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EFFECT OF DETERGENT, HERBS AND SPICES ON THE GROWTH OF Vibrio parahaemolyticus BIOFILM IN SEAFOOD

ELEXSON NILLIAN

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By

ELEXSON NILLIAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

September 2014

Dedicated to God for the love, wisdom and strength

Dedicated to my family and my relatives for their unconditional love and endless support

Dedicated to my friends for the wonderful friendship, love and joy

Dedicated to everyone whom have invested their lives in my life

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

EFFECTOF DETERGENT, HERBS AND SPICES ON THE GROWTH OF *Vibrio parahaemolyticus* BIOFILM IN SEAFOOD

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September 2014

Chairman: **Professor Son Radu, PhD**
Faculty: **Food Science and Technology**

The aim of this study was to investigate the effects of detergent, herbs, and spices on antimicrobial activity and specificity to mitigate the growth of *V. parahaemolyticus* biofilm. A total of 394 samples were collected from wet markets (n=201) and hypermarkets (n=193), consisting of cockles (*Anadara granosa*), clams (*Mya arenaria*), shrimps (*Penaeous spp.*) and squids (*Loligo opalescens)*. The prevalence of 9.14% (36/394) of positive *toxR* genes, 63.8% of positive isolates contained virulent genes of *V. parahaemolyticus tdh+*, 22.2% of *V. parahaemolyticus trh+*, and 5.56% (2/36) of positive isolates in both *V. parahaemolyticus tdh+* and *trh+* had been detected. The thirty six (n=36) isolates were further tested with antibiotic susceptibility test and they werefound to be resistant to at least seven out of eighteen antibiotics. *V. parahaemolyticus* isolates (97.2%) showed Multiple Antibiotics Resistance index > 0.2, indicating that these isolates might have originated from high risk source. Next, RAPD-PCR was carried out to investigate the relationship between genetic diversity and clonal among the strains. As dendogram was generated among the four groups, group Type B was the largest group that possessed 92.86% (13/14) of isolates from bivalve seafood, such as cockles and clams. The samples were derived from wet markets and they are closely related. RAPD-PCR fingerprinting and antibiotic resistance profiling indicated multiple antibiotics resistant *V. parahaemolyticus* were wide-spread among the groups. The isolates were examined in the assessment of biofilm producer at different temperatures. *V. parahaemolyticus* could form biofilm and presence of biofilm was detected at 37 ºC (optimum temperature), followed by room temperature (25 ºC), and 4°C (chiller temperature). The representatives of clonal from each group, which wereresistant antibiotics of*V. parahaemolyticus*, were examined for effect of detergents. Out of the three detergents; Detergent 1 (Linear Alkylbenzene sulphonate based), Detergent 2 (Quaternary Ammonium based), and Detergent 3 (Sodium Hydroxide based) with minimum concentration of 100 mg/ml (100,000 µg / ml), Detergent 1 was found to be the most effective. As for Detergent 1, the Minimum Inhibition Concentrations (MICs) ranged from 97.656 - 1562.5 µg/ml, while the Minimal Bactericidal

Concentrations (MBCs) were at 781.25 - 3125 µg/ml. Meanwhile, inhibition time assay showed that the bactericidal activity of detergent was fast-acting against antibiotics resistant *V. parahaemolyticus* at 8× MIC within 1 hr and the reduction in CFU/ml was 3 log units (99.9%) with P value < 0.05 was considered statistically significant. The growth of antibiotics resistant *V. parahaemolyticus* biofilm was inhibited at 1562.5 – 6250 µg/ml and was eradicated at 3125 – 12500 mg/ml. Then, the study was furthered by using natural antimicrobial herbs and spices in inhibiting and in preventing the growth of *V. parahaemolyticus* biofilm. Out of the twenty herbs and spices, four of them, whichwere cloves, star anise, lemon leaves, and curry leaves, showedsome activities towards the antibiotics resistant isolates. At a minimum of 10 mg/ml (1000 µg/ml), clove was the most effective as it showed MICs at 19.531-78.125 µg/ml and MBC at 78.125-625 µg/ml. The killing time activity with cloves was fast-acting against antibiotic resistant *V. parahaemolyticus* at 8× MIC in 0.5hr time, while 1 hr, 4 hr, and 8 hr with star anise, lemon leaves, and curry leaves extracts. The reduction in CFU/ml was 3 log units (99.9%). Besides, cloves demonstrated concentration that ranged from 78.125 – 156.25 µg/ml, and 312.5 – 625 µg/ml to inhibit and to totally eradicate the growth of antibiotic resistant *V. parahaemolyticus* biofilm. Thus, these findings can be used to mitigate the resistance of biofilm growth in any food supply chain related to seafood in future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KESAN DETERGEN, HERBA DAN REMPAH RATUS KE ATAS PERTUMBUHAN *Vibrio parahaemolyticus* BIOFILEM DALAM MAKANAN LAUT

Oleh

ELEXSON NILLIAN

September 2014

Pengerusi : Profesor Son Radu, PhD
Fakulti : Sains dan Teknologi Makanan

Penyelidikan ini bertujuan untuk mengenalpasti kesan detergen, herba dan rempah ratus dengan aktiviti mikrobial yang tinggi dan spesifikasi khusus terhadap pengurangan pertumbuhan bio filem *V. parahaemolyticus* . Sejumlah 394 sampel telah di kumpul daripada pasar borong (n=201) dan pasaraya (n=193) yang terdiri daripada kerang (*Anadara granosa*), kepah (*Mya arenaria*), udang (*Penaeous spp.*) dan sotong (*Loligo opalescens)*. Kelaziman sebanyak 9.14 % (36/394) positif gen t*oxR*, 63.8% kehadiran gen virulen *V. parahaemolyticus tdh+*, 22.2% *V. parahaemolyticus trh+*, dan 5.56% (2/36) pencilan *V. parahaemolyticus* mempunyai kedua-dua *tdh+* and *trh+* gen. Sejumlah tiga puluh enam (n= 36) pencilan tersebut telah di uji terhadap ujian keberkesanan antibiotik dan kepelbagaian rintangan kepada sekurangnya terhadap tujuh daripada lapan belas antibiotik yang di kenalpasti.Pencilan *V. parahaemolyticus* (97.2%) menunjukkan Kepelbagaian rintangan indek > 0.2, menunjukkan pencilan ini kemungkinan bersal daripada sumber yang berisiko tinggi. RAPD - PCR telah di lanjutkan dengan mengenal pasti kepelbagaian genetik dan pengkaitan klon di antara pencilan. Setelah dendogram di hasilkan, daripada empat kumpulan, kumpulan jenis B adalah kumpulan terbesar mengandungi 92.86% (13/14) pencilan terdiri daripada makanan laut seperti kerang dan kepah. Dimana diperolehi daripada pasar borong dan berkait rapat. Kaedah RAPD-PCR cap jari dan kepelbagain rintangan antibitotik profil menunjukkan pengandaan kepelbagaian rintangan antibiotik *V. parahaemolyticus* tersebar secara meluas di antara kumpulan. Pencilan diperiksa dengan mengakses penghasilan bio filem di dalam suhu yang berbeza *V. parahaemolyticus* boleh di bentuk dan terdapat pertumbuhan bio filem di 37 º C (suhu optimum), di ikuti oleh suhu bilik (25 ºC) and with 4° C (suhu pendingin). Wakil dari setiap klon daripada setiap kumpulan yang mempunyai pengandaan rintangan antibiotik *V. parahaemolitikus* telah di periksa ke atas kesan detergen. Daripada tiga pencuci, detergen 1, (berasaskan Linear Alkylbenzene sulphonate), detergen 2 (berasaskan Quaternary Ammonium) dan detergen 3 (berasaskan Sodium Hydroxide) mempunyai kepekatan minimum

sebnayak 100 mg/ml (100000 µg/ml), detergen 1 di dapati paling efektif. Untuk detergen 1, Perencatan kepekatan minimum (MICs) diantara 97.656 - 1562.5 µg/ml manakala minimum kepekatan penbunuhan (MBCs) di antara 781.25 - 3125 µg/ml. Assay masa membunuh menunjukkan aktiviti pembunuhan pencuci adalah bertindak pantas melawan rintangan antibiotik *V. parahaemolyticus* di 8× MIC di dalam 1 jam dan pengurangan di CFU/ml adalah 3 log units (99.9%) dengan nilai $P < 0.05$ adalah di anggap tidak ada perbezaan signifikan statistik. Pertumbuhan bio filem rintangan antibiotik *V. parahaemolyticus* boleh di rencat di 1562.5 – 6250 µg/ml di basmi di 3125 – 12500 mg/ml. Kemudian, kajian telah di lanjutkan untuk memeriksa menggunakan herba dan rempah ratus untuk merencat dan menghalang pertumbuhan *V. parahaemolyticus* bio filem. Daripada dua puluh herba dan rempah ratus, empat termasuk cengkih, bunga lawang, daun limau dan daun kari menunjukkan aktiviti terhadap pencilan rintangan antibiotik. Di kepekatan minimum 10 mg/ml (1000 µg/ml), cengkih adalah paling efektif menunjukkan MICs di 19.531 - 78.125 µg/ml dan MBC 78.125 - 625 µg/ml. Aktiviti masa membunuh cengkih adalah pantas bertindak terhadap rintangan antibiotik *V. parahaemolyticus* di 8× MIC dalam 0.5 jam manakala 1 jam, 4 jam and 8 jam oleh ekstrak bunga lawang, daun limau dan daun kari, Pengurangan di CFU/ml adalah 3 log units (99.9%). Cengkih demontrasi kepekatan di antara 78.125 – 156.25 µg/ml dan 312.5 – 625 µg/ml untuk merencat dan membasmi sepenuhnya pertumbuhan bio filem *V. parahaemolyticus*. Maka, penemuan ini boleh digunakan untuk mengurangkan pertumbuhan kerintangan bio filem di dalam rantaian bekalan makanan berhubung dengan makanan laut di masa hadapan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Son Radu, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Yaya Rukayadi, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Nor Ainy binti Mahyudin, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Name and Matric No: Elexson Nillian (GS33477)

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LIST OF ABBREVIATIONS

µl	-	Microliter
µm	-	Micrometer
µg	-	Microgram
ADI	-	Acceptable daily intake
ATCC	-	American Type Culture Collection
BAM	-	Bacteriological Analytical Manual
bp	-	base pair
CDC	-	Centre for Disease Control
D1		Detergent 1
D2	-	Detergent 2
D3	-	Detergent 3
DNA	-	Deoxynucleic acid
dNTP	-	deoxyribo nucleoside triphospate
EDTA	-	Ethylene diamine tetra-acetic acid
FAO	-	Food and Agricultural Organization
FDA	-	Food and Drug Administration
HIV	-	Human immunodeficiency virus
ICMSF	-	International Commission on Microbiological
MAR	-	Multiple Antibiotic Resistance
mg	-	milligram
$MgCl_2$	-	Magnesium Chloride
MIC	-	Minimum inhibitory concentration
MBC	-	Minimum inhibitory concentration
MBEC	-	Minimum bactericidal eradication concentration
mM	-	milli Molar
MOH	-	Ministry of Health
MPN	-	Most probable Number
MRO	-	Multiple resistant organism
NaCl	-	Sodium chloride
NCCLS	-	National Committee for Clinical Laboratory Standard
OD	-	Optical Density
PCR	-	Polymerase Chain Reaction
RAPD	-	Randomly Amplified Polymorphic DNA
SPB	-	Salt Polymyxin Broth
SMIC	-	Sessile Minimum inhibitory concentration
Taq	-	*Thermus aquaticus*
TBE	-	Tris-Boric acid-EDTA
TSA	-	Trypticase Soy Agar
TSB	-	Tryptic soy broth
U	-	Unit
U. S	-	United States
UV	-	Ultra violet
V	-	Volt
WHO	-	World Health Organization
WTO	-	World Trade Organization
x g	-	unit gravity

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

In nature, most bacteria exist as plankton (free floating) cells or suspended cells (sessile cells), and some exist on surfaces. According to the National Institute of Health (NIH) in the United States, 80 % of microbial can form biofilm and this includes food borne pathogens, such as *Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Campylobacter* spp., *Escherichia coli*, and *Listeria* spp. These bacteria are of special significance in ready-to-eat and minimally processed food products (Kim et al., 2006). Bacteria have the capability to attach themselves and to colonize surfaces in many natural and artificial surfaces, which are called biofilms.

Biofilm is known as a complex community that consists of microorganisms embodied in a matrix of extracellular polymeric material that they have generated. Thus, a mass or biofilm is established when bacteria adhere to the surface and produce extracellular polysaccharides (EPS). The EPS matrix consists of not only polysaccharides, but also proteins, which may be the major component in environmental and waste water biofilms and nucleic acids. This environment increases the resistance of bacteria to detergents and antibiotics as the dense extracellular matrix and the outer layer of cells protect the interior of the community (Merill, 2010).

Vibrio parahaemolyticus is a bacterium that is dangerous because it is one of the main causes of contamination in seafood, which can cause gastroenteritis worldwide (Lee, & Pogliano, 2007). *V. parahaemolyticus* is marine seafood borne pathogen, which causes gastroenteritis in humans (Miwatani, & Takeda, 1976). It has been recognized as a common cause of food borne illnesses in many Asian countries, including Japan, Taiwan, and China. According to Su and Liu (2007), it is the leading cause of human gastroenteritis in the United States and it is an unsafe seafood borne pathogen throughout the world.

Polymerase Chain Reaction (PCR) was adopted due to its specificity, easily automated, and its ability to amplify the amount of sample within few minutes if food poisoning outbreak occurs. Besides, in order to plan and to monitor targeted intervention strategies at preserving the therapeutic efficacy of antimicrobial agents, surveillance of antibiotic resistance is a key element to provide the latest information on the magnitude and the trends in resistance. On top of that, RAPD-PCR analysis was used to determine the relationship between antibiotic and isolates that were recovered from different habitats based on their RAPD-PCR fingerprints. Antibiotic resistance and RAPD proved to be

effective tools in characterizing and in differentiating the *V. parahaemolyticus* strains (Lesley et al., 2005; Tunung et al., 2007).

In addition, food processing environments provide a variety of conditions, which might favor the formation of biofilm with the presence of moisture, nutrients, and inocula of microorganism from raw materials. Bacterial colonization of food processing equipment and facilities is the main concern and is a potential source of contamination of foods that may lead to spoilage or transmission of food borne pathogens. Moreover, when biofilm detaches from the surfaces, individual microorganisms can easily spread.

In seafood food supply chain from pre harvest to marketable level, safe and effective detergent applied on the food contact surfaces and food facilities or equipment will ensure an acceptable decrease in microbial levels without the presence of toxic residuals or causing any damage for humans and useful forms of life. Natural antimicrobials nowadays are favorable concern to several issues pertaining to microorganisms. These include those that reduce the need of antibiotics and monitor microbial contamination in food. Due to the generally recognized as safe (GRAS) properties, herbs and spices have appeared to be natural antimicrobial, which could meet consumer demands for safe and healthier food consumption without any undesirable effect when applied in the food system.

In seafood industrial practice, it is necessary to develop a complete and a cost-effective cleaning program, which will inhibit the accumulation of both particulates and sessile bacterial cells present on the surfaces of food and processing line. Thus, in order to get rid of this problem, this study was carried out to investigate the effects of detergent, herbs, and spices on the growth of *V. parahaemolyticus* biofilm. Therefore, the objectives of this study were:

1. To determine the prevalence of *Vibrio parahaemolyticus,* regulator gene *toxR* gene, and virulence gene (*trh, tdh*) from seafood using polymerase chain reaction.

2. To profile *V. parahaemolyticus* based on antibiotics resistance and Random Amplified Polymorphism DNA–PCR fingerprinting.

3. To compare the formation of *V. parahaemolyticus* biofilms with varying temperatures: chiller temperature (4 ºC), room temperature (25 ºC), and optimum temperature (37 ºC).

4. To determine the effect of detergents against growth of antibiotic-resistant *V. parahaemolyticus* biofilm.

5. To determine the effects of herbs and spices against growth of antibiotic-resistant *V. parahaemolyticus* biofilm.

REFERENCES

Abdullah, M. (2010). Biopotency role of culinary spices and herbs and their chemical constituents in health and commonly used spices in Nigerian dishes and snacks. *African Journal of Food Science* 5(3):111-124

Abraham, T. J., Manly, R., Palaniappan, R. and Dhevendaran, K. (1997). Pathogenicity and antibiotic sensitivity of luminous *Vibrio harveyi* isolated from diseases penaeid shrimp. *Journal of Aquaculture Tropical* 3(12): 1-8.

Adams, M. R. and Moss, M. O. (2002). Food Microbiology. United Kingdom: *The Royal Society of Chemistry* 6: 142-144.

Adeleye, A., Vivian, E., Rita, N., Stella, S. and Emmanuel, O. (2008). Antimicrobial susceptibility of potentially pathogenic halophilic*Vibrio* species isolated from seafoods in Lagos, Nigeria. *African Journal of Biotechnology* 7 (20): 3791-3794

Aditya, M., Vishhal, G., Hemant, N. and Vishal, S. (2013). Protective Effect of (Curry Leaf) Leaves Extract Against Genotoxicity Induced by Cyclophosphamide in Mouse Bone Marrow Cells.*Global Veterinaria* 10 (2): 128-133

Alam, M. J., Tomochika, K., Miyoshi, S., Shinoda, S. (2002). Environmental investigation of potentially pathogenic *Vibrio parahaemolyticus* in the Seto-Inland Sea, Japan. *Federation of European Microbiological Societies (FEMS) Microbiology Letters* 20: 83-87

American Heart Association (2013). Accessed on 26th June 2013. http://www.heart.org/HEARTORG/GettingHealthy/NutritionCenter/Fish-101_UCM_305986_Article.jsp

Amit, P. and Parul, S. (2011). Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens. *Asian Journal of Plant Science and Research* (2):69-80

Anderson, D. L. and Gibb, A. J. (1994). Identification and phylogeny of spore-cyst fungi (*Ascosphaera* spp.) using ribosomal DNA sequences. *Mycological Research* (102)5: 541–547

Annous, B. A., Fratamico, P. M., and Smith, J. L. (2009). Scientific status summary. *Journal of Food Science* 74, R24-37

Aqil, F., Beg, A. and Ahmad, I. (2000). *in vitro* testing of essential oil against soil fungi, *Journal Medicine Aroma*tic *Plant Science* 22(1): 44-46

Araujo, P., Lemos, M., Mergulhao, F., Melo, L. and Simoes, M. (2011)..*In antimicrobial resistance to disinfectants in biofilm* (Ed) Mendez, V. Science against Microbial pathogens: communicating current research and technological advances

Armstrong, J., Gibbs, A., Peakall, R., and Weiller, G. (1990). RAPD Distance Programme. Australian National University, Canberra, Australia

Augustin, M., Ali-Vehmas, T., and Atroshi, F. (2004). Assessment of enzymatic cleaning agents and disinfectants against bacterial biofilms. *Journal of Pharmacy and Pharmaceutical Sciences* 18, 55–64

Austin, B. (2009). Vibrios as causal agents of zoonoses. *Veterinary Microbiology*, doi:10.1016/j.vetmic.2009.03.015 (In Press)

Australian and New Zealand Food safety, (2014), Accesed on 25th June 2014. http://www.foodstandards.gov.au/Pages/default.aspx

Baggaley, (2008).Antimicrobial Resistance in Developing Countries.. Springer New York Dordrecht Heidelberg, London

Barry, A. L. (1976). The Antimicrobial Susceptibility Test: Principles and Practices. In V. Lorian, ed. Antibiotics in Laboratory Medicine, 2nd ed. William & Wilkins, Baltimore. Cited P.M. Davidson and M.E. Parish. 1989. Methods for Testing the Efficacy of Food Antimicrobials. *Food Technology* (1): 148-155

Bartolini, F., Gallerani V., Raggi M., Viaggi D (2005). Contact design and targeting for the production of public goods in agriculture: the impact of the 2003 CAP reform. Proceeding of 11th EAAE congress: "The future of Rural Europe in the global agrifood system". Copenhagen. 24-27 August 2005

Bauer, A. W., Kirby, W. M. M., Sheris, J. C., Turck, M. (1966). Antibiotics susceptibility testing by standardized single disk method. *American Journal of Clinical Pathology* 45: 493-496

Bhattacharya, M., Choudhury, P. and Kumar, R. (2008). Antibiotic and metal-resistant strains of *Vibrio parahaemolyticus* isolated from shrimps. *Microbiology of Drug Resistant* 6: 171-172

Birkenhauer, J.M., and Oliver, J.D. (2003). Use of diacetyl to reduce the load of *Vibrio vulnificus* in the Eastern oyster, Crassostrea virginica. *Journa Food Protection* 66: 38–43

Bina, L. J., Tista, P., Anjana, S. and Kayo, D. Y. (2010). Study of antimicrobial activity of lime juice against *Vibrio cholera. Scientific World* Vol. 8, No. 8: 7

Blascek H. P., Wang. H. A. (2007). Biofilm in the Food environment (Eds) Willey Blackwell, Ames, Oxford, Carlton

Bostock J. C. (2009). Use of information technology in aquaculture. In New technologies in aquaculture: improving production efficiency, quality and environmental management, ch. 35 (eds Burnell G., Allan G., editors.), 1064–1118 Oxford, UK: Woodhead Publishing

Brooks, J.D. and Flint, S.H. (2008). Biofilms in the food industry: problems and potential solutions. *International Journal of Food Science and Technology* Vol.43, pp. 2163-2176

Burt, S.A. (2004). Essential oils: Their antibacterial properties and potential applications in foods: A review. *International Journal Food Microbiology* 94: 223-253

Bhaskar, N., Setty, T.M.R., Mondal, S., Joseph, M.A., Raju, C.V., Raghunath, B.S. and Anantha, C.S. (1998). Prevalence of bacteria of public health significance in the cultured shrimp (*Penaeus monodon*). *Food Microbiology* 15: 511-519

Boeije, G. M., Schowanek, D. R. and Vanrolleghem, P. A. (2000). Incorporation of biofilm activity in river biodegradation modeling: a case study for linear alkylbenzene sulphonate (LAS). *Water Research 34*(5): 1479-1486

Bradley, D. (2005). Star role for bacteria in controlling flu pandemic. *Nature reviews. Drug discovery* 4 (12): 945–946

Broberg, C. A., Calder, T. J. and Orth, K. (2011). *Vibrio parahaemolyticus* cell biology and pathogenicity determinants. *Microbes and Infection* 13: 992-1001

Brooks, J.D. and Flint, S.H. (2008). Biofilms in the food industry: problems and potential solutions. *International Journal of Food Science and Technology*, Vol.43, pp. 2163-2176

Canillac, N. and Mourey, A. (2001). Antibacterial activity of the essential oil of Picea excelsa on *Listeria*, *Staphylococcus aureus* and coliform bacteria. *Food Microbiology* 18: 261–2

Carson, C.F., Cookson, B.D., Farrelly, H.D. and Riley, T.V. (1995). Susceptibility of methicillin-resistant *Staphylococcus aureus* to the essential oil of Melaleucaalternifolia. *Journal of Antimicrobial Chemotherapy* 35: 421–424

Cassell, G. H. (1995). ASM Task Force urges broad program onantimicrobial resistance. ASM News 61: 116–120

Centers for Disease Control and Prevention (CDC) (2005). Outbreak of *Vibrio parahaemolyticus* Infection Associated with Eating Raw Oysters and Clams Harvested from Long Island Sound, Connecticut, New Jersey, and New York, Morbidity and Mortality Weekly Report 47: 129-131

Centers for Disease Control and Prevention (CDC), (2013). Vibrio illnesses after Hurricane Katrina multiple states. *Morbidity Mortality Week Rep*ort 54: 928-931

Chai, L. C.,Tunung, R., Usha, M. R., Jurin, W.G., Fatimah, A.B. Farinazleen, M. G., Son, R., and Malakar, P. R. (2007): Thermophylic*Campylobacter* spp. in salad vegetables in Malaysia. *International Journal of Food Microbiology* 117: 106-111

Chai, L. C., Ghazali, F.M. , Bakar, F.A., Lee, H. Y., Suhaimi, R. L., Talib, S. L., Nakaguchi, Y. Nishibuchi, and Son, R. (2009). Occurrence of thermophilic *Campylobacter* spp. contamination on vegetable farms in Malaysia. *Journal of Microbiology and Biotechnology* 19 (11): 1415-1420

Chaignon, P., Sadovskaya, I., Ragunah, Ch., Ramasubbu, N., Kaplan, J. B., and Jabbouri, S. (2007). Susceptibility of *staphylococcal* biofilms to enzymatic treatments depends on their chemical composition. *Applied Microbiology and Biotechnology* 75, 125–132

Chakraborty, M. A. (2008). The antioxidant and antimicrobial properties of the methanolic extract from *Cocus nuciferamesocarp*. *Food Chemical* 107: 994-999

Chang, C. M,, Chiang, M.L. and Chou, C. C. (2000). Responses of heat-shocked *Vibrio parahaemolyticus* to subsequent physical and chemical stresses. *Journal of Food Protection* 67: 2183-2188

Chao, G. X., Jiao, X. N., Zhou, X. H., Wang, F., Yang, Z.Q., Huang, J. L., Pan, Z. M., Zhou, L. P. and Qian, X. Q. (2009). Distribution of genes encoding four pathogenicity Islands VPaIs., T6SS, Biofilm, and type I Pilus in food and clinical strains of *Vibrio parahaemolyticus* in China. *Foodborne Pathogen Dis*covery 76: 649-658

Chapman, J. S. (2003). Biocide resistance mechanisms. *International Biodeterioration and Biodegradation* 51(2): 133-138

Chen, Y. T., Chang, H. Y., Lai, Y. C., Pan, C. C., Tsai, S. F. and Peng, H. L. (2004). Sequencing and analysis of the large virulence plasmid pLVPK of *Klebsiella pneumoniae* CG43. Gene 337: 189–198

Chmielewsky, R.A. and Frank, J. F. (2006). Biofilm formation and control in food processing facilities. *Comprehensive Reviews in Food Science and Food Safety* 2: 22 - 32

Clinical and Laboratory Standards Institute (CLSI) (2009). Document M7- A6, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Guideline, Wayne, PA

Clinical and Laboratory Standards Institute (2012). Document M7- A6, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Guideline, Wayne, PA.

Colwell, R. R. (1984). Polyphasic taxonomy of the genus *Vibrio*: numerical taxonomy of *Vibrio cholerae, Vibrio parahaemolyticus* and related *Vibrio* species. *Journal of Bacteriology* 104: 410-433

Colwell, R. R. (2006). A global and historical perspective of the genus *Vibrio*. In *The Biology of Vibrios*, ed. F. L. Thompson, B. Austin, and J. Swings, pp. 3-11. Washington D. C.: ASM Press

Commission of European Committees (1994). Report of the scientific committee for food.11th series.

Conte, M. P., Longhi, C., Polidoro, M., Petrone, G., Buonfiglio, V., Di Santo, S. (1996). Iron availability affects entry of *Listeria monocytogenes* into the enterocyte like cell line Caco-2. *Infection and Immunity* 64, 3925–3929

Cook. W. H. and Canning, P. (1990). Inheritance is not subtyping. In *Proc. ACM Symp. on Principles of Programming Languages.*

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Rev*iew 12: 564-582.

Cox, J. M. and Fleet, G. H. (2003). New directions in the microbiological analysis of foods. In, *Foodborne Microorganisms of Public Health Significance*, 6th Edition (eds) A.D. Hocking, et al. AIFST (NSW) Food Microbiology Group, Sydney pp.103-162

Cutler, H., G. (1995). Natural product flavor compounds as potential antimicrobials, insecticides, and medicinals. *Agro Food Industry Hi- Technolgy* 6; 19–23

Cressy, H.K., A.R. Jerrett, C.M. Osborne and P.J. Bremer. (2003). A novel method for the reduction of numbers of *Listeria monocytogenes* cells by freezing in combination with an essential oil on bacteriological media. *Journal Food Protection* 66 (3): 390-395

Daniels, N. A., Ray, B., Easton, A., Marano, N., Kahn, E., McShan, A. L. 2nd, Del Rosario, L., Baldwin, T., Kingsley, M. A., Puhr, N. D., Wells, J. G., Angulo, F. J. (2000). Emergence of a new *Vibrio parahaemolyticus* serotype in raw oysters: A prevention quandary. *Journal of the American Medical Association (JAMA)*284 (12): 1541-1545

Dao, H. N., Rayner, D. S. and Alvin, W. G. (1999). Marketing and export of marinebased food products. Department of Fisheries, Sabah

Das, A. K., Rajkumar, V. and Dwivedi, D. K. (2010).Antioxidant effect of curry leaf (*Murraya koenigii*)powder on quality of ground and cooked goat meat.*International Food Research Journal* 18: 563-569

Davidson, F. M (1997). Chemical preservatives and natural antimicrobial compounds. In: Doyle, M., L.R. Beuchat, T. J. Montville, (Eds). Food Miccrobiology: Fundamentaland Frontiers, ASM Press, Washington, D. C. 6: 520-556

Deans, S. G. and Ritchie, G. (1987). Antibacterial properties of plant essential oils. *International Journal of Food Microbiology* 5: 165– 180

Delaquis, P. J. K. Stanich, B., Girard, K. and Mazza, G. (2002). Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *International Journal Food Microbiology* 74 (1/2): 101-109

Deepanjali, A., Kumar, H. S., and Karunasagar, I. (2005). Seasonal variation in abundance of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters along the southwest coast of India. *Applied Environment Microbiology* 71: 3575–3580

DePaola, A., Capers, G. M., Alexander, D. (1994). Densities of *Vibrio vulnificus* in the intestines of fish from the US Gulf Coast. *Applied and Environmental Microbiology* 60: 984-988

Desmarchelier, P. M. (1997). Pathogenic Vibrios. In: Foodborne Microorganisms of Public Health Significance. 5th ed., AIFST (NSW Branch), Food Microbiology Group, North Sydney, NSW: 285-312

Desmarchelier, P. M. (2003). Pathogenic vibrios. In *Foodborne Microorganisms of Public Health Significance*, ed. Hocking, A.D., pp: 333-358

Dewanti, R., and Wong, A. C. (1995). Influence of culture conditions on biofilm formation by *Escherichia coli*. *International Journal Food Microbiol*ogy 26**:**147-164

Djordjevic, D, Wiedmann, M. and Sborough, L. A. (2002). Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. *Journal Applied Microbiol*ogy 68: 2950-2958

Dileep, V., Kumar, H. S., Kumar, Y., Nishibuchi, M., Karunasagar, I. and Karunasagar, I. (2003). Application of polymerase chain reaction for detection of *Vibrio parahaemolyticus* associated with tropical seafoods and coastal environment. *Letters in Applied Microbiology* 36: 423-427

Dong, Y.-H., Gusti, A. R., Zhang, Q., Xu, J.-L., and Zhang, L.-H. (2002). Identification of quorum-sensing N-acyl homoserine lactonases from *Bacillu*s species. *Applied and Environmental Microbiology* 68, 1754–1759

Donlan, R. M. (2002). Biofilms: microbial life on surfaces. *Emerging infectious diseases*8 (9): 881

Dorman, H. J. D. and Deans, S. G. (2009). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal Applied Microbiology* 88: 308- 316

Duan, J. and Su, Y. C. (2005). Occurrence of *Vibrio parahaemolyticus* in two Oregon oyster-growing bays. *Journal of Food Science* 70: 58-63

Dvorak, G. (2008). Disinfection 101.*Journal of Food Microbiology* 9; 110-111

Dwivedi, B. K., Pardley, R. L., Pardley, G., Pant, H. L., and Logani, L. (2002): Evaluation of *angiospermi*c plant extracts against *Rhizopusstoionifer* and *Gloesporiumpsidii* of guava. *Biodiversity* 13: 129-134.

Eckert, K. A., Kunkel, T. A. (1990). High fidelity DNA synthesis by the Thermusaquaticus DNA polymerase. *Nucleic Acids Research* 18 (13): 3739-3744

Eloff , J. N. (1998).Which extractant should be used for the screening and isolation of antimicrobial components from plants. *Journal of Ethnopharmacology* 60: 1–8

Endzt, H., Ang, C. W., Van den Braak, N., Duim, B., Rigter, A., Price, L. J. (2000). Molecular characterization of *Campylobacter jejuni* from patients with Guillain-Barré and Miller Fisher syndromes. *Journal of Clinical Microbiology* 38: 2297-2301

European Commission, (2000). Scientific committee on veterinary measures relating to Public Health on *V. vulnificus* and *V. parahaemolyticus* in raw or undercooked seafood.

Faruque, S. M., Nair, G. B. (2006). Epidemiology. In *The Biology of Vibrios*, ed. F.L. Thompson, B. Austin, and J. Swings, pp. 385-398. Washington D. C.: ASM Press

Fett, W. F. (2010). Naturally occurring biofilm on alfalfa and other types of sprouts. . *Journal of Food Protecttion* 63 : 624-632

Fleming, T. H. (1998). The short-tailed fruit bat: a study of plant-animal interactions. University of Chicago Press, Chicago

French G. L. J. (2006).Inhibitory and Bactericidal Activities of Daptomycin, Vancomycin, and Teicoplanin against Methicillin-Resistant *Staphylococcus aureus* Isolates Collected. *Antimicrobiology Chemother*aphy 58:1107—1117

Food and Agriculture Organization of the United Nations (FAO)(2005). A Regional Survey of the Aquaculture Sector in West Asia. Aquaculture Development and Coordination Programme, Rome. ADCP/REP/89/35: 4-5

Food and Agriculture Organization of the United Nations (FAO). (2010). A Regional Survey of the Aquaculture Sector in West Asia. Aquaculture Development and Coordination Programme, Rome. ADCP/REP/89/35: 4-5

Food Safety and Inspection isolation method (FSIS) (2012), Microbiology Laboratory Guidebook. http://www.fsis.usda.gov/wps/portal fsis/topics/science/

Forsythe, S. J., and Hayes, P. R. (1998). Food hygiene, microbiology and HACCP (3rd ed.).Aspen Publishers

Fukushima, H., and Seki, R. (2003). Ecology of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in brackish environments of the Sada River in Shimane Prefecture, Japan. *FEMS Microbiology Ecology* 48: 221-229

Gopal, S. Otta, S. k., Kumar, S., Karunasagar, I., Nishibuchi, M. (2005): The occurrence of *Vibrio* species in tropical shrimp culture environments; implications for food safety. *International Journal of Food Microbiology* 102: 151-159

Greg, R. (2009). Controlling Contamination in Water Treatment Equipment. *CWS-VI Water Conditioning and Purification*, Vol (1) 667-668

Guardabassi, L., Andreas, P., John, E., and Anders, D. (1998). Antibiotic Resistance in Acinetobacter spp. Isolated from Sewers Receiving Effluent from a Hospital and a Pharmaceutical Plant. *Applied and Environmental Microbiology* 64: 3499-3502

Goutam, M. P. and Purohit, R. M. (1974). Antimicrobial activity of the essential oil of the leaves of *Murraya koenigii Spreng* (IndianCurry Leaf), *Indian Journal of pharmacology* 36 (1), 11

Gwendelynne, B. T., Son, R., Nishibuchi, M., Raha, A. R., Suhaimi, N., Lesley, M., Jurin, W. G. (2003). Characterization of *Vibrio parahaemolyticus* isolated from coastal seawater in Peninsular Malaysia. *The Southeast Asian Journal of Tropical Medicine and Public Health* 36 (4): 940-945

Hafidh, R., Ahmed, S., Law, S.W., Fatimah, A. B., Faridah,A., Fatemeh, J. and Zamberi S. (2011). Inhibition of Growth of Highly Resistant Bacterial and Fungal Pathogens by a Natural Product.*The Open Microbiology Journal* 5:96-106

Hamendra, S. P. and Anand, K. (2007): Antidiabetic potential of *Citrus sinensis* and *Punicagranatum* peel extracts in alloxan treated male mice. *Bio Factors* 31: 17–24

Han, F., Walker, R. D., Janes, M. E., Prinyawiwatkul, W. G. B. (2007). Antimicrobial susceptibilities of *Vibrio parahaemolyticus* and *Vibrio vulnificus* isolates from Louisiana Gulf and retail raw oysters. *Applied and Environmental Microbiology* 73 (21): 7096-7098

Han, S. T. (1998). Medicinal Plants in the South Pacific, World Health Organization, (WHO), Regional Publications West Pacific Series, No. 19, Manila

Hara-Kudo, Y., Nishina,T., Nakagawa, H., Konuma, H., Hasegawa, J., Kumagai, S. (2001). Improved method for detection of *Vibrio parahaemolyticus* in seafood. *Applied and Environmental Microbiology* 67 (12): 5819-5823

Harvey, J., Keenan, K. P. and Gilmour, A. (2007). Assessing biofilm formation by *Listeria monocytogenes* strains. *Food Microbiology* 24: 380–392

Hlady, W. G., and Klontz, K. C. (1996). The epidemiology of *Vibrio* infections in Florida (1981-1993*). Journal of Infectious Diseases* 173: 1176-1183

Honda, T. and Iida, T. (1993). The pathogenicity of *Vibrio parahaemolyticus* and the role of the thermostable direct haemolysin and related haemolysin. *Review in Medical Microbiology* 4: 106-113

Hood, S. K. and Zottola, E. A. (1995). Biofilms in food processing. *Food Control* 6: 9-18

Hosseini, H., Cheraghali, A. M., Yalfani, R., and Razavilar, V. (2004). Incidence of *Vibrio* spp. in shrimp caught off the South Coast of Iran. *Food Control* 15: 187-190

Hwang, J. K., Shim,J..S and Chung. J.Y. (2004). Anticariogenic activity of some tropical medicinal plants against *Streptococcus* mutans. *Fitoterapia*75:596–598

International Commission on Microbiological Specification for Food (ICMSF). (1995). Roberts TA, Baird-Parker A C and Tompkin RB (eds.). Microorganisms in foods 5. Blackie Academic and Professional, London

Ito, C., Itoigawa, M., Miyamoto, Y., Onoda, S., Rao, K.S., Mukainaka, T., Tokuda, H., Nishino, H. and Furukawa, H. (2003). Polyprenylatedbenzophenones from Garciniaassugu and their potential cancer chemopreventive activities. *Journal of Natural Products* 66(2): 206–209

Jay, J. M. (2000). Modern Food Microbiology. 6th ed., APAC Publisher, Singapore: 425-568

Jiang, X. and Chai, T.J. (1996). Survival of *Vibrio parahaemolyticus* at Low Temperatures under Starvation Conditions and Subsequent Resuscitation of Viable, Nonculturable Cells. *Applied Environment Microbiology* 62(4):1300

Jin, Y., Samaranayake, L. P., Samaranayake, Y. and Yip, H. K. (2004). Biofilm formation of Candida albicans is variably affected by saliva and dietary sugars. *Archieves Oral Biology* 49: 789–798

Joshi, D. D. (2012). Herbal Drugs and Fingerprints: Evidence Based Herbal Drugs. Springer India

Kaleta, E.F. (2013). Disinfection in poultry medicine - aims and means. *Veterinary Journal* 48 (2) 112-115

Kawasaki S, Horikoshi, N., Okada, Y. (2005). Multiplex PCR for simultaneous detection of *Salmonella spp., Listeria monocytogenes*, and *Escherichia coli O157:H7* in meat samples. *Journal Food Pro*tection 68 : 551–556

Kaysner, C., DePaola, A.J. (2004): U.S. Food and Drug Administration: Bacteriological Analytical Manual: Methods for specific pathogens: Chapter 9: *Vibrio*. Accessed on 24 May 2007. Available at http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070830.htm.

Kim, Y. B., Okuda, J., Matsumoto, C., Takahashi, Nakabayashi, Y., Kumagai, K., (1999). Molecular Identification of *Vibrio parahaemolyticus* strains at the direct hemolysin (TDH) and TDH-related hemolysin of species level by PCR targeted to the toxR gene. *Journal of Clinical Microbiology* 37 (4): 1173-1177

Koburger, J. A., Lazarus, C. R. (1974). Isolation of *Vibrio parahaemolyticus* from salt springs in Florida. *Applied Microbiology* 27: 435-436

Kraa, E. (1995). Surveillance and epidemiology of foodborne illness in NSW, Australia. *Food Australia* 47: 418-423

Krämer, M., Bongaerts, J., Bovenberg, R., Kremer, S., Müller, U.; Orf, S., Wubbolts, M., Raeven, L. (2003). Metabolic engineering for microbial production of shikimic acid. *Metabolic Engineering* **5** (4): 277–283

Krumperman, P. H. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied and Environmental Microbiology* 46: 165-170

Krupesha, S., Shankar, K. M., Sathyanarayana, M. L ,Sahoo, A. K. , Patil, R., Narayanaswamy, HD. and Rao, S. (2010). Evaluation of immune response and resistance to diseases in tiger shrimp, Penaeusmonodon fed with biofilm of *Vibrio alginolyticus*. *Fish & Shellfish Immunology* 29: 724-732

Kudva, I. T., Jelacic, S., Tarr, P. I., Youderian, P., and Hovde, C. J. (1999). Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. *Applied and Enviromental Microbiology* 65, 3767–3773

Laksamana, S. P., Srinivasa, R.T.C.H.H., Srinivasa, L., Prasad, A.K.C. (1997). *In vitro* antibacterial activity of culinary spices Aniseed, Star Anise and Cin.namon against bacterial pathogens of fish. *International Journal of Pharmacy and Pharmaceutical Sciences* Vol 4(3) : 221-228.

Lapornik B, Prosek M, Wondra,A. G. (2005).Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of Food Engineering* 71: 214–222.

Le, X. T., Munekage, Y. and Kato, S. (2005). Antibiotic resistance in bacteria from shrimp farming in mangrove areas. *Science of the Total Environment*, vol.349, pp.95-105.

Lee, H. C., and Pogliano, K. (2007). *Vibrio parahaemolyticus*: Microbe Wiki. Accessed on 4 January 2010. Available at http://microbewiki.kenyon.edu/index.php/Vibrio_parahaemolyticus.

Lee, J. (2009). Laboratory studies of growth conditions of *Vibrio parahaemolyticus* in Pacific Oyster (*Crassostreagigas*) with international considerations to shellfish-associated illnesses in South Korea. Bachelor Thesis. Oregon State University.

Lesley, M. B., Son, R., Bahaman, A. R., Raha, A. R., Suhaimi, N., Michael, W. C. V. L., Gwendelynne, B. T. and Nishibuchi, M. (2005). Detection of *Vibrio parahaemolyticus* in cockle *(Anadara granosa)* by PCR. *Federation of European Microbiological Societies (FEMS) Microbiology Letter*s 252: 85-88

Lelieveld, H. L, Mostert, M. A, Holah, J. and White, B. (2003). Hygiene in Food Processing, Cambridge, Woodhead Publishing Limited.

Lindow, S.E. and Brandl, M.T. (2003). Microbiology of the phyllosphere. *Applied and Environmental Microbiology* 69: 1875-1883

Lorian, V. (2005). Antibiotics in Laboratory Medicine, 5th ed., *Lippincott Williams and Wilkins, London*

López-Malo, A., Alzamora, S.M., and Argaiz, A. (1997). Effect of natural vanillin ongermination and radial growth of moulds in fruit-based agar systems. *Food Microbiology* 12: 213-219

Magnus, P., Jaakkola, J.J., Skrondal, A., Alexander, J., Becher, G., Krogh, T., (1999). Water chlorination and birth defects. *Epidemiology* 10(5):513–517

Mahmud, Z. H., Kassu, A., Mohammad, A., Yamato, M., Bhuiyan, N. A., Nair, G. B., and Ota, F. (2005). Isolation and molecular characterization of toxigenic *Vibrio parahaemolyticus* from the Kii Channel, Japan. *Microbiological Research*. Available online at www.elsevier.de/micres

Mallavarapu, G. R., Ramesh, S., Syamasunday, K.V., Chandrasekhara, R.S. (1999). Composition of Indian curry leaf oil. *Journal Essential oil Res*earch 11: 176-178

Mandrell, R. E. and Wachtel, M. R. (1999). Novel detection techniques for human pathogens that contaminate poultry. *Current Opinion Biotechnolgy,Review.* 10(3)**:**273-278

Mao, Z. J.,Yu, L., You,Z.Q., Wei, Y.W. and Liu, Y. (2007). Cloning, expression and immunogenicty analysis of five outer membrane proteins of *Vibrio parahaemolyticus* zj2003. *Fish Shellfish Immunology* 23, 567-575

Maruti J. Dhanavade, Chidamber B. Jalkute, Jai S. Ghosh and Kailash D. Sonawane Study Antimicrobial Activity of Lemon (*Citrus lemon L.)* Peel Extract. *British Journal of Pharmacology and Toxicology* 2(3): 119-122

Mata, A. I., Blanco, M. M., Dominguez, L., Fernandez-Garayzabal, J., Gibello, l. (2004). Development of a PCR assay for Streptococcus iniae based on the

lactate oxidase (lctO) gene with potential diagnostic value. *Veterinar Microbiology* 101(2): 109-116

Matsumoto, C., Okuda, J., Ishibashi, M., Iwanaga, M., Garg, P., Ramamurthy,T., Wong,H., Depaola, A., Kim. Y. B. (2000). Pandemic spread of an O3:K6 clone of *Vibrio parahaemolyticus* and emergence of related strains evidenced by arbitrarily primed and toxRS sequence analyses. *Journal Clinical Microbiology* 38: 578–585

McCarter, L. (1999). The multiple identities of *Vibrio parahaemolyticus*, J*ournal of Microbiol*ogy *Biotechnology* 1: 51-57

McCarter, L. L. (2006). Motility and Chemotaxis. In *The Biology of Vibrios*, ed. F.L. Thompson, B. Austin, and J. Swings, pp. 115-132. Washington D. C.: ASM Press

McCarthy S.A.,(1992). Attachment of *Listeria monocytogenes* to chitin and resistance to biocides. *Food Technology* 46, 84–88.

McKeon, D.M., Calabrese, J.P. and Bissonette, G. K. (1995) Antibiotic resistant gram-negative bacteria in rural groundwater supplies. *Water Research* 29: 1902–1908

McLaughlin, J. B., DePaola, A., Bopp, C. A, Martinek, K. A., Napolilli, N., Allison, C, Murray, S. L., Thompson, E. C., Bird, M. M,, Middaugh, J.P. (2005). Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. *New England Journal of Medicine*353 (14): 1463-1470

Mereghetti, L., Quentin, R., Marquet-Van Der Mee, N., Audurier, A. (2000). Low sensitivity of *Listeria monocytogenes* to quaternary ammonium compounds. *Applied Environmental Microbiology* 66 5083–5086

Merrill, A. Biel (2010). Biofilm Infections. Photodynamic Therapy of Bacterial and Fungal FEMS Microbiology Letters 232

Miller, K., O'Neill, A. J. and Chopra, I. (2002). Response of *Escherichia coli* hypermutators to selection pressure with antimicrobial agents from different classes. *Journal AntimicrobologyChemotheraphy* 49:925-934

Mills-Robertson, F., Addy, M. E., Mensah, P., and Crupper, S. S. (2002). Molecular characterization of antibiotic resistance in clinical *Salmonella typhi* isolated in Ghana. *FEMS microbiology Letters* 215: 249-253

Minakshi, De, Amit Krishna, De, Parimal Sen and Arun, B. (2002). Antimicrobial Properties of Star Anise (*Illicium verum Hook* f). *Phototherapy research* 16, 94–95

Miwatani, T. and Takeda, Y. (1976). *Vibrio parahaemolyticus* - a causative bacterium of food poisoning . *National Library of Australia* 6 :110-136

Mizunoe, Y., Wai, S. N., Ishikawa, T., Takade, A., and Yoshida, S. (2000). Resuscitation of viable but nonculturable cells of *Vibrio parahaemolyticus* induced at low temperature under starvation. *FEMS Microbiology Letters* 186: 115-120

Molina-Aja, A., Garcia-Gasca, A., Abreu-Grobois, A., Bolan-Mejia, C., Roque, A., and Gomez-Gil, B. (2002). Plasmid profiling and antibiotic resistance of *Vibrio* strains isolated from cultured penaeid shrimp. *FEMS Microbiology Letters* 213: 7-12

Monfort, P., Piclet, G. and Plusquellec, A. (2000). *Listeria innocua* and *Salmonella panama* in estuarine water and seawater: a comparative study. *Water Residue* 34: 983-989

Nakamura, C.V., Ishida, K., Faccin, L.C. Filho, B.P. Cortez, D. A., Rozental, S., de Souza, W. and Ueda-Nakamura, T. (2004). In vitro activity of essential oil from Ocimumgratissimum against four *Candida* species. *Research Microbiology* 155: 579-586

National Committee for Clinical Laboratory Standards (NCCLS)(2001). Performance standards for antimicrobial susceptibility testing: eleventh informational supplement. Wayne (PA) *(NCCLS document no. M-100-S11)*

National Environment agency (2013). Sustaining our environment. National Environment Agency Annual Report 2012/13

Negi, P. S., A.S. Chauhan, G.A. Sadia, Y.S. Rohinishree and R.S. Ramteke (2005). Antioxidant and antibacterial activities of various seabuckthorn (Hippophaerhamnoides L.) seed extracts. *Food Chemistry* 92: 119-124

Nishibuchi, M., Kaper, J. B. (1995). Thermostable direct haemolysin gene of *V. parahaemolyticus*: a virulence gene acquired by a marine bacterium. *Infection and Immunity* 63: 2093-2099

Noube, N. S., Afolayan, A. J., and Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology* 7 (12): 1797-1806

Ngwoke, K. G., Odimegwu, D. C., Esimone, C. O. (2011). Antimicrobial natural products. In: Mendez-Vilas A, editor. Science against microbial pathogens:

communicating current research and technology advances. Badajoz, Spain: FORMATEX pp. 1011.

Odugbemi, T. O. (2006).Outlines and pictures of medicinal plants from Nigeria, University of Lagos Press, Lagos, Nigeria, pp. 91

Oliver, J. D. and Kaper, J. B. (1997). *Vibrio* species. In: Doyle, M, Beuchat, L, R, Montville, T.J., (Eds), *Food Microbiology: Fundamental and Frontiers*, ASM Press, Washington D. C., pp. 228-264

Oliver, J. D., Hite, F., McDougald, D., Barrett, T., Glover, L. A., Prosser, J. I. (1995). Entry into and resuscitation from, the viable nonculturable state by Vibrio vulnificus in an estuarine environment. *Applied and Environmental Microbiology* 61: 2624-2630.

Onayade, D. A., and Adebujo, A. C. (2000). Composition of the leaf volatile oil of *Murrayakoenigii* growing in Nigeria. *Journal Herbs, Spices Medicine Plants* 7(4): 59-66

O'Toole, G. H. B., Kaplan, R., Kolter, H. (2000). Biofilm formation as microbial development. *Annula Review Microbiology* 54:49–79.

Pandey, R. R, Dubey, R. C, Saini, S. (2010). Phytochemical and antimicrobial studies on essential oils of some aromatic plants. *African Journal of Biotech*nology 9(28):4364-68

Pankey, G. A., Ashcraft, D. S. (2010). In vitro antibacterial activity of tigecycline against resistant Gram-negative *bacilli* and enterococci by time–kill assay. *Diagnostic Microbiology Infect*ion *Disease* 64: 300—304

Palaniswamy, U. R. (2007). Asian horticultural crops and human dietetics. *HortTechnology*11:504–509

Pavithra, G. (2014). Effect of Spices on Bacteria -A Short Review. *Journal of Pharmacy Science* Vol. 6(8), 268-270

Perret, C., Tabin, R., Marcoz, J. P., Lor, J., Cheseaux, J. J., (2011). Apparent life-threatening event in infants: think about star anise intoxication! *Archives de Pédiatrie* 18(7): 750–75

Phillip, J.(1981). Curryleaf and its issues. *World crops*,33:125-127

Piera Serra, G. (2003). Estudio de biofilms: Formación y consecuencia.

Prudent, D., F. Perineau, J.M. Bessiere, G.M. Michel and J.C. Baccou (2005). Analysis of the essential oil of wild oregano from Martinique (*Coleus*

aromaticus Benth.)—evaluation of its bacterioatatic and fungistatic properties. *Journal Essential Oil Res*earch 7: 165– 173

Pruthi, J.S. (1976). Spices and condiments. New Delhi: National Book Trust, India

Rajkowski, K. T. (2009). Biofilms in fish processing. In P. M. Fratamico, B. A. Annous, and N. W. Gunther (Eds.), Biofilms in the food and beverage industries . Woodhead Publishing pp. 499 - 516

Raman, M, (2001). Malaysia: Each prawn produced represents a teardrop. *World Rainforest Movement Bulletin* 51: 1-2

Rasmussen, T. B., Skindersoe, M. E., Bjarnsholt, T., Phipps, R. K., Christensen, K. B., Jensen, P. O., et al. (2005). Identity and effects of quorum-sensing inhibitors produced by *Penicillium* species. *Microbiology* 151, 1325–1340

Reeves, M. W., Evins, G. M., Heiba, A. A., Plikaytis, B. D., Farmer, J. J. (1989). Clonal nature of *Salmonellatyphi*and its genetic relatedness to other *Salmonellae* as shown by multilocus enzyme electrophoresis, and proposal of *Salmonella bongori.* J*ournal Clinical Microbiology 27:* 313-320

Reddy, V., Urooj, A. and Kumar, A. (2005). Evaluation of antioxidant activity of some plant extracts and their application in biscuits. *Food Chem*ical 90: 317-321

Reisch, J., Goj, O., Wickramasinghe, A. and Herath, H.M.T.B. (1992). Carbazole alkaloids from seeds of*Murrayakoenigii*. *Phytochemistry* 31: 2877-2879

Reichelt, J. L., Baumann, P., Baumann, L. (1976). Study of genetic relationships among marine species of the genera *Beneckea* and *Photobacterium*by means of in vitro DNA/DNA hybridization. *Archives of Microbiology* 110: 101-120

Ronner, A. B., and Wong**,** A. C**.** (1993). Biofilm development and sanitizer inactivation of *Listeria monocytogenes* and *Salmonella typhimurium* on stainless steel and Buna-n rubber.. *Journal Food Prot*ection 56**:**750-758

Roth, L.S. (2006). Mosby's Handbook of Herbs and Natural Supplements. 3rd Ed. Mosby, StLouis

Rukayadi, Y., Lee, K., Han, S., Yong, D., Hwang, J. K. (2009). In Vitro Activities of Panduratin A against Clinical *Staphylococcus* Strains. *Antimicrobial Agents Chemother*aphy 53: 4529—4532

Rukayadi, Y., Han, S., Yong, D. and Hwang, J.K. (2010). *In Vitro* Antibacterial Activity of Panduratin A against Enterococci Clinical Isolates. Biology *Pharmacy Bull*etin 3(9): 1489-1493

Sachse, K. and Frey, J. (2003). PCR Detection of Microbial Pathogens. Humana Press, Totowa, New Jersey 216: 3-80

Sakazaki, R, Tamura, K., Kato, T. O., Yamai, S., Hobo, K. (1968). Studies on the enteropathogenic facultatively halophilic bacteria *Vibrio parahaemolyticus*: III. Enteropathogenicity. *Japanese. Journal of Medical Science and Biology* 21, 325–331

Saumendu, D.R., Ratnali, B., Jashabir, R. (2012). Pharmacognostic, phytochemical, physicochemical property and antimicrobial activity studies of lemon peel oil. *Journal National Production Plant Resourceses* 2 (3):431-435

Sandoe, J. A. T., Wysome, A. P., West, J., Heritage, M. H,, Wilcox (2006). Measurement of ampicillin, vancomycin, linezolid and gentamicin activity against enterococcal biofilms. *Antimicrobial Chemother*aphy 57, 767—770

Sarkar, B. L., Nair, G. B., Banerjee, A. K., Pal, S. C. (1985). Incidence and level of *Vibrio parahaemolyticus* in freshwater environs and in association with freshwater fishes in Calcutta. *Applied and Environmental Microbiology* 46: 132-136

Scates, P., Moran, L., Madden, R. H. (2003). Effect of incubation temperature on isolation of *Campylobacter jejuni* genotypes from foodstuffs enriched in Preston broth. *Applied and Environmental Microbiology* 69: 4658-4661

Schwach, T.S. and Zottola, E.A. (1982). Use of scanning electron microscopy to demonstrate microbial attachment to beef and beef contact surfaces. *Journal Food Science* 47*:* 1401–1405

Settanni, L. and Corsetti, A. (2007).Application of bacteriocins in vegetable food biopreservation. *International Journal of Food Microbiology* 121; 123–138

Sharma, M., and Anand, S. K. (2002). Characterization of constitutive microflora of biofilms in dairy processing lines. *Food Microbiology* 19, 627–636

Shelef, L.A. (1980). Antimicrobial effects of spices. *Journal of Food Safety* 6: 29–44

Shinoda, S., Honda, T., Takeda, Y., Miwatani, T. (1974). Antigenic difference between polar monotrichous and peritrichous flagella of *Vibrio parahaemolyticus*. *Journal of Bacteriology* 120: 923-928

Shinoda, S., Kariyama, R., Ogawa, M., Takeda, Y., Miwatani, T. (1976). Flagellar antigens of various species of genus *Vibrio* and related genera. *International Journal of Systematic Bacteriology* 26: 97-101

Simoes, M., Simoes, L.C., Viera. M.J. (2010). A reveiew of current and emergent biofilm control strategies. *LWT-Food Sciences and Technology* 43(4): 573-583

Smack, D. P., Harrington, A.C., Dunn, C., Howard, R. S., Szkutnik, A. J., Krivda, S. J. Caldwell, J. B. and James, W. D. (1996). Infection and allergy incidence in ambulatory surgery patients using white petrolatum vs bacitracin ointment. A randomized controlled trial *JAMA : the journal of the American Medical Association*276 (12): 972–7. PMID 8805732

Sonia, S., Aparna, D.B., Gupta, G., Saksham, G. (2013). Antimicrobial activity of spices against isolated foodborne pathogens. *International Journal of Pharmacy and Pharmaceutical Sciences* 5 (1): 118-235

Sorum, H., L., Abee, T. and Lund, T. M. (2002). Antibiotic resistance in food-related bacteria – a result of interfering with the global web of bacterial genetics. *International Journal of Food Microbiology* 78: 43-56

Sommer, P., Martin-Rouas, C. and Mettler, E. (1999). Influence of the adherent population level on biofilm population, structure and resistance to chlorination. *Food Microbiology* 16: 503–515

Spann, C. T, Taylor, S. C., Weinberg, J. M. (2004). "Topical antimicrobial agents in dermatology". *Disease-a-month* : DM 50 (7): 407–21

Sujeewa, A. K. W., Norrakiah, A. S. and Laina, M. (2009). Prevalence of toxic genes of *Vibrio parahaemolyticus* in shrimps (*Penaeus monodon*) and culture environment. *International Food Research Journal* 16: 89-95

Srivastava, N. (2005). Antibacteria Activity of Crude Ethanolic Extacts and Essential Oils of Spices Against *Salmonella* and other *Enterobacteria*. KMITL *Science Technology Journal* 5 (3) :6-12

Staronline (2013). Accessed on 12 February 2014. http://www.frim.gov.my/frim-conducts-course-on-plant-diseases-pests/?wppa-album=87&wppa-cover=0&wppa-occur=1&wppa-photo=311

Sutherland, I. W. (2001). The biofilm matrix – an immobilized but dynamic microbial environment. *Trends in Microbiology* 9, 222–227

Su, Y. C. and Liu, C. A. (2007). *Vibrio parahaemolyticus*: a concern of seafood safety, *Food Microbiol*ogy 24: 549-558

Sybiya, V., Packiavathy, I. A., Agilandeswari, P., Syed Musthafa, K., Pandian, S. K., Ravi, A.V.(2012). Antibiofilm and quorum sensing inhibitory potential of

Cuminumcyminum and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens. *Food Research International* 45: 85-92

Szczuka, E. and Kaznowski, A. (2004). Typing of clinical and environmental Aeromonas sp. Strains by random amplified polymorphic DNA PCR, repetitive extragenic palindromic PCR, and enterobacterial repetitive intergenic consensus sequence PCR. *Journal of Clinical Microbiology* 42:220-228

Tada, J., Ohashi, T., Nishimura, N., Shirasaki, Y., Ozaki, H., Fukushima, S., Takano, J., Nishibuchi, M. and Takeda, Y. (1992). Detection of thermostable direct hemolysin gene (trh) and thermostable direct hemolysin-related hemolysin gene (trh) of *Vibrio parahaemolyticus* by polymerase chain reaction. *Molecular and Cellular Probes* 6: 477-487

Tendencia, E. A., and Pena, L. D. (2001). Antibiotic resistance of bacteria from shrimp ponds. *Aquaculture* 195: 193-204

Thiem and Goslinska, (2001).Antimicrobial activity of *Rubuscha maemorus* leaves. *Fitoterapia* 75:93–95

Thompson, F. L., Iida, T., Swings, J. (2004). Biodiversity of vibrios. *Microbiology and Molecular Biology Reviews* 38: 403-431

Tomatake, H., Koga, T.,Yamato, M., Kassu, A. and Ota, F. (2006). Antibacterial activity of citrus fruit juices against Vibrio species. *Journal Nutrition Science Vitaminol*ogy 52(2):17-160

Tu, T. H. Silvestre, F. Bernard, A. Douny, C. Phuong, T.N. Tao, T.C. Maghuin-Rogiste, G. and Kestemont, P. (2008). Oxidative stress response of black tiger shrimp (*Penaeus monodon*) to enrofloxacin and to culture system. *Aquaculture* 285, pp.244-248

Tunung, R., Chai, L. C., Usha, M. R., Lee, H. Y., Fatimah, A. B., Farinazleen, A. B., Son, R. (2007). Characterization of *Salmonella*enterica isolated from street food and clinical samples in Malaysia. ASEAN *Food Journal* 14: 161-173

Tsukamoto, K. K, Oyaizu, H. , Nanba, K., Simidu, U. (1993). Phylogenic relationship of marine bacteria, mainly members of the family Vibrionaceae, determined on the basis of 16S rRNA sequences. *International Journal System Bacteriology* 43:8–19

Urakawa, H., Rivera, I. N. G. (2006). Aquatic Environment. In *The Biology of Vibrios*, ed. F.L. Thompson, B. Austin, and J. Swings, pp. 175-189. Washington D. C.: ASM Press

United States Department of Food and Agriculture (USDA). (2005). Most Probable Number Procedure and Table. In Laboratory Guidebook. United States Department of Agriculture, Food Safety and inspection Service, Office of Public Health Science. pp. 1-8

Valle, J., Re, D. S., Henry, N., Fontaine, T., Balestrino, D., Latour-Lambert, P. (2006). Broad-spectrum biofilm inhibition by a secreted bacterial polysaccharide. *Proceedings of the National Academy of Sciences* USA, 103, 12558–12563

Vandamme, P., Pot, B., Gillis, M., de Vos, P., Kersters, K., Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematic. *Microbiological Reviews* 60: 407-438

Wan, J., Wilcock, A. and Coventry, M.J. (1998) The effect of essential oils of basil on the growth of *Aeromonas hydrophila* and *Pseudomonas* fluorescens. *Journal of Applied Microbiology* 84, 152–158

Wang, R., Huang, J., Zhang, W., Lin, G., Lian, J., Jiang, L., Lin, H., Wang, S., Wang, S. (1997). Detection and identification of *Vibrio parahaemolyticus* by multiplex PCR and DNA-DNA hybridization on a microarray. *Journal of Genetics and Genomic 38*(3):129-135

Wekell, M. M., Manger, R., Kolburn, K., Adams, A.a dn Hill, W.(1994). Microbiological quality of Seafoods: viruses, bacteria and parasites. InShahidi F., &Botta R. J (Eds). *Seafoods Chemistry, Processing Technology and Quality* pp : 220-232

Welsh, J. and McClelland, M. (1990). Fingerprinting genome using PCR with arbitrary primers. *Nucleic Acid Research* 18: 7213-7218

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. (1990). DNA polymorphisms applied by arbitrary primers useful as genetic markers.*Nucleic Acid Research* 18: 6531-6535

Yearsley, G.K., Last, P.R. and Ward R.D. (eds) (1999). Australian seafood handbook: an identification guide to domestic species, CSIRO, Hobart. ISBN 0643 06194 0

Yoshitsugu Nakaguchi (2013). MHContamination by *Vibrio parahaemolyticus* and Its Virulent Strains in Seafood Marketed in Thailand, Vietnam, Malaysia, and Indonesia. Tropical Medicine and Health 41 No. 3, 2013, pp. 95-10

Zhang, L., D. Pornpattananangkul, C. M. and Huang. C. M. (2010). Development of nanoparticles for antimicrobial drug delivery. *Current Medicinal Chemistry* 17(6): 585-594

Zink, D. L. (1997). The impact of consumer demands and trends on food processing. *Emerging Infectious Diseases*, 3(4), 467-469. http://dx.doi.org/10.3201/eid0304.970408

Zottola E.A. and Sasahara K.C. (1994): Microbial bio− films in the food−processing industry - should they be a concern. *International Journal of Food Microbiology*, 23: 125–148

Zulkifli, Y., Alitheen, N. B., Raha, A. R., Yeap, S. K., Marlina, Son, R. and Nishibuchi, M. (2009). Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from cockles in Padang, Indonesia. *International Journal of Food Research* 16: 53-58

Zulkifli, Y., Alitheen, N. B., Son, R., Yeap, S. K., Lesley, M. B. and Raha, A. R. (2009). Identification of *Vibrio parahaemolyticus* isolates by PCR targeted to the *toxR* gene and detection of virulence genes. *International Food Research Journal 16*: 289-296.