

**PHENOTYPIC AND GENOTYPIC
CHARACTERISTICS OF FOODBORNE
BACTERIA ASSOCIATED WITH BIOFILM
FORMATION IN FRESH *ULAM***

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**MASTER OF FOOD SCIENCE
UNIVERSITI MALAYSIA TERENGGANU**

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**Thesis Submitted in Fulfilment of the Requirement for the Degree of
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DEDICATION

This thesis is dedicated to my father, Bahri Chik who taught me that the best kind of knowledge is the one that is learned for its own sake. It is also dedicated to my mother, Sakinah Ismail who taught me that even the largest task can be accomplished if it is done one step at a time.

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

PHENOTYPIC AND GENOTYPIC CHARACTERISTICS OF FOODBORNE BACTERIA ASSOCIATED WITH BIOFILM FORMATION IN FRESH *ULAM*

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Faculty : Faculty of Fisheries and Food Science

Ulam are important food among the Malays because of their nutritional values, and their consumption is increasing. However, the foodborne outbreak which is associated with eating raw *ulam* may pose health risks to the consumers by increasing cross-contamination, antibiotic-resistant bacteria and biofilm formation. The objectives of this study were to determine the phenotypic characterisation and genotypic identification of *E. coli* and *Salmonella* isolated from *ulam* and their association with biofilm. A total of 32 samples of *ulam* were randomly collected from wet markets and supermarkets in Kuala Terengganu. The microbiological study was done to enumerate microbial counts and to isolate *E. coli* and *Salmonella* using biochemical tests and API20E kit. They were further assessed for phenotypic characteristics such as antibiotic susceptibility testing, colony morphology and biofilm formation. Polymerase chain reaction (PCR) was performed to determine the genotypic identification associated with tetracycline resistance and biofilm formation in *E. coli* and *Salmonella* isolates. The microbiological quality of *ulam* samples were in the range of 6.36-8.83; 4.14-7.48; 0-8.16; 3.94-6.45 log₁₀ CFU/g for aerobic mesophilic bacteria, coliforms, *Salmonella* and yeast and moulds, respectively. *E. coli* and *Salmonella* were detected in 31.3% and 9.4% of *ulam* samples, respectively. All of the selected isolates were confirmed as *E. coli* and *Salmonella enterica* based on the molecular identification using the 16S rRNA and *invA* gene, respectively. Overall, it was found that the *E. coli* isolates were highly resistant to cephalothin (78.3%), followed by ampicillin (52.2%), tetracycline (52.2%) and streptomycin (30.4%).

However, only one *Salmonella* isolate showed resistant to antibiotics. In addition, 47.8% of *E. coli* and 16.7% of *Salmonella* were classified as multiple drug resistance (MDR). The *E. coli* and *Salmonella* also showed multiple antibiotic resistance (MAR) index ranging from 0.06-0.48 and 0.00-0.19, respectively. The findings revealed that 69.6% of *E. coli* and 66.7% of *Salmonella* isolates were strong biofilm producers. The *E. coli* isolates were correlated with the expression of red, dry and rough (rdar) morphotype, while all *Salmonella* isolates expressed intermediate morphotypes. The presence of *csgA* gene in *E. coli* demonstrates the ability of strong biofilm and rdar morphotype. With the absence of the *csgA* gene in *Salmonella*, the organism lacks rdar morphotype, but still able to demonstrate strong biofilm formation. This study also revealed that the tetracycline resistance was confirmed with the presence of the *tetA* gene in the selected *E. coli* isolate. Majority of the *E. coli* and *Salmonella* isolates were classified as strong biofilm producer. These findings confirmed that *ulam* could serve as a host for foodborne transmission to humans due to *E. coli* and *Salmonella* associated with *ulam* have the ability to form biofilm and withstand antimicrobial therapy.

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**PENGENALPASTIAN FENOTIP DAN GENOTIP BAKTERIA BAWAAN
MAKANAN YANG BERKAITAN DENGAN PEMBENTUKAN BIOFILM DI
DALAM ULAM SEGAR**

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Fakulti : Fakulti Perikanan dan Sains Makanan

Ulam adalah salah satu makanan penting dalam kalangan orang Melayu disebabkan oleh kandungan nutrisinya dan pengambilannya sedang meningkat. Walau bagaimanapun, wabak bawaan makanan yang berkaitan dengan memakan ulam yang segar mungkin menimbulkan risiko kesihatan kepada pengguna dengan peningkatan pencemaran silang, bakteria rentan terhadap antibiotik dan pembentukan biofilm. Objektif kajian ini adalah untuk menentukan pencirian fenotip dan pengenalpastian genotip *E. coli* dan *Salmonella* yang dipencilkan dari ulam dan kaitannya dengan pembentukan biofilm. Sebanyak 32 sampel ulam telah dikumpul secara rawak dari pasar basah dan pasar raya di Kuala Terengganu. Kajian mikrobiologi dilakukan untuk menghitung jumlah mikrob dan memencilkan *E. coli* dan *Salmonella* dengan menggunakan ujian biokimia dan kit API20E. Selanjutnya, *E. coli* dan *Salmonella* telah dinilai untuk ciri-ciri fenotip seperti ujian kerentanan terhadap antibiotik, morfologi koloni dan pembentukan biofilm. Kaedah tindak balas rantai polimerase (PCR) telah dilakukan untuk pengenalpastian genotip yang berkaitan dengan kerentangan terhadap tetrasiklina dan pembentukan biofilm dalam *E. coli* dan *Salmonella*. Kualiti mikrobiologi sampel ulam berada dalam lingkungan 6.36-8.83; 4.14-7.48; 0-8.16; 3.94-6.45 log₁₀ CFU/g untuk bakteria mesofil aerobik, koliform, *Salmonella*, yis dan kulapuk. *E. coli* dan *Salmonella* telah dikesan di dalam 31.3% dan 9.4% sampel ulam. Semua kultur yang dipilih telah disahkan sebagai *E. coli* dan *Salmonella enterica* berdasarkan pengenalpastian molekul menggunakan kaedah gen

16S rRNA dan *invA*. Secara keseluruhan, *E. coli* didapati sangat rentang terhadap cefalotin (78.3%), diikuti oleh ampisilin (52.2%), tetrasiklina (52.2%) dan streptomisin (30.4%). Walau bagaimanapun, hanya satu *Salmonella* telah menunjukkan kerentanan terhadap antibiotik. Di samping itu, 47.8% *E. coli* dan 16.7% *Salmonella* telah dikelaskan sebagai MDR. *E. coli* dan *Salmonella* juga telah menunjukkan indeks MAR di antara 0.06-0.48 dan 0.00-0.19. Hasil kajian menunjukkan bahawa 69.6% *E. coli* dan 66.7% *Salmonella* telah menghasilkan biofilm yang tinggi. *E. coli* yang membentuk biofilm yang tinggi telah dikaitkan dengan dengan penghasilan morfotip merah, kering dan kasar (rdar), sementara semua *Salmonella* menunjukkan morfotip pertengahan. Kehadiran *csgA* gen di dalam *E. coli* menunjukkan keupayaan untuk membentuk biofilm yang tinggi dan morfotip rdar. Dengan ketiadaan gen *csgA* di dalam *Salmonella*, organisma tersebut tidak membentuk morfotip rdar, tetapi masih dapat menunjukkan pembentukan biofilm yang tinggi. Kajian ini juga menunjukkan bahawa rintangan tetrasiklina telah disahkan dengan adanya gen *tetA* dalam *E. coli* yang telah dipilih. Majoriti *E. coli* dan *Salmonella* diklasifikasikan sebagai pengeluar biofilm yang kuat. Penemuan ini mengesahkan bahawa ulam boleh berfungsi sebagai hos untuk penyebaran bakteria makanan kepada manusia kerana keupayaan *E. coli* dan *Salmonella* yang dipencilkan daripada ulam berupaya membentuk biofilm dan menjadi rentan terhadap rawatan antimikrob.