BACTERIAL COMMUNITY PROFILES ON THE SURFACE OF ALUMINUM TRAY FOLLOWING CONTACT WITH BEEF, CHICKEN, AND PORK

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

July 2022

FPSK (m) 2022 45

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Professor Syafinaz Amin Nordin, MBChB, MPath, MHEd
Faculty : Medicine and Health Sciences

Meat products are frequently linked to outbreaks of foodborne diseases. Bacteria have long been used in food products as a hygienic marker. As a result, increasing knowledge about the contribution of bacteria in food can impact determining proper food safety guidelines. Based on 16S rRNA amplicon metagenomic sequencing analyzed using the Illumina MiSeq system, this study aimed to determine the functional bacterial community structure, diversity, biomarkers, and taxa co-occurrence on food-associated surfaces used in industrial food preparation areas, i.e., aluminum tray, following contact with beef, pork, and chicken. *Acinetobacter*, *Enterobacteriaceae* family, *Empedobacter*, and *Pseudomonas* were the most abundant taxa on the beef-contact surface. *Shewanella*, *Acinetobacter*, *Pseudomonas*, and *Aeromonas* were prevalent in chicken-contact trays, whereas *Acinetobacter*, *Myroides*, *Pseudomonas*, and *Serratia* were prevalent in pork-contact trays. Trays that had been in contact with chicken had the highest species evenness and richness, followed by pork and beef. The diversity between chicken, beef, and pork-contacted trays was significantly different. *Kocuria*, *Soonwooa*, *Myroides*, *Bacillus*, *Escherichia-Shigella*, *Rummeliibacillus*, in beef, *Alteromonadales*, *Klebsiella*, *Flavobacterium*, and *Aquitalea* in chicken, while *Comamonadaceae*, *Xanthomonadaceae*, *Flavobacterium*, *Staphylococcus*, *Wohlfahrtiimonas*, *Weissella*, and *Vitreoscilla* genera in pork, were detected as hub nodes. Most of the hub nodes could cause human or animal diseases, and some of them were antibiotic resistant. In addition, bacterial markers were identified in each meat type, and some of them were pathogens such as *Shewanella* in chicken, *Klebsiella* in pork, and Staphylococcus in beef. The prediction of functional metagenomic content demonstrated that the enzymes, KEGG orthologs, and biochemical pathways in the various meats were similar. Some proteins, however, were discriminant between meat groups. Methyl-accepting chemotaxis protein was significantly more abundant in chicken samples than in beef samples, which is beneficial in bacteria colonization. The enzymes beta-glucosidase and glutamine synthetase, which are commonly associated with sepsis, were significantly higher in chicken than in pork. In conclusion, bacterial pathogens were found on the aluminum trays despite applying usual storage procedures, indicating that proper hygienic practice and regular cleaning of food preparation areas and utensils are required to avoid cross-contamination. This study has provided detailed information about the bacterial taxa found on the surface of trays after contact with meats, which will be useful in developing appropriate guidance for safer food handling in the future. Further research into the differences in the bacterial community in other meats can be conducted.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
memenuhi keperluan untuk ijazah Master Sains

PROFIL KOMUNITI BAKTERIA DI ATAS PERMUKAAN DULANG ALUMINIUM SELEPAS BERSENTUHAN DENGAN DAGING LEMBU, AYAM, DAN KHINZIR

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Produk daging biasanya dikaitkan dengan wabak penyakit bawaan makanan. Bakteria telah sekian lama digunakan dalam produk makanan sebagai penanda kebersihan. Akibatnya, peningkatan pengetahuan mengenai sumbangan bakteria dalam makanan memberikan impak bagi menentukan panduan keselamatan makanan sebenar. Berdasarkan analisa jujukan metagenomik amplikon rRNA 16S menggunakan sistem Illumina MiSeq, kajian ini bertujuan untuk menentukan struktur komuniti bakteria fungsian, diversiti, biopenanda, dan keterjadian bersama taxa ke atas permukaan berkaitan makanan yang digunakan dalam kawasan penyediaan makanan industrial, iaitu, dulang aluminum, akibat kontak dengan daging lembu, khinzir, dan ayam. *Acinetobacter*, famili *Enterobacteriaceae*, *Empedobacter*, dan *Pseudomonas* merupakan taxa paling berlebihan di atas permukaan berkontak daging lembu. Shewanella, *Acinetobacter*, *Pseudomonas*, dan *Aeromonas* adalah ketara dalam dulang berkontak daging ayam, manakala *Acinetobacter*, *Myroides*, *Pseudomonas*, dan *Serratia* adalah ketara dalam dulang berkontak daging khinzir. Dulang yang berkontak dengan daging ayam mempunyai spesies keserataan dan kekayaan tertinggi, diikuti oleh khinzir dan lembu. Diversiti antara dulang berkontak ayam, lembu, dan khinzir adalah berbeza dengan signifikan. *Kocuria*, *Soonwooa*, *Myroides*, *Bacillus*, *Escherichia-Shigella*, *Rummeliibacillus*, dalam daging lembu, *Alteromonadales*, *Klebsiella*, *Flavobacterium*, dan *Aquitalea* dalam ayam, manakala *Comamonadaceae*, *Xanthomonadaceae*, *Flavobacterium*, *Staphylococcus*, *Wohlfahrtiimonas*, *Weissella*, dan *Vitreoscilla* genera dalam khinzir, telah dikesan sebagai noda hub . Kebanyakan noda hub menyebabkan penyakit manusia atau haiwan dan sebahagian daripada mereka adalah kerintangan antibiotik. Di samping itu, penanda bakterial telah dikenal pasti dalam setiap jenis daging, dan antaranya ialah patogen seperti *Shewanella* dalam ayam, *Klebsiella* dalam khinzir, dan *Staphylococcus* dalam lembu. Ramalan kandungan metagenomik fungsian memperlihatkan bahawa enzim, ortolog KEGG, dan laluan biokimia dalam pelbagai daging adalah serupa. Beberapa protein, walau bagaimanapun, merupakan diskriminan antara kumpulan daging. Protein kimotaksis penerima Metil secara signifikan adalah lebih banyak dalam sampel daging ayam daripada sampel daging lembu, yang berguna dalam kolonisasi bakteria . Enzim beta glukosidasa dan sintesis glutamina, yang biasanya dikaitkan dengan sepsis, adalah secara signifikan lebih tinggi dalam daging ayam daripada daging khinzir. Kesimpulannya, patogen bakteria telah ditemui di atas dulang aluminium walaupun selepas mengaplikasi prosedur penyimpanan biasa, menunjukkan bahawa amalan kebersihan yang betul dan pembersihan berkala pada kawasan dan peralatan penyediaan makanan diperlukan untuk mengelakkan pencemaran silang. Kajian ini memberikan maklumat mendalam mengenai taxa bakteria di atas permukaan dulang selepas kontak dengan daging, yang berguna dalam membangunkan panduan yang sesuai bagi pengendalian makanan yang lebih selamat pada masa hadapan. Kajian selanjutnya mengenai perbezaan komuniti bakteria di dalam daging lain wajar dijalankan.

ACKNOWLEDGEMENTS

This project would not have been possible without the support of my supervisor, Prof. Dr. Syafinaz Amin Nordin. I appreciate your patience, guidance, and support. Your wealth of knowledge has benefited me greatly. You are a great leader and an excellent role model. I am grateful that you accepted me as a student. I am proud of and appreciative of the opportunity to work with you.

Also, I am indebted to my co-supervisor, Dr. Amalia Binti Mohd Hashim, who provided invaluable feedback and support on my project in the early morning and late nights, during holidays and weekends. Your levels of expertise, ingenuity, and patience are something that I will always strive to attain. Thank you for always being a helper.

Also, I greatly appreciate Dr. Noor Azira Abd Mutalib for her great feedback, guidance, and help. A special thanks also to Dr. Nur Fadhilah Khairil Mokhtar, Dr. Nurul Asyifah Mustapha, and Dr. Toshinari Maeda for your support and guidance.

Last but not least, I am indebted to my parents for their endless love and support, which keep me confident and motivated. My achievements and success are a result of their faith in me and support. Without exception, you have always been by my side. My brother, I want to express my heartfelt gratitude for taking care of everything in my absence. Also, I owe my deepest gratitude to my wife, Yasaman. I will be extremely thankful for the constant love and support I have received throughout my project and daily. And finally, your family also deserves gratitude for their support.

This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

NGS	Next Generation Sequencing
WHO	World Health Organization
CDC	Centers for Disease Control and Prevention
E. coli	*Escherichia coli*
AMR	Antimicrobial-resistance
MDR	Multidrug-resistant
MRSA	Methicillin-resistant Staphylococcus aureus
SSO	Specific Spoilage Organisms
FPE	Food Processing Environments
MLVA	Multiple-locus Variable
MLST	Multilocus Sequence Typing
WGS	Whole Genome Sequencing
SNP	Single Nucleotide Polymorphism
OTU	Operational Taxonomy Units
PCR	Polymerase Chain Reaction
NCBI	National Centre for Biotechnology Information
LEFSe	Linear Discriminant Analysis Effect Size
PICRUST	Phylogenic Investigation of Communities by Reconstruction of Unobserved States
LDA	Linear Discriminant Analysis
MCP	Methyl-accepting chemotaxis
E. rhusiopathiae	*Erysipelothrix rhusiopathiae*
C. jejuni	*Campylobacter jejuni*
L. monocytogenes	*Listeria monocytogenes*

NCBI	National Centre for Biotechnology Information
U.S.	United States
S. aureus	*Staphylococcus aureus*
A. butzleri	*Arcobacter butzleri*
MCP	Methyl-accepting chemotaxis

CHAPTER 1

INTRODUCTION

1.1 Background

Bacteria have been used in the food industry as a hygienic indicator since they are one of the primary causes of different illnesses in people and animals. Bacterial contamination of food can result in death and disease and negative economic and public health implications. Foodborne disease outbreaks are typically associated with meat products that may include pathogens or antibiotic-resistant bacteria. Enhancing our understanding of bacterial diversity and its functions in food can substantially impact the development of appropriate standards, disease identification, and meat products risks assessment.

Food-producing animals (such as cows, pigs, and chickens) serve as the principal reservoir for various foodborne pathogens, including *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli*. The zoonotic capability of foodborne illnesses, as well as their capacity to inflict illness or death, show the necessity to emphasize the problem. Between 1996 and 2000, beef was reportedly responsible for 7% of the 1.7 million foodborne disease incidents recorded in Wales and England. (Heredia & García, 2018). *Campylobacter*, *Yersinia enterocolitica*, *Salmonella*, and *Listeria monocytogenes* are some of the most common foodborne pathogens associated with livestock, such as beef, chicken, and pork. These pathogens can cause symptoms such as nausea, vomiting, diarrhea, bacteremia, and gastrointestinal diseases (Chlebicz & ŚLiżewska, 2018).

Aluminum is widely utilized by individuals and companies daily, including in the food and beverage packaging industry (Rahimzadeh et al., 2022). Various studies were focused on the bacterial community of the food trays and their effects on the transmission of pathogens. For example, the *Clostridium perfringens* were identified on tray surfaces (Xu et al., 2021). The presence of antibiotic-resistant bacteria on egg tray surfaces was also studied in another investigation (Sultana, 2018). This study employed an aluminum tray as the model surface since it is often used in food preparation and handling (Dordevic et al., 2019). However, the purpose of this study was not to investigate the effects of aluminum trays, and they were not considered a variable in this study. These findings emphasize the importance of studying the aluminum surfaces utilized in the food industry for the benefit of human health and well-being.

Numerous factors, including those found in the environment (water from various sources and animal products), as well as those associated with humans (butchering, processing, and storage procedures), may contribute to contamination (Heredia & Garcia, 2018). Water, dust, air, equipment, insects, and humans contribute significantly to pollution. Various factors can lead to the contamination of raw materials, such as live animals, surfaces in the manufacturing facility, and even the internal organs of slaughtered animals (Marriott et al., 2018).

Human, animal, and environmental health are all at risk due to the spread of Antimicrobial Resistance (AMR). Because of this, Multidrug-resistant (MDR) bacteria have emerged, spread, and remained persistent. MDR pathogens reside inside animals, humans, and the environment, and these sources are tightly linked (Aslam et al., 2018). According to the Ministry of Health Malaysia, antibiotic resistance has been rising in recent years (Institute for Medical Research Malaysia, 2020). These bacteria are spread from food to people via raw material processing as well as cross-contamination and re-contamination across food items at the distribution, production, and consumer levels (Jans et al., 2018).

One of the often-used approaches for studying microbiota is 16S rRNA amplicon metagenomics, which is based on Next Generation Sequencing (NGS) on the Illumina MiSeq platform. This strategy surpasses the culture-dependent approach in speed, sensitivity, and cost-effectiveness. The method is not dependent on the cultivability of the bacteria in a sample, which is one of the primary advantages (Gupta et al., 2019).

Investigation of biological information has entered a new era thanks to the remarkable improvements in computing power and memory capacity. The sequencing of genomes of bacteria has increased hopes for improved bacteria management. In the last ten years, bioinformatics has quietly taken up the responsibility of value data processing and improved our knowledge of bacterial genomes. Bioinformatics science and its various instruments could be used to make NGS output data more understandable and interpretable. Bioinformatics, while being a new science, has aided biomedical sciences like microbiology via the creation of discoveries, tools, and algorithms (Christensen, 2019).

This investigation should result in a better knowledge of meat's bacterial ecology and health. Additionally, determining the number, connections, and functions of bacteria on the surface of meat trays and their contamination level can aid in developing more effective hygiene instructions for the various phases of meat preparation, from manufacturing to consumption.

1.2 Problem statement and significance of the study

According to a WHO study in 2015, approximately 600 million infections were reported in 2010 due to contaminated food, with pathogenic bacteria responsible for nearly 350 million of those (Chlebicz & ŚLiżewska, 2018). According to a Centers for Disease Control and Prevention (CDC) report (CDC, 2018), yearly, one in every six American people (or 48 million people) falls ill, 3,000 people die, and 128,000 people are hospitalized as a result of foodborne infections. Meat products have been discovered as a critical source of infections found in food (Morales-Partera et al., 2017). In Malaysia, the actual number of foodborne disease cases is unclear because of insufficient effort to address this problem (Saw et al., 2020). However, it has been shown that the trend is increasing since water and foodborne diseases rose by 13% in 2019, with food infection accounting for 97% of cases (Abdul Mutalib et al., 2022).

As the human population expands, meat consumption, particularly chicken and pork, increases among people (Godfray et al., 2018). There has been an increase in the importance of meat health and cleanliness, and the need to educate people as meat consumption has increased. By 2030, animal product consumption is expected to reach 376 million tons (Heredia & García, 2018).

Numerous investigations have been done to ascertain the bacterial population in various meats. However, there is a dearth of knowledge on this subject in Malaysia, particularly regarding the bacterial community associated with food trays, e.g., aluminum-based, following contact with raw meats such as beef, chicken, and pork.

1.3 Research Objectives

This study generally aimed to identify the bacterial community of the trays following contact with beef, chicken, and pork samples. Specifically,

The specific objectives of the study were:

1. To identify the bacterial communities and their putative functions on the aluminium tray's surface following contact with beef, chicken, and pork meats.

2. To identify the dominating genera, differentially abundant biomarkers, co-occurrence network of bacteria in the samples, and pathogens on the tray surfaces.

3. To predict the metagenomic functions of the bacterial community.

1.4 Research hypotheses

Generally, it was hypothesized that by determining the bacterial community of the tray surfaces following contact with beef, chicken, and pork, pathogenic bacteria would be determined. Specifically:

1. There were bacteria population on the tray surfaces after contact with beef, chicken, and pork with pathogenic functions.

2. Some of the bacteria were dominated and were biomarkers in each meat type. In addition, there were some correlations between the co-occurrence of the bacteria, and some of them were pathogens.

3. Bacteria that exist on tray surfaces had metagenomic functions.

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