tile in steak tartar a la turca (1 isolate), wood cutting place of kokoreç (1 isolate) (Table 1).

Fig. 2 shows the Gram staining characteristic of *Bacillus cereus* among the bacteria identified. In addition, Figure 3 displays the colony sizes, shapes, structures, edge characteristics and pigmentation of the identified bacteria.

The DNA extracted from the isolates was visualized by agarose gel electrophoresis for molecular diagnosis (Fig. 4). The DNA samples, whose purity was confirmed, were subjected to PCR analysis. DNA sequencing analysis was performed by the GTCA Company (Germany). The obtained DNA sequences were compared with the data in GenBank using the nBLAST program (https://blast.ncbi.nlm.nih.gov/) and the molecular diagnosis was performed. The resulting 67 bacterial species are listed in Table 1.

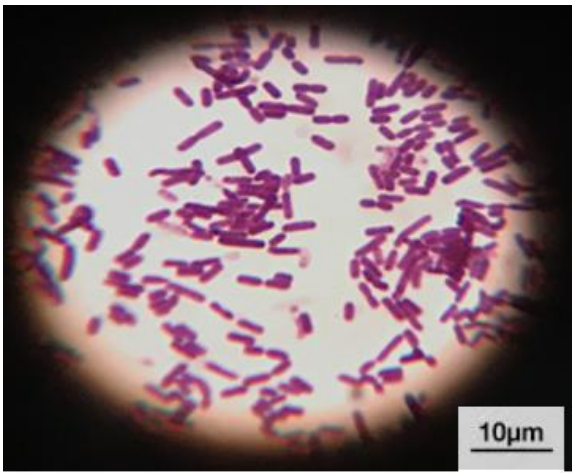

Fig. 2. Gram staining image of *Bacillus cereus* (100 ×).

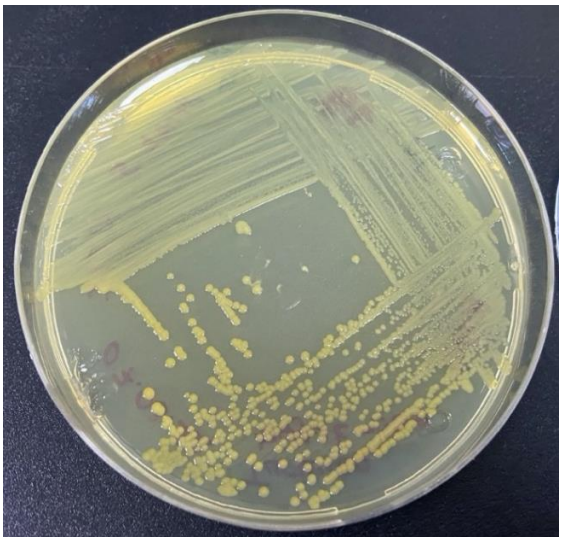

Fig. 3. Bacterial culture grown on TSA medium.

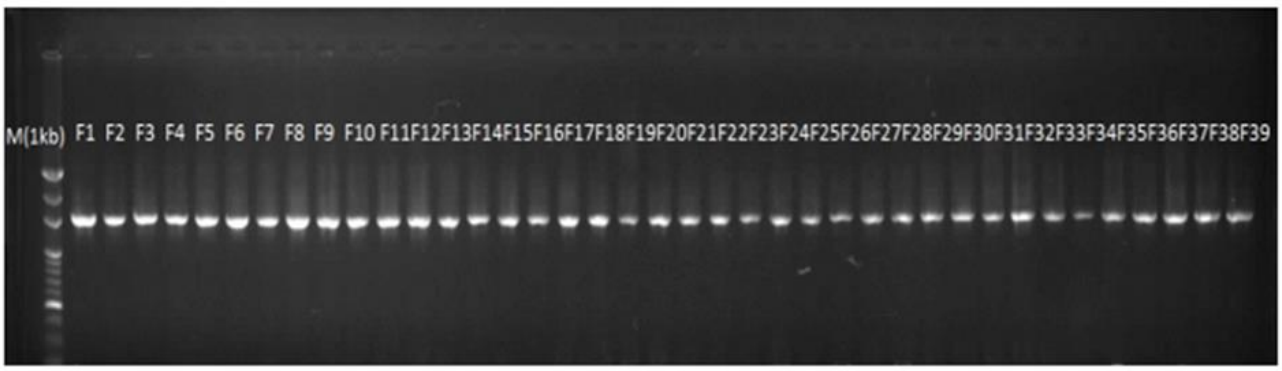

Fig. 4. Imaging of the isolated bacterial DNA in gel electrophoresis (M:1kb DNA marker. Genmark).

Table 1. Bacterial strains identified by molecular data.

Sample	Bacteria	Accession No	Similarity (%)
Chicken doner	*Enterobacter ludwigii strain D8*	MT374261.1	99
	Enterobacter ludwigii strain CEB04	CP039741.1	99
	Enterococcus faecalis strain 2623	MT611645.1	99
	Enterobacter ludwigii strain KCOM 1206	MT239508.1	99
	Enterococcus faecalis strain 2623	MT611645.1	99
Chicken metal stall	*Kurthia gibsonii strain KH2*	MN453416.1	99
	Morganella morganii strain MP63	CP048806.1	99
	Bacillus subtilis strain CFR07	MT641226.1	99
	Bacillus licheniformis strain AG-RA	MG662177.1	100
	Morganella morganii strain 17YB9	MN807694.1	100
	Proteus mirabilis strain MPE0108	CP053614.1	100
	Proteus mirabilis strain MPE5139	CP053684.1	99
	Proteus mirabilis strain HN2p	CP046048.1	100
	Kurthia gibsonii strain KH2	MN453416.1	99
Chicken	*Enterococcus fecalis strain 2358*	MT604811.1	100
	Proteus mirabilis strain MPE4069	CP053718.1	100
	Enterococcus fecalis strain 2358	MT604811.1	100
	Proteus mirabilis strain MPE4069	CP053718.1	100
	Proteus mirabilis strain MPE4069	CP053718.1	100
Chicken doner stall	*Enterobacter ludwigii strain D8*	MT374261.1	100
Meat doner stall	*Bacillus subtilis strain DY10-1*	KU862326.1	100
	Streptococcus gallolyticus strain TDGB428	JQ912071.1	99
	Streptococcus gallolyticus strain VTM4R20	KP009828.1	100
	Bacillus licheniformis strain VRKPCH23	KJ958501.1	100
	Enterococcus gallinarum strain CCFM8325	KJ803882.1	99
Fish stall	*Alcaligenes faecalis* strain PS25	MT471003.1	99
Mussel stall	*Escherichia coli* strain EcPF7	CP054232.1	100
	Enterobacter hormaechei RB18	LC386024.1	100
Inner surface of mussel shell	*Bacillus subtilis* strain HR-4	MT645613.1	100
Outer surface of mussel shell	*Bacillus velezensis* strain HSB1	MT626060.1	100
	Staphylococcus epidermidis strain3039	MT613456.1	100
	Bacillus cereus strain TBMAX51	MK834690.1	99
	Bacillus velezensis strain HSB1	MT626060.1	100
Simit stall	*Klebsiella pneumoniae strain KP20194a*	CP054780.1	99
	Klebsiella pneumoniae strain sks1	HM007813.1	99
	Enterobacter asburie strain NPKC2	MN691841.1	99
	Bacillus cereus strain PJA1.5	MT337533.1	99
	Klebsiella pneumonie strain MS14393	CP054303.1	100
Cheese stall	*Klebsiella pneumoniae strain WSHvKP*	CP054063.1	100
	Klebsiella pneumoniae strain 7609	MT516162.1	100
	Escherichia coli strain Y4-2	MT192517.1	100
	Klebsiella pneumoniae strain 2484	MT634697.1	100
	Pseudomonas aeruginosa strain MLTBM2	MT646431.1	100
	Enterobacter hormaechei strain RHBSTW-00012	CP058191.1	100
	Escherichia coli strain N15-1	MT192520.1	100
	Enterococcus faecalis strain *2358*	MT604811.1	99
Tulum cheese	*Morganella morganii strain OF8*	MN547625.1	100
	Morganella morganii strain RIMI3	MN158172.1	99
	Citrobacter freundii strain E2WCTM1	MH985222.1	99
	Escherichia coli strain NF73	MT649856.1	100
Metal spoon in olive bowl	*Klebsiella pneumoniae strain 7609*	MT516162.1	100
	Bacillus amyloliquefaciens strain A13	MT591294.1	99
Plastic olive bowl	*Bacillus velezensis strain KKLW*	CP054714.1	100
	Acinetobacter calcoaceticus strain CA32	MT197389.1	100
	Klebsiella pneumoniae strain 7609	MT516162.1	99
Olive	*Bacillus subtilis strain CFR08*	MT641227.1	99
	Acinetobacter schindleri strain RP1	MG461636.1	98

Table 1. Bacterial strains identified by molecular data (Continued).

Sample	Bacteria	Accession No	Similarity (%)
Tulumba dessert	*Bacillus cereus* strain GD1	MT434695.1	100
Şambali dessert	*Enterococcus faecium* strain CAU1957	MF424369.1	100
	Staphylococcus warneri strain DK131	MT642942.1	100
Tiriliçe dessert	*Pantoea conspicua* strain B6	MF083088.1	100
Wooden stall in toaster	*Bacillus velezensis* strain DH8043	CP047268.1	100
	Enterococcus faecium strain 4525	MT545041.1	100
Plastic stall in toaster	*Klebsiella michiganensis* strain F107	CP024643.1	99
	Klebsiella michiganensis strain W14	MT572941.1	100
Ceramic tile in steak tartar a la turca	*Bacillus velezensis* strain HSB1	MT626060.1	100
Wood cutting place of Kokoreç	*Bacillus amyloliquefaciens* strain BV2007	MT613661.1	100

Qualitative analysis of biofilm formation

To determine the qualitative analysis of biofilm formation, the Congo Red Agar method was used. Black-colored colonies were considered biofilm-positive, and red-pink colored colonies were considered biofilm-negative (Fig. 5 and 6). According to the qualitative analysis, out of 67 bacterial species, only 9 bacterial species showed biofilm-positive results. Quantitative analysis was performed for these biofilm-positive isolates.

Fig. 5. A view of a representative biofilm negative isolate (*Enterococcus faecium*) on CRA.

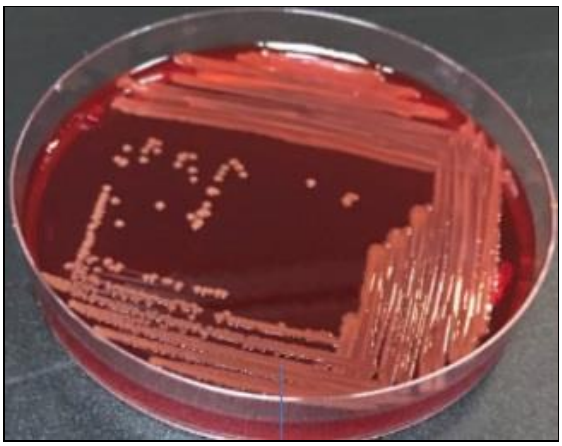

Fig. 6. A view of representative biofilm positive isolate (*Klebsiella pneumoniae*) on CRA.

Quantitative analysis of biofilm formation

Microplate method was applied for quantitative analysis to the 9 bacterial species that showed positive results for biofilm formation. The analysis results are presented in Table 2. Strains with an optical density value of ≥0.240 were considered as strong biofilm producers, strains with a value of 0.120-0.240 were considered as moderate biofilm producers (Keskin *et al*. 2017).

According to the results of the biofilm tests, 13% of the isolated bacteria were found to have the ability to form biofilms. The bacterial strains that were found to produce biofilms were *Enterococcus faecalis* strain 3, *Bacillus cereus* strain 12, *Citrobacter freundii* strain 25, *Staphylococcus epidermidis* strain 42, *Bacillus cereus strain* 43, *Enterococcus gallinarum* strain 66, *Enterococcus faecium* strain 72, and *Pantoea conspicua* strain 74.

Table 2. Biofilm-forming bacterial species and their adherence values.

Bacteria	Sample	Adherence
Enterococcus faecalis strain 2623	0.612	**Strong**
Bacillus cereus strain PJA1.5	0.32	**Strong**
Citrobacter freundii strain E2WCTM1	0.282	**Strong**
Staphylococcus epidermidis strain3039	0.287	**Strong**
Bacillus cereus strain TBMAX51	0.935	**Strong**
Pantoea conspicua strain B6	0.293	**Strong**
Enterococcus faecium strain 4525	0.384	**Strong**
Enterococcus gallinarum strain CCFM8325	0.142	**Moderate**
Enterococcus faecium strain CAU1957	0.176	**Moderate**

Optical Density Value of Negative Control: 0.065, Positive Control: 0.086 (Negative Control: Medium, Positive Control: *Staphylococcus aureus* ATCC 25923) (Strong: ≥0.240, Moderate: 0.120-0.240, Week: ≤0.120).

Discussion

The data obtained from our study are similar to previous study data. There are many studies on the detection of biofilm-forming bacteria in different food samples and materials. Çoban &Yaman (2023) investigated the biofilm formation abilities of bacteria isolated from fish and fish stalls and showed that 36 isolates in total within the genera *Citrobacter, Staphylococcus, Lactococcus, Leuconostoc, Enterococcus*, and *Proteus* produced biofilms. In the same study, only *Alcaligenes faecalis* strain PS25 was isolated from the fish counter and it was found to not produce biofilm (Çoban & Yaman 2023). Uyanık *et al*. (2022) investigated the ability of bacteria isolated from various food samples (milk, dairy products, ready-to-eat foods) and slaughterhouse environments to form biofilms. For this purpose, Congo Red Agar was used for qualitative detection, and microplate method was used for quantitative detection. It was determined that 12.5% of the isolates produced biofilms, and according to the quantitative analysis, *Enterococcus faecium* and *E. faecalis* strains were strong biofilm producers, *Listeria monocytogenes* (serotype 1/2a) strain was a moderate biofilm producer, and *Escherichia coli*, *Staphylococcus aureus*, and *L. monocytogenes* (serotypes 1/2a and 4b) strains were weak biofilm producers. However, all *Salmonella* spp. strains were found to be negative for biofilm production. Gürkan *et al*. (2021) isolated 80 bacteria from raw milk, cheese, raw chicken meat, and raw meat, and identified 70% *Enterococcus faecalis* and 30% *Enterococcus faecium* bacteria. The biofilm-forming ability of the identified *Enterococcus* species was screened. In the qualitative analysis, it was observed that 60 *Enterococcus* strains produced 2 strong, 22 moderate, and 36 weak biofilms on Congo Red Agar. In the quantitative analysis, 2 strong, 8 moderate, and 32 weak biofilm formations were seen in *E. faecalis* strains, while 1 moderate and 17 weak biofilm formations were determined in *E. faecium*, but no strong biofilm formation was detected. In our study conducted on surfaces in contact with food in a roasted chicken and chicken doner restaurant, *Enterococcus faecalis* strain 3, *Enterococcus faecium* strain 78 showed strong biofilm formation while *Enterococcus faecium* strain 72 showed moderate biofilm formation. Yanıkan (2020) identified several bacteria including *Acinetobacter baumannii*, *Staphylococcus warneri*, and *Escherichia coli* on various surfaces in contact with food in a meat processing facility. In our study, *Escherichia coli* strains were isolated from the cheese counter and mussel surface, *Staphylococcus warneri* from the sausage, and *Acinetobacter calcoaceticus* and *Acinetobacter schindleri* from the surface of olives.

Chen *et al*. (2020) evaluated the biofilm-forming ability of *Staphylococcus aureus* strains isolated from food samples, and identified 97 *S. aureus* isolates of which 72% formed biofilm. Of the isolates identified, 54.64% formed weak biofilm, 14.43% formed moderate biofilm, and 3.1% formed strong biofilm. *Staphylococcus epidermidis* strain 3039 was isolated from the mussel shell sample, and *Staphylococcus warneri* strain DK131 was isolated from the sweet sausage sample. It was observed that *Staphylococcus epidermidis* strain 3039 formed a strong biofilm.

Samples were taken from nine wooden cutting boards used in meat sales at the nine wet-markets in Hong Kong Island, including Ap Lei Chau Market (AL), North Point Market (NP), Sai Ying Pun Market (SY and CSF), Shek Tong Tsui Market (ST), Sheung Wan Market (SW), Smithfield Market (SF) and Wan Chai Market (WC and TW) and bacterial isolation was performed (Lo *et al*. 2019). *Klebsiella pneumoniae* was the most isolated bacteria from the surface of the cutting boards, while *Enterobacteriaceae, Escherichia*, and *Shigella* spp. were the most frequently encountered bacteria in the gaps between the cutting boards (Lo *et al*. 2019). In the study, it was also shown that the scraping method did not provide the necessary hygiene (Lo *et al*. 2019). In addition, it was determined that the bacteria that formed biofilm in the gaps in the cutting boards created a shield against sanitizing agents, and the traditional cleaning method of scraping was insufficient for sanitation (Sekoai *et al*. 2020). Sekoai *et al*. (2020) isolated *Lactococcus garvieae*, *Weissella hellenica* and *Kurthia gibsonii* from the wooden cutting board.

Zhao *et al*. (2017) found that the surfactin compound produced by *Bacillus subtilis* prevented the biofilm formation of *Salmonella enterica* and *Escherichia coli* strains. In our study, *Escherichia coli* strains were isolated from the mussel counter, cheese counter, and tulum cheese samples, but they were not observed to form biofilm. It is thought that the negative biofilm formation of *Escherichia coli* strains may be due to the compound produced by other bacteria in the environment. Ayhan (2016) reported that no biofilm formation was observed on the stainless-steel surface of a dairy farm after disinfection, but only bacterial cell attachment was observed. In our study, bacterial species belonging to *Morganella, Citrobacter, Escherichia, Klebsiella, Pseudomonas*, and *Enterococcus* were detected from cheese and cheese counters, and only *Citrobacter freundii* strain 25 was found to form a strong biofilm.

Gündogan & Ataol (2012) investigated the biofilm-forming abilities of *Staphylococcus aureus* isolated from minced beef and chicken legs obtained from various supermarkets. As a result of the study, a total of 56 *S. aureus* strains were isolated from minced beef and 41 strains were isolated from chicken legs, most of which were found to form biofilms due to sanitation deficiencies during food preparation. *Enterobacter* and *Enterococcus* species were found in chicken doner samples, while *Proteus* and *Enterococcus* species were found in chicken samples.

Conclusion

In the present study, it was determined that bacteria isolated from samples of chicken doner, simit counter, tulum cheese, mussel shell, tirilice dessert, and grilled

cheese sandwich counter formed strong biofilms. In addition, bacteria isolated from samples of shambali dessert and doner kebab counter were found to form moderate biofilms. Based on these findings, it was concluded that sufficient sanitation measures were not being taken in various food samples sold in open markets and on the street, as well as on the counters where these foods are placed. It was also observed that the utensils (knives, spatulas, etc.) and hand hygiene used by sellers were not properly implemented. We believe that the data obtained in this study is important for providing insights to researchers working on this topic and for its contribution to the existing literature.

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Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: All data are available within the study.

Author Contributions: Conception/Design: E.P.Ç., Data Acquisition: M.O., Data Analysis/Interpretation: E.P.Ç., M.O., Drafting Manuscript: E.P.Ç., Critical Revision of Manuscript: E.P.Ç., Final Approval and Accountability: E.P.Ç., Technical or Material Support- E.P.Ç., M.O.

Conflict of Interest: The authors have no conflicts of interest to declare.

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